Szent István University

Doctoral School of Environmental Science

Improvement and Application of Analytical and Ecotoxicological Methods Suitable for Judgement of Surface Waters and Sediments

Abstract of the PhD thesis

Gábor Endre Halász

Gödöllő
2010

The Doctoral School

Name: Szent István University, Doctoral School of Environmental Science

Discipline: Environmental Science

Leader: Dr. György Heltai
Professor, Head of Department, Doctor of the Hungarian Academy of Science
SZIU Faculty of Agricultural and Environmental Science
Department of Chemistry and Biochemistry, Institute of Environmental Science

Supervisor: Dr. György Heltai
Professor, Head of Department, Doctor of the Hungarian Academy of Science
SZIU Faculty of Agricultural and Environmental Science
Department of Chemistry and Biochemistry, Institute of Environmental Science

....

Approval of the School Leader Approval of the Supervisor

1. INTRODUCTION AND OBJECTS

A new trend has formed in the research of environmental risk assessment of heavy metal pollution since the 1990s. Methods dealing with the qualitative and quantitative determination of individual chemical forms of the elements in question have pushed conventional assessment based on the determination of total concentration into the background. This intensive progress has led to a new terminology. Numerous papers were published using terms like "chemical speciation", "operational speciaton" etc. in different meanings. In order to use a consistent terminology in this field the IUPAC has proposed the usage of the following terms (Templeton et al. 2000):

- *Chemical species.* Specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure
- *Speciation analysis.* Analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample
- *Speciation of an element; speciation.* Distribution of an element amongst defined chemical species in a system
- *Fractionation.* Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties. (referred to as operational speciation earlier, a simple and relevant alternative for total chemical speciation)

Research and methodological studies in the field of environmental risk assessment with regard to heavy metals have been performed at the Department of Chemistry and Biochemistry of the SZIU for several decades. To complement the sequential extraction fractionation recommended by the EU BCR. which is based on application of aggressive solvents, a new method has been developed, that preserves original species for further ecotoxicological investigation. As a PhD student I joined these projects. My activity was primarily connected with the survey of environmental status of rivers and lakes and the development of methods for this purpose. I have taken part in the following projects:

- Assessment of the environmental status of the Rákos stream and the attached fish pond system
- Methodological study on the development of a monitoring network according to the EU Water Framework Directive (based partly on the foregoing work)
- Study of the status of the Tisza and Szamos rivers in German-Hungarian cooperation after the contamination of the rivers in 2000
- Improvement of sequential extraction for fractionation of easily mobilisable element content of surface water sediments, soils and gravitation dust sediments (in connection with the foregoing projects)

In my activity I have set myself to improve and apply new methods connected with the above fields of research:

1. Improvement and optimisation of the novel three-step extraction, that applies supercritical CO_2, subcritical H_2O and their mixture for fractionation of easily mobilisable element content of surface water sediments, soils and gravitation dust sediments and was elaborated at our department (Heltai et al. 2002), for extraction of samples with different $CaCO_3$ content.
2. Connection of simple bioassays for the assessment of ecotoxicological potential of surface water sediments with different sediment extracts, comparison of the applicability of various bioassays (Pollen Tube Growth Test, Ecotoxicological Stable Isotope Metabolic Assay, cell viability assays).
3. Application of the above methods in the assessment of the status of the Rákos stream and Tisza river connected with the monitoring according to the EU Water Framework Directive.

2. MATERIALS AND METHODS

2.1 Studies on the environmental load of rivers and lakes

2.1.1 Monitoring on the Rákos stream water system

Studies on the environmental status of the Rákos stream and the fish pond system between Gödöllő and Isaszeg have been in progress since the 1990s at our department. Fekete (2002) studied the pollution ot the ponds with plant nutrients and heavy metals using sediments as documents of the ponds' history, she also estimated the rate of sedimentation and age of polluted levels in the sediments. She produced a valuable database, the collected sediment samples were used for latter studies. In 2004 the project "Development of a complex monotoring system and data base for small water catchment areas in Hungary according to the Water Framework Directives of the European Union" submitted by a consortium of the SZIU Department of Chemistry and Biochemistry, Budapest University of Technology and VITUKI, was supported by the Hungarian Ministry of Eduction. My task was primarily the arrangement of the monthly monitoring of Rákos stream, the processing of its results and the study of point sources (treated sewage inlets). Four water bodies were designated on the stream: Four water bodies were identified: the first one from the source as far as the first fish pond, the second one includes the fish ponds, the third one spreads from Isaszeg to the border of Budapest, and the last one inside Budapest to the Danube. Fifteen sampling sites were involved in the monthly monitoring programme between May 2004 and April 2005, it included on site (water output, temperature, O_2 saturation, pH, conductivity, redox potential) measurements and water sampling for laboratory investigation. Mass flows of some components were estimated on the basis of water output and concentration. Loads of some pollutants were also estimated using the self-control data of sewage treatment plants.

2.1.2 Studies on the Tisza and Szamos rivers

In June 2000 experts of the UFZ (Centre for Environmental Research Leipzig-Halle) performed a sampling expedition on the Tisza and Szamos rivers. Water and sediment samples for analytical and ecotoxicological studies were taken at thirteen sites. Our department has joined the project. Water samples were analyzed in Magdeburg, ecotoxicological tests were performed in Leipzig. My task was the study of aqueous sediment extracts with plant bioassays. Sampling was repeated in March 2001 and May 2002.

2.2 Samples

2.2.1 Surface water sediments

River sediment samples have been taken within the scope of the UFZ sampling expeditions. The upper 10 cm layer of the sediment was sampled. Samples were transported in plastic boxes at 4°C.

Lake sediments were from the fish pond system in the Rákos valley between Gödöllő and Isaszeg. Core and mixed samples were taken in July 1995 (Heltai et al. 1998). Samples were transported in polyethylene boxes or clingfilm in a cool box into the laboratory. Wet core samples were cut to segments of 5 cm. Mixed samples were taken in September 2002, which were used partly to gain porewater or freeze-dried.

2.2.2 Soils

Soil samples used in the kinetic study of H_2O/CO_2 extraction were from the collection of the Department of Soil Science and Agrochemistry. Their $CaCO_3$ content was determined by Scheibler's calcimetry:

Sample No. 107 from Mosonmagyaróvár (Danube alluvium, loam), 22.1% $CaCO_3$, humus 2-3%
Sample No.124 from Nagyhörcsökről (loam built on loess), 4.8% $CaCO_3$, humus 3-3.5%
Sample No.126 from Kecskemétről (sand, Danube/Tisza alluvium), 3.8% $CaCO_3$, humus 1%

2.2.3 Gravitation dust sediment

Gravitation dust sediment sample was collected by the Technical University of Košice with the Bergerhoff method in Košice city. The content of the samplers was transferred to platinum pots, dried on water bath, finally homogenised in an agate mortar (Remeteiová et al. 2007).

2.3 Extraction

2.3.1 Gaining of pore water

100 grams of the wet sediment was weighed in centrifuge tubes and centrifuged with 8600 min^{-1} for 30 minutes. The supernatant was decanted and filtered through filter paper.

2.3.2 Cold water leaching

25 grams of air-dried or freeze-dried sediment samples and 100 cm^3 bidistilled water were weighed into 500 cm^3 screw capped Schott-Duran flasks. The flasks were shaken at 10 min^{-1} for 24 hours in a átfordítós shaker at room temperature. Decanted extracts were filtered through filter paper.

2.3.3 Sequential extraction in a supercritical fluid extractor

Three-step extraction developed earlier at our department was performed in a Jasco supercritcal fluid extractor consisting of two HPLC pumps (one of them was cooled for liquid CO_2 transport), a column oven and a back pressure regulator (restrictor). Liquid CO_2 was taken from a bottle with a dip-tube. The extractions were performed under the following conditions:

Adjustment	1st step	2nd step	3rd step
Column oven temperature, °C	80	80	80
Pressure, MPa	27	27	27
CO_2 flow rate, cm^3 min^{-1}	1	0	0,1
H_2O flow rate, cm^3 min^{-1}	0	1	1
Restrictor temperature, °C	60	60	60
Duration, min	30-60	30-60	30-90

The soil or sediment samples were mixed with SiO_2 prior to filling into the columns, which are stainless steel tubes with screw-caps on both ends, with a built-in filter in the cap at the bottom. First 1 g of SiO_2 was filled into the column, then the mixture of the sample and SiO_2, finally the residual volume was filled with SiO_2 again. The extracts were collected into plastic flasks containing 0.5 mol dm^{-3} HNO_3. Kinetic study on the third step of the extraction was performed in experiments with a duration of 4 hours, sampling frequency was 10 minutes (into polypropylene centrifuge tubes).

2.3.4 Elemental analysis

Sediment extracts were analysed with a Jobin Yvon JY 24 inductive coupled plasma optical emission spectrometer (ICP-OES), operational parameters were set according to the manufacturer's instructions. Calcium concentration of the extract series in the kinetic study was measured with a Buck 200 AAS atomic absorbtion spectrometer in emission mode at a wavelength of 422.7 nm. Determination of the element contents of the extracts gained by modified three-step extraction was done by Dr. Éva Széles at the University of Debrecen and at the Institute of Isotopes of the HAS, respectively. Concentration of Al, Ca, Fe, K, Na, P, S was measured with a Perkin Elmer Optima 3300 DV ICP-OES equipment, whereas concentration of As, B, Ba, Be, Cd, Ce, Co, Cr, Cu, Er, Gd, Ge, Hg, Ho, In, La, Li, Lu, Mg, Mn, Mo, Ni, Pb, Sc, Se, Sr, Ti, V, Y, Yb, Zn was determined with a Thermo Elemental X series ICP mass spectrometer.

2.4 Ecotoxicological studies

2.4.1 Pollen Tube Growth Test (PTG)

General ecotoxicological testing of aqueous extracts was performed using Pollen Tube Growth Test (PTG) in the UFZ, Leipzig under the supervision of Prof. Dr. habil Klaus Jung. Pollen were isolated from anthera of sterile *Nicotiana sylvestris* variants and kept at -18°C. The principle of the method is that pollen is suspended in a medium and pollen tubes begin to grow, this process is influenced by the composition of the medium (e.g. pollutants). After 18 hours exposition pollen tube growth is terminated by adding Alcian Blue dye to the medium, which is adsorbed on the surface of the pollen tubes. Finally, the adsorbed dye is redissolved with citric acid and measured photometrically (Kristen et al. 1999).

Data processing was done using Microsoft Excel. Outliers were identified with Dixon's test at 95% confidence level. Mean of the absorbances of the blanks was subtracted from the samples' absorbances. The corrected absorbances were averaged for each treatment, finally inhibition of pollen tube growth in percent of the control was calculated. Results were depicted as bar graphs showing confidence intervals at 95% confidence level. Response of the method against clean solutions with different concentrations of Cu, Pb, Cd and As was also studied, IC_{50} values and their confidence intervals were calculated by Graphpad Prism software.

2.4.2 Ecotoxicological Stable Isotope Metabolic Assay (ESIMA)

Several phytotoxicity assays based on ^{15}N stable isotope tracing technique were developed in the UFZ. I have used garden cress *(Lepidium sativum)* in hydroculture and pea *(Pisum arvense)* epicotyl assays for the study of aqueous extracts. In the cress test 5 days old seedlings that grew on nitrogen-free medium were incubated with a mixture of the extracts and medium containing $^{15}NH_4Cl$ for 5 hours, then the sprouts were cut and digested with H_2SO_4 in a Kjeldahl apparatus. Finally, excess of ^{15}N, which is in proportion to the incorporated quantity of nitrogen, was measured with a NOI-7 emission spectrometer. In case of the pea test epicotyl segments of ethiolated, 2 days old seedlings were shaken with a mixture of the extracts and medium containing $^{15}NH_4Cl$ for 5 hours. Digestion and measurement of ^{15}N excess were performed as described above. Data processing was done as described by the PTG test.

2.4.3 Cell viability assays with *Epithelioma papillosum cyprini* (EPC) cell line

I performed the experiments with cell cultures in the UFZ under the supervision of Dr. Kristin Schirmer. Cells were grown to confluent monolayer in culture flasks in Minimal Essential Medium (MEM) containing 10 *V/V*% Fetal Bovine Serum (FBS) at 18°C. Cell viability assays were carried out on 96-well culture plates, 150.000 cells were placed into each well. 24 hours later the cells were treated with the test solutions for 48 hours. Treatments were done in 5 parallels. Series of solutions containing copper were diluted from sterile 600 mmol dm^{-3} (38,13 g dm^{-3}) $CuCl_2$ with MEM with or without FBS. Benzo[*a*]pyrene (BaP) test solutions were prepared from a stock solution in dimethyl sulfoxide in concentrations 200-times higher than test concentrations in the wells, then 1 µl of the test solution was added into each well. After the treatments cell viability was studied with alamar Blue, carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) and neutral red fluorescent dyes. Fluorescence of the wells was measured with a SpectraMAX Gemini microplate reader.

Data processing was done with Microsoft Excel. Mean and standard deviation of parallels were calculated, then mean of the blank was subtracted from the treatments' means. Finally, corrected means and standard deviations of the treatments were expressed in percent of the control and plotted as a function of Cu^{2+} or BaP concentration. EC_{50} values and their confidence intervals were calculated with Graphpad Prism.

3. RESULTS

3.1 Sequential extraction with CO_2/H_2O solvents

3.1.1 Sediment analysis

Total element content of the sediment samples collected on the Tisza and Szamos rivers and fish pond No. VII between Gödöllő and Isaszeg was determined by ICP-OES after microwave assisted digestion with HNO_3/H_2O_2. Results were compared to analysis data of the core sample taken in 1995 from the fish pond and to threshold limits for soils and sewage sludges for agricultural application (KöM-EüM-FVM-KHVM Decree No. 10/2000. and Government Decree No. 50/2001, Appendix No. 5). From the results it can be stated that:

- Zinc and cadmium concentration in sediment samples from Tiszaadony and Olcsva and cadmium concentration in sample from Tiszavalk were significantly higher than the threshold limits for soils, concentrations of the other four elements in study were under the limits.
- Upper layer of the sediment from fish pond No. VII (1995) was highly contaminated: all six elements' concentrations were over the limits, in case of zinc and cadmium with more than an order of magnitude. Concentrations of chromium and cadmium almost reached (total chromium, 0-5 cm layer) or exceeded (total chromium, 0-10 cm mixed, cadmium in both samples) the limits for field deposition of sewage sludges . Element concentrations in the layers below this contaminated one were under the threshold limits for soils.
- Pollution level of the sediment from the fish pond No. VII (2002) is similar to the samples from Tisza and Szamos rivers, although its zinc content is 53%, cadmium contend 32% higher than the threshold limit for soils.

In order to estimate the easily mobilisable element content of the sediment samples taken in 2002, the samples were extracted with the novel three-step sequential ($CO_2/H_2O/CO_2+H_2O$) procedure (Heltai et al. 2002). Duration of each step was 30 min. Concentration of zinc, cadmium, lead, nickel, chromium and copper in the extracts was measured with ICP-OES módszerrel. Easily mobilisable concentrations compared to pseudototal concentrations showed that mobility of zinc is the highest among the six elements (as found by BCR fractionation as well), however, mobility which was estimated with the new three-step method is much lower than the mobility calculated from the results of the BCR's 1st step fraction.

3.1.2 Kinetic study on the extraction with CO_2/H_2O mixture in the supercritical fluid extractor

The kinetic study of the third step of the novel three-step extraction, which was developed at our department, was performed using three soil samples with different $CaCO_3$ content. This step is modelling the mobilisation of the element content bound to carbonates, and it was assumed that the time needed for total dissolution of this fraction is influenced by the $CaCO_3$ content of the original sample. Sample amounts of 0.5 g and 5 g were weighed in (mixed with SiO_2), respectively. Total time of extraction was 4 hours, extracts were collected in 10-minute fractions. Calcium concentration of the samples was measured by atomic emission spectrometry. Mass of the extracted calcium and the rate of dissolution was calculated and plotted as a function of time. The results showed that by the extraction of 5 g sample (sample-SiO_2 ratio cca. 1:1) the rate of dissolution is constant (and identical) for cca. 80 min in case of the samples from Mosonmagyaróvár (with a very high $CaCO_3$ content) and Nagyhörcsök. In these cases the dissolution is controlled basically by the system parameters (solvent composition and flow rate, pressure, temperature). This phase determined the whole time of extraction in case of the Mosonmagyaróvár sample, by the Nagyhörcsök sample the rate of dissolution fell after 80 min, whereas in case of the Kecskemét sample it was falling permanently from the beginning of the extraction. After the reduction of the sample weight to 0.5 g (sample-SiO_2 ratio cca. 1:20), the rate of calcium dissolution had a decreasing tendency from the start of the extraction by all three samples. Extraction of the Nagyhörcsök and Kecskemét samples was completed after 90 min, by the Mosonmagyaróvár sample 210 min were necessary.

3.1.3 Modified sequential extraction of soil, sediment and gravitation dust sediment samples

On the basis of the observations of the kinetic study the duration of the steps and the sample weight in the original three-step H_2O/CO_2 extraction (that was developed for extraction of samples with moderate $CaCO_3$ content) were modified. With the modified procedure the easily mobilisable element contents of the soil sample from Nagyhörcsök, the sediment sample from the fish pond No. VII between Gödöllő and Isaszeg and the gravitation dust sediment sample from Košice were fractionated. Duration of the first and second steps (extraction with supercritical CO_2 and subcritical H_2O, respectively) was 60 min, the third one (90% H_2O, 10% CO_2) lasted 90 min. Sample weight was 0.5 g. A procedure blank extraction was performed on a column filled with 10 g SiO_2. Analysis of the extracts was performed by Dr. Éva Széles at the University of Debrecen and the Institute of Isotopes of HAS, respectively with ICP-OES and ICP-MS. On the basis of the distribution of the lead, copper, zinc and cadmium (as elements that can be determined with certified values with the BCR sequential extraction) in the different fractions it can be stated that mobilisable lead, copper and zinc content of the gravitation dust is much higher than in the other samples, its cadmium content is close to the sediment's. In case of the dust sample the carbonate-bound fraction is dominant for all four elements The sediment sample has significant zinc and cadmium content in the carbonate-bound fraction, whereas most of its lead content is in the CO_2-soluble (organic-bound) fraction. These observations agree with the results of former studies on the fish pond sediments with BCR extraction. Soil sample has similar lead content as the sediment, but mainly in the carbonate-bound fraction. Ascendent order of the pollution level for the three samples: soil, sediment, gravitation dust. The correction with blank measurements resulted in negative concentrations in case of some elements and samples, therefore the determination of these elements was not reliable. Boron could only be detected from the CO_2 extract of the dust sample, copper from the H_2O and H_2O/CO_2 extracts of the dust sample, nickel from the H_2O/CO_2 extract of the sediment. (Arsenic, barium, calcium, manganese, phosphorus and titan was detectable from the H_2O and H_2O/CO_2 extracts of all three samples.) Uncertainty of the determination is increased by the dilution of the sample with SiO_2 and the dilution of the extracts because of the longer extraction times. Further efforts should be made to minimize the effects of these phenomena.

3.2 Ecotoxicological tests

3.2.1 PTG model experiments with single toxic elements

Effects of different toxic elements in the PTG assay was studied with solutions containing different concentration of arsenic, cadmium, lead and copper. Results showed that dose-response relationships are well detectable in simple systems. The four elements inhibited the pollen tube growth to a different extent, ascendent order of the inhibition effect was $Cu^{2+} \approx Pb^{2+} < Cd^{2+} <$ As(III).

3.2.2 Bioassays with extracts of sediments collected in June 2000 on the Tisza and Szamos rivers

Ecotoxicological testing of extracts made with cold water leaching of sediments was performed using three assays: PTG, ESIMA with pea epicotyls and cress in hydroculture. The pollen strains responded differently to the extracts, significant inhibition could not be detected. The sample from Tiszaadony seemed to be more toxic than other samples with a slight inhibition effect of 20% (Csenger, Olcsva, Tiszavalk, Kisköre, Tiszasziget), however, this difference is not significant due to the relatively high uncertainty. Six samples had negative inhibition, which means they stimulated the pollen tube growth. In ESIMA with pea epicotyls extracts of sediments from Olcsva and Tivadar were slightly toxic, their inhibition of nearly 30% is significantly higher than by the other samples (although four of them were stimulating). In the cress test sediment extract from Csenger caused the highest inhibition which was significantly higher than by six other samples (Tiszaadony, Tokaj, Tiszalök, Kisköre, Tiszakécske, Mindszent; four of them were stimulating). Toxicity of the sediments from Csenger and Olcsva can be supported by the enrichment of some toxic elements found in these samples.

3.2.3 Bioassays with extracts of sediments collected in March 2001 on the Tisza and Szamos rivers

Ecotoxicological investigation of extracts made with cold water leaching of sediments was carried with PTG and ESIMA with pea epicotyls. There was no unambiguous difference between the pollen strains in the tests. Similar to the results with the samples taken in 2000, no significant inhibition could be detected. Four samples seemed to be slightly toxic with 20-25% inhibition (Olcsva, Tiszaadony, Tiszakeszi, Tiszasziget), three samples (Tiszavalk, Tiszasziget, Mindszent) caused slightly higher inhibition, but the differences were not significant. In the pea epicotyl tests the sample extract from Tiszalök showed the highest inhibition, which was significantly higher than by the other samples except for Mindszent and Tápé. These latter samples from the lower Tisza region were slightly more toxic than the other ones, but these differences were not significant.

3.2.4 Bioassays with extracts of sediments collected in September 2002 on the Szamos and Tisza rivers and fish pond No. VII by Gödöllő

Pore water samples, extracts made by cold water leaching or subcritical water extraction were tested with PTG, ESIMA with pea epicotyls and cress in hydroculture. In the PTG test there was no difference between the two pollen strains. Toxicity of cold water leachates was in all three cases (Olcsva, Tiszavalk, Kisköre) lower than that of pore water samples. Subcritical water extract of sediment from Olcsva was significantly more toxic than its cold water leachate. The inhibition caused by cold water extracts was similar two those from 2000 and 2001, it can be assumed that toxicity of the sediments didn't change significantly. Pea epicotyl tests showed that cold water extracts had a similar or lower inhibition effect to the foregoing years, no significant difference could be detected between the samples. Pore water from Olcsva seemed to be slightly toxic with 20% inhibition, other two samples were stimulating. In the cress test the inhibition of pore water samples was higher than that of cold water extracts (just as by PTG), but only in case of the sediment from Tiszavalk was the difference significant (its cold water extract was stimulating). There was no significant difference between the extracts of the same type. Results of both ESIMA tests point to no changes in the sediment toxicity compared to the foregoing years.

3.2.5 Model experiments with EPC cell line

Cotoxicity of heavy metals and polyaromatic hydrocarbons (PAH) in EPC cell line was studied in simple experiments. Copper (as sterile $CuCl_2$ solution) was uses as heavy metal and benzo[*a*]pyrene (BaP) as PAH. Both substances were applied independently and together. Effect of fetal bovine serum (FBS) in the medium was also investigated. Cell viability was studied by fluorimetric measurement of the uptake or transformation of three dyes (CFDA-AM, alamar Blue, neutral red).

Protecting effect of FBS in the medium was verified by the dose-response relationships recorded with different $CuCl_2$ concentrations. Decrease in cell viability was observed by concentrations about twice higher if the medium contained FBS according to all three assays. Cytotoxitity of benzo[*a*]pyrene could only be detected with neutral red assay in medium without FBS. Simultaneous application of $CuCl_2$ and BaP was performed in two experiments in medium without FBS. In the first one each substance was applied in one concentration independently and together (Cu^{2+}: 19.07 mg dm^{-3}, BaP: 378.5 μg dm^{-3}). Simultaneous application of the substances resulted a decrease in cell viability according to the three assays, the greatest increase in the inhibition was observed with CFDA-AM. In the second experiment five different concentrations of $CuCl_2$ in the range 12.71-25.42 mg dm^{-3} alone and in the presence of 189.2 μg dm^{-3} BaP. The toxicity of copper was increased by BaP in this case too, however, this effect was less significant.

3.3 Chemical status of Rákos stream and effect of point sources

3.3.1 Chemical status on the basis of the 12-month monitoring

Mean and relative standard deviation of basic chemical parameters of the water samples collected during the surveillance monitoring 2004-2005 were calculated for each water body. High values of relative standard deviation represent the spatial and temporal variability together in case of some parameters. A special sampling that involved 13 sites along the stream inside Budapest was performed for analysis of the spatial variability of chemical and physico-chemical parameters. After the chemical analysis of the water samples Eszter Szilágyi has studied the correlation between the relative error and the number of the samples by estimation ot the confidence intervals (László et al. 2006). The results showed that one sampling point is theoretically enough for the characterisation of a water body for some parameters (chloride, chemical oxygen demand (COD), total hardness). However, more changeable parameters can require an extremely high number of sampling points (e.g. at least 9 points for ammonium) to get statistically satisfactory information about the water body. Total and dissolved heavy metal concentrations in the water samples were near or under the detection limits. Anthropogenic load can be presented in relatively high concentrations of nitrate, ammonium, chloride, phosphate and COD.

3.3.2 Temporal variability of chemical parameters

The monthly frequency of sampling, that was applied in the surveillance monitoring, has not proved to be sufficient for the reliable assessment of the temporal variability of the studied components. Further model studies are required in order to determine the adequate sampling frequency for each component, which makes the reliable characterisation of a given water body possible in the five-graded classification according to the WFD.

3.3.3 Effect of point sources on the chemical status

Effects of the sewage treatment plants in Gödöllő, Isaszeg and Pécel – as known point sources – on the water quality of the Rákos stream were studied. Mass flows of some components in the stream were calculated from the water output values (measured on site) and the concentrations in water samples in the period May 2004 – April 2005.. These mass flows were compared to the loads calculated from the self-control data of the sewage treatment plants. Maximums in the mass flow of total phosphorus were found in May and June, highest values were found by the sampling sites in Budapest, Pécel and Isaszeg. Mass flows by the sites in Gödöllő were in every month well below them of the other ones. Phosphorus load with the treated waste water from Gödöllő plant presumably increases the mass flow by the sampling sites farther away, the other two plants have probably a smaller effect on the water quality. However, classification of all water samples with regard to total phosphorus resulted in “excellent” quality in the whole period. Estimated mass flows of three nitrogen forms were more varied than that of total phosphorus. Nitrate dominated among the nitrogen forms studied in the stream except for June. From November until March classification of the water samples with regard to nitrate resulted in “polluted” quality. From May to October and in April samples from Isaszeg, Pécel and Gödöllő were of “tolerable” or “good” quality, samples from Gödöllő were worse. Nitrogen input with the treated waste water from Gödöllő was much higher than other mass flows, it was on average 30-50-times higher than load of the Isaszeg and Pécel plants and cca. four times higher than the mass flow in the stream inside Budapest. Quantity of the very toxic nitrite in the treated waste water from Gödöllő plant was significant in some months, especially in November. This problem can also be seen in the classification of the water samples taken farther away: in June and July several samples were of “very polluted” quality, and only the samples from Gödöllő taken in June were better than “tolerable”. In June (and partly in July) ammonium was also significant in the total nitrogen mass flow. Classification of the water samples from Isaszeg, Pécel and partly they from Budapest with regard to ammonium resulted in “very polluted” quality in May, June, July, February and March.

3.3.4 Estimation of the load from the sewage treatment plant in Gödöllő based on former sediment studies

The load of the fish pond No. I with phosphorus, lead, nickel and copper was estimated using the data of Fekete (2002), which is connected with the input of the treated sewage, as it was lead directly into this pond earlier. Now the treated sewage reaches the Rákos stream after the pond chain through a canal, so that its load can be found at the sampling site in Isaszeg. Mass flows of the four components in the stream by the last sampling site before the pond chain were subtracted from the mass flows in the stream by Isaszeg to estimate the load caused by the treated sewage. These values were compared to them calculated from sediment studies, in some cases the values were of the same order.

Excess kg/month *calculated from*	**P**	**Pb**	**Ni**	**Cu**
Water analysis	939	4.9	4.3	0.9
Sediment analysis	107	3	5.7	7

4. NEW SCIENTIFIC RESULTS

1. The novel sequential extraction procedure developed at our department, which is based on the application of supercritical CO_2, subcritical H_2O and their mixture as non-toxic and cheap solvents, was improved according to kinetic studies. It was established that element content bound to carbonates, that can be easily mobilised if concentration of free CO_2 increases, can be dissolved completely if the sample weight is lower and the extraction time is longer than in case of the original procedure. The method was capable of fractionating the water soluble and carbonate-bound heavy metal content of soils, sediments and gravitation dusts separately when coupled with an analytical technique of good detection power (ICP-MS). However, the increase in the solid dilution of the sample and in the dilution of the extracts due to the longer extraction time resulted in higher blank values, which increases the uncertainty of the determination of element concentrations. Element content of a lake sediment, a soil sample and an urban gravitation dust was fractionated with the optimised sequential extraction. The easily mobilisable heavy metal content was the highest in the dust sample, the least in the soil sample.

2.a Well measurable dose-response relationship was found in the Pollen Tube Growth test (PTG) in simple model systems using single toxic elements (copper, lead, cadmium, arsenic). Arsenic was found to be the most toxic, whereas copper and lead were the least toxic.

2.b Ecotoxicity of different aqueous extracts of surface water sediments exposed to heavy metal contamination (pore water, extracts gained by cold water leaching and subcritical water extraction) was studied with PTG and Ecotoxicological Stable Isotope Metabolic Assay (ESIMA). PTG as a simple and quick method was found to be capable of assessing general ecotoxicity of sediments. However, in contrast to the model study, close correlation between the detected toxicity of the extracts and the heavy metal content of the sediments could not be found.

2.c Results of the more complex ESIMA tests refer to higher toxicity in case of some samples. Assessment of the effects of real samples, which are exposed to several kinds of contamination, is made more difficult because the response measured in the test is a result of many physiological processes, thus it is more complex than in case of PTG. Sediment extracts can contain plant nutrients as well, so that their stimulating effect can compensate the inhibition caused by pollutants. Effects of the heavy metal pollution of Tisza and Szamos rivers in 2000 on the toxicity of sediments could not be verified with ESIMA tests, either.

2.d Pore water was found to be the most suitable for ecotoxicological tests among the differnt kinds of extracts, applicability of extracts gained by cold water leaching is limited.

2.e Cotoxicity of heavy metals and PAHs in aquatic environment was studied with model experiments. Cell viability assays (neutral red, CFDA-AM, alamar Blue) with cell cultures of EPC cell line showed that toxicity of copper chloride was increased by the model PAH benz[*a*]pyrene, which was hardly cytotoxic if applied alone.

3.a Extracts of sediment samples collected after the heavy metal pollution of the Tisza and Szamos rivers in 2000 were studied with ESIMA bioassays. Samples from Csenger and Olcsva (Szamos) and Tivadar (Upper-Tisza region) were found to be more toxic than other ones, this phenomenon can be connected with element accumulations detected in the sediments from Csenger and Olcsva. Unambigouos correlation between the results of the bioassays with sediments taken in 2001 and 2002 and the preceding heavy metal pollution could not be established.

3.b My studies within the 12-month surveillance monitoring on the Rákos stream made the calculation of the proper spatial frequency of sampling (according to the requirements on confidence of the EU WFD) possible for various components. It was also verified that measurements on one sampling site are not satisfactory for characterisation of a water body, except for a few parameters. Mass flow of several components and the load from sewage treatment plants as known point sources were compared. Effects of the treated sewage from the plant in Gödöllő on the mass flow of total phosphorus and nitrogen forms was demonstrated. Moreover, this was supported with calculation of the load with total phosphorus and heavy metals based on former sediment studies.

5. CONCLUSIONS AND PROPOSALS

The novel sequential extraction procedure, that is based on the application of supercritical CO_2, subcritical H_2O and their mixture as solvents, proved to be suitable for the estimation of easily mobilisable element content of surface water sediments, soils and gravitation dust sediments. Widening of the application of the method to other kinds of solid environmental samples (biofilm, sewage sludge etc.) and further kinetic studies on the second and third steps are planned, if an elemental analytical method with a reasonable detection limit is available.

Assessment of the applicability of bioassays was possible in case of the Rákos stream (as a stream expposed to general urban and industrial loads) and the Tisza river (as a river exposed to havaria-like pollution). The results showed that the measured response is influenced by factors other than the presumed pollution in case of real samples, therefore their role in the assessment of the chemical status is limited, they cannot replace the chemical fractionation as a risk assessment tool. Pollen Tube Growth test is simple, quick and cost-effective, thus it can be proposed for testing of sediments polluted with heavy metals (also for monitoring purposes). On the basis of the foregoing studies it is expected to be applicable in ecotoxicological studies with other kinds of samples (surface water, sewage etc.) as well.

Increase in toxicity as a result of simultaneous occurance of heavy metals and PAHs was well detectable with cell viability assays using EPC cells in the copper-benzo[a]pyrene model. Complex study of the problem with other metallic species and PAHs as well as real samples is suggested, not only in cell viability assays. Long-term target of the studies can be the clearing up of this phenomena on the molecular level.

Loose correlation found between the mass flows calculated from water studies and the self-control data of the sewage treatment plants show that more accurate investigation of the effects of point sources is necessary in case of streams with low water output and fluctuating water regime, therefore higher temporal frequency of sampling should be provided for the water body and the sources of pollution as well.

6. REFERENCES

10/2000. (VI. 2.) KöM-EüM-FVM-KHVM együttes rendelet a felszín alatti víz és a földtani közeg minőségi védelméhez szükséges határértékekről. (Decree of ministries about the threshold limits for the protection of ground water and geological medium, in Hungarian.)

50/2001. (IV.3.) Korm. rendelet a szennyvizek és szennyvíziszapok mezőgazdasági felhasználásának és kezelésének szabályairól. (Decree of the Hungarian Government about the agricultural application and treatment of sewage and sewage sludge, in Hungarian.)

Fekete I. (2002): A környezetterhelés komplex értékelése a Gödöllő és Isaszeg közötti tórendszerben. (Complex evaluation of the environmental loads in case of the pond chain Gödöllő-Isaszeg, in Hungarian) PhD thesis, SZIU Gödöllő.

Heltai Gy., Fehér B., Percsich K., Barabás B., Fekete I. (2002): Application of sequential extraction with supercritical CO_2, subcritical H_2O, and an H_2O/CO_2 mixture for estimation of environmentally mobile heavy metal fractions in sediments. *Anal. Bioanal. Chem.*, **373**, 863–866.

Heltai Gy., Fekete I., Gémesi Z., Percsich K., Flórián K., Tarr Zs. (1998): Environmental evaluation of a local lake chain affected by wastewater by means of spectrochemical analytical methods. *Microchem. J.*, **59**, 125-135.

Kristen U., Jung K., Pape W., Pfannenbecker U., Rensch A., Schell R. (1999): Performance of the Pollen Tube Growth test in the COLIPA Validation Study on Alternatives to the Rabbit Eye Irritation Test. *Toxicol. In Vitro*, **13**, 335-342.

László B., Szilágyi F., Szilágyi E., Heltai Gy. (2006): Kisvízfolyások Víz Keretirányelvnek megfelelő monitorozó rendszerének megalapozó vizsgálata fiziko-kémiai és kémiai minőségi elemekre. Magyar Hidrológiai Társaság XXVI. Vándorgyűlése, Pécs, 2006 július 5-6. (CD melléklet, 1szekcio\240104.doc)

Remeteiová D., Sminčáková E., Flórián K. (2007): Study of the chemical properties of gravitation dust sediments. *Microchim. Acta*, **156**, 109–113.

Templeton D.M., Ariese F., Cornelis R., Danielsson L.G., Muntau H., Van Leeuwen H.P., Lobinski R. (2000): Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches. *Pure Appl. Chem.*, **72**, 1453– 1470.

7. PUBLICATIONS LINKED TO THE THESIS

Papers published in international journals in English

Heltai Gy., Percsich K., Halász G., Jung K., Fekete I. (2005): Estimation of ecotoxicological potential of contaminated sediments based on a sequential extraction procedure with supercritical CO_2 and subcritical H_2O solvents. *Microchem. J.*, **79**, 231-237. IF: 1.806

Halász G., Szlepák E., Szilágyi E., Zagyva A., Fekete I. (2007): Application of EU Water Framework Directive for monitoring of small water catchment areas in Hungary, II. Preliminary study for establishment of surveillance monitoring system for moderately loaded (rural) and heavily loaded (urban) catchment areas. *Microchem. J.*, **85**, 72-79. IF: 1.800

Rusnák R., Halász G., Horváth M., Remeteiová D. (2009): Intensification of the BCR sequential extraction with sonication for sediments, soils, and gravitation dust sediment samples. *Toxicological & Environmental Chemistry*, accepted for publication.

Proceedings

Halász G**.**, K. Jung, Heltai Gy. (2003): Előkísérletek növényi biotesztek alkalmazására üledékek toxicitásának vizsgálatában. (Preliminary studies for application of plant bioassays to assess sediment toxicity, in Hungarian.) Scientific meeting of the Institute for Chemistry, University of Western Hungary, Sopron, 7. November 2002, pp. 19-21.

Fekete I., Halász G., Percsich K., Heltai Gy. (2003): A Gödöllő-Isaszeg közötti tórendszer üledékében felhalmozódott nehézfémek mobilitásának becslése szekvens extrakciós és ökotoxikológiai módszerekkel. (Estimation of the mobility of heavy metals accumulated in the sediments of the Gödöllő-Isaszeg pond chain, in Hungarian.) 46th Hungarian Symposium on Spectrochemistry, 30. June-2. July 2003, Szeged, pp. 57-60.

Halász G., Fekete I., Heltai Gy. (2005): Környezetterhelési transzport számítások a Gödöllő-Isaszeg tórendszerben az üledék és víz elemanalitikai vizsgálata alapján. (Calculations on environmental load transport of the Gödöllő-Isaszeg pond chain based on elemental analytical studies of sediment and water, in Hungarian.) 48th Hungarian Symposium on Spectrochemistry, Hajdúszoboszló, pp. 127-130.

Kruppiné Fekete I., Halász G., Szlepák E., Szilágyi E., Zagyva A., Csányi B., Heltai Gy. (2006): Előtanulmányok mérsékelten terhelt vidéki és erősen szennyezett városi vízgyűjtő területek felügyeleti monitoringjának tervezéséhez. (Preliminary studies on planning surveillance monitoring of moderately loaded rural and heavily polluted urban water catchment areas, in Hungarian.). XXIV. National Symposium on Hydrology, Pécs, 5-6. July 2006 (CD-ROM).

Abstracts

Halász G**.**, Heltai Gy., Schüürmann G., Schirmer K. (2001): The use of fluorescent microplate assays and a carp cell line to study the toxicological effects of copper and benzo[a]pyrene individually and in combination, X Hungarian-Italian Symposium on Spectrochemistry, Eger, 1-5 October 2001, p. 95.

Halász G**.**, Jung K., Szlepák E., Heltai Gy. (2001): Analytical and toxicological characterization of lake and river sediments by means of SFE sequential extraction and phytotoxicological tests, X Hungarian-Italian Symposium on Spectrochemistry, Eger, 1-5 October 2001, p. 96.

Hafner C., Halász G., Tümpling W., Jung K., Schüürmann G. (2002): Ecotoxicological investigations with sediments of the rivers Tisza (Hungary) and Saale (Germany). GDCh Jahrestagung 2002 Umweltchemie und Ökotoxikologie, Braunschweig, 2002. október 6-8. (ÖKO-11).

Halász G., K. Jung, Heltai Gy., Percsich K. (2003): Plant bioassays and heavy metal analysis of river sediments and sediment extracts. SETAC Europe 13th Annual Meeting, 27.04-01.05.2003, Hamburg, p. 146.

Heltai Gy., Kristóf J., Horváth E., Fehér B., Halász G. (2003): Fractionation of heavy metals in sediments. Colloquium Spectroscopicum Internationale XXXIII, Granada, Spain, 7-12 September 2003, p. 690.

Heltai Gy., Percsich K., Halász G. (2003): Fractionation of environmentally mobile heavy metals in sediments by means of sequential extraction with supercritical CO_2 and subcritical H_2O. XI Italian-Hungarian Symposium on Spectrochemistry, Venice (Italy), October 19-24, 2003, p. 49.

Halász G., Fekete I., Heltai Gy., Percsich K., Jung K. (2004): Heavy metal mobility and ecotoxicity relationships in lake sediments. SETAC Europe 14th Annual Meeting, 19-22.04.2004, Prague, p. 283.

Halász G., Szlepák E., Fekete I., Heltai Gy. (2004): Fractionation of heavy metal content in sediments by combination of subcritical H_2O and supercritical CO_2 solvents, 2nd International FESTEM Symposium on Trace Elements and Minerals in Medicine and Biology, 13-15.05.2004, Neuherberg (München), p. 59.

Halász G., Fekete I., Heltai Gy. (2005): Berechnungen des Belastungstransports aufgrund elementaranalytischer Untersuchungen von Sediment und Wasser am Rákos Bach (Ungarn), SETAC-GLB, 10. Tagung, Basel, p. 208 (2005).

Halász G., Fekete I., Szilágyi E., Csányi B., Heltai Gy. (2005): Application of the EU Water Framework Directive for monitoring of small water catchment areas in Hungary III. Streams in heavily loaded urban areas. XII Hungarian-Italian Symposium on Spectrochemistry: Environmental Pollution and Human Health, Pécs, p. 81..

Heltai G., Flórián K., Halász G., Fekete I., Rusnák R., Remeteiová D., Boková V., Horváth M. (2008): Comparative studies of different sequential extraction schemes for characterization of environmental mobility of heavy metals in soils, sediments and gravitation dusts. European Geosciences Union General Assembly 2008, Vienna, Austria, 13–18 April 2008, EGU2008-A-04153, Geophysical Research Abstracts CD-ROM, Volume 10, ISSN 1029-7006.

Halász G., Rusnák R., Fekete I., Horváth M., Flórián K., Heltai G. (2008): Optimization of subcritical H_2O/CO_2 extraction for soils, sediments and gravitation dusts. XIII Italian-Hungarian Symposium on Spectrochemistry, Environmental Contamination and Food Safety, April 20-24 2008, Bologna, Italy, ISSN 0393-5620, p. 101.

Fekete I., Halász G., Rusnák R., Boková V., Remeteiová D., Flórián K., Heltai G. (2008): Comparison of information content of BCR fractionation with subcritical H_2O/supercritical CO_2 fractionation. XIII Italian-Hungarian Symposium on Spectrochemistry, Environmental Contamination and Food Safety, Bologna, Last Minute Abstracts.

Rusnák R., Boková V., Fekete I., Halász G., Remeteiová D., Heltai G., Flórián K. (2008): Acceleration of BCR sequential extraction procedure by ultrasonic treatment for soils, sediments and gravitation dust samples. XIII Italian-Hungarian Symposium on Spectrochemistry, Environmental Contamination and Food Safety, April 20-24 2008, Bologna, Italy, ISSN 0393-5620, p. 103.

Other publications not directly linked to the thesis

Albert L., Németh Zs.I., Halász G., Koloszár J., Varga Sz., Takács L. (1999): Radial variation of pH and buffer capacity in the red-heartwooded Beech (Fagus sylvatica L.) wood. *Holz Roh Werkst.*, **57**, 75-76. IF: 0.256

Abulikemu A., Halász G., Csámpai A., Gömöry Á., Rábai J. (2004): Improved synthesis of perfluorooctylpropyl amine. *J. Fluorine Chem.*, **125**, 1143-1146. IF: 1.364

Albert L., Németh Zs.I., Halász G., Bidló A., Koloszár J., Varga Sz., Takács L. (1998): Eltérések a vörös gesztű bükk (Fagus sylvatica L.) faanyagának kémiai paramétereiben. (Differences in the chemical parameters of the red-heartwooded beech (Fagus sylvatica L.) wood, in Hungarian.) *Faipar*, **46** (1), 36-37.

Albert L., Németh Zs.I., Halász G., Koloszár J., Varga Sz., Takács L. (1998): A szabad és kötött savtartalom sugárirányú változása a vörös gesztű bükk (Fagus sylvatica L.) faanyagában. (Radial variation of the free and bound acid content in the red-heartwooded beech (Fagus sylvatica L.) wood, in Hungarian.) *Faipar*, **46** (2), 23-24.