

HEAVY METAL CONTAMINATION AND ENVIRONMENTAL RISK ASSESSMENT BY MEANS OF ANALYTICAL METHODS

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ABSTRACT. The environmental mobility and biological impact of heavy metals in environmental systems are determined by their specific chemical forms. Biological risk assessment of contaminated areas therefore in principle requires the total speciation of all chemical elements present in the system. However, to solve such analytical task in practice is impossible. As a compromise fractionation by extraction with a series of solvents with increasing chemical aggressivity is applied for characterization of environmental mobility of heavy metals. Methodologies developed for this purpose are based on the application of solvents modelling the different environmental conditions sequentially or one by one mode. Sequential extraction scheme generally proposed by EU for investigation of aquatic sediments can distinguish four fractions by gradual decomposition of chemical structures of sediment: 1. Water soluble and carbonate-bound; 2. Bound to reducible Fe- and Mn-oxides; 3. Bound to organic matter and metal-sulphides; 4. Residual (soluble in strong oxidative acids). Recently the pollution events connected with the mining and metallurgical industry (Tisza-2000, Red mud-2010) enhanced the interest for development of fractionation methodology suitable for rapid and longterm risk assessment. In present paper an overview will be given on the progress in these methodologies extension of applicability to different environmental sample matrices (e.g. soils, gravitation dust, biofilms, red mud), solving of calibration and validation problems connected with application of multielemental spectrochemical methods in this field.

Key words: *heavy metals, speciation, sequential extraction*

ELEMENTAL SPECIATION AND BIOLOGICAL IMPACT

Potentially toxic elements which are in the environmental chemistry literature frequently called as heavy metals are the most important group of the persistent inorganic pollutants. The role of different chemical elements in concern of food production is one of the most important questions of the agricultural chemistry research since the middle

of the XIXth century. Numerous methodologies were elaborated for estimation of nutrition capacity of soils by determination of biologically available fraction of plant nutrition element content (Heltai et al. 1992). Similar questions were generated since the eighties of the XXth century due to the increasing environmental pollution problems, however in this field, the estimation of human - and the ecotoxicological impact became the main problem. This claim has brought new requirements for the methodology and has led to the development of the speciation analytical chemistry which is the precondition of the evaluation of toxicological effects of elemental species present in the system (Gunn et al. 1988, Templeton et al. 2000). In these procedures during the sampling the intact species should be preserved and then separated before the analytical detection (Fig. 1).

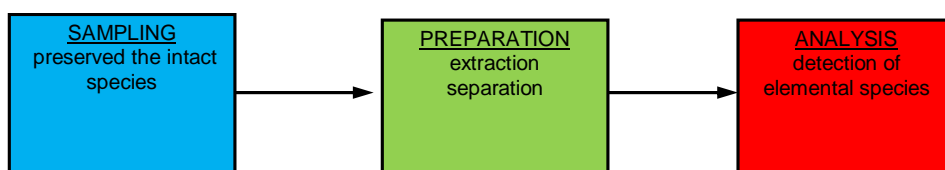


Fig. 1. *The scheme of speciation analysis procedures*

As a result of these intensive researches in the EU was established the European Virtual Institute for Speciation Analysis in 2002 for promoting cooperation, collection, reviewing, discussion of information and knowledge related to chemical speciation analysis (www.speciation.net).

The researches have called attention to the fact, that the environmental mobility and biological impact of heavy metals in environmental systems are determined by their chemical species forms. Biological risk assessment of contaminated areas therefore in principle should require the total speciation of all chemical elements present in the system, however, due to complex structure of solid environmental samples (e.g. soils, sediments, gravitation dust, mining waste, etc.), this analytical task in practice cannot be completely solved.

FRACTIONATION AND ENVIRONMENTAL MOBILITY

As a compromise fractionation of heavy metal content according to environmental mobility and estimation of biological availability by sequential extraction procedures became the main research trend. The papers published and cited in this field exponentially increased in the period of 1995-2013: papers from 200/year to 700/year; related citations from 1000/year to 19000/year (Web of Science 2013). Primary risk assessment of heavy metal contamination in the soil/water/atmosphere system was based on fractionation of metallic element content of environmental samples according to their environmental mobility. The term "fractionation" was recommended by the IUPAC in 2000 (Templeton et al. 2000). Mobility of an element according to different solubility of different binding

classes can be estimated by solvents sequentially applied which contain increasingly more and more aggressive reagents (Caroli 1996, Kersten and Förstner 1995). In agricultural chemistry the serial single step strategy (Fig. 2), in environmental chemistry the strategy of sequential extraction became dominant (Fig. 3).

The first sequential extraction procedure for characterization of mobility of heavy metals in aquatic sediments was developed by Tessier et al. (1979) who classified the metal content of sediments according to solubility and binding forms of metallic species as it is presented in the following scheme (Fig. 4).

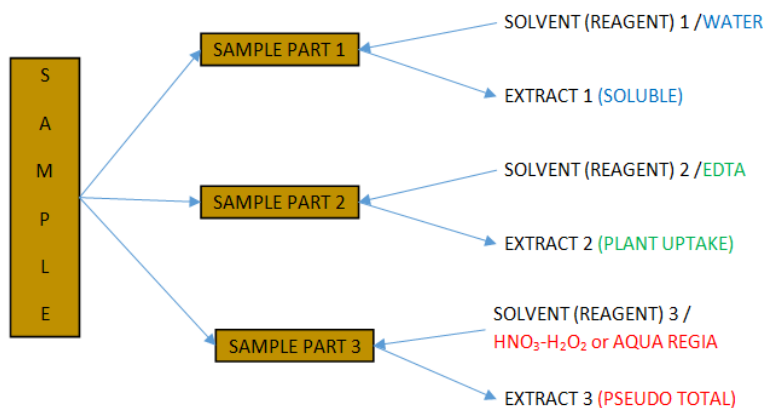


Fig. 2. Strategy of serial single-step extractions.

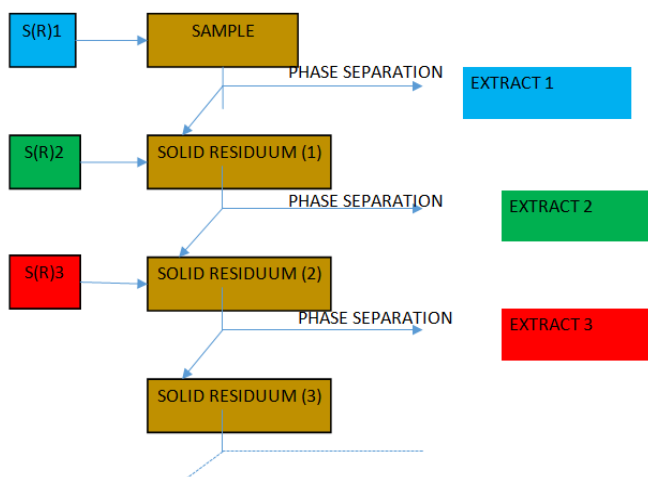


Fig. 3. Strategy of sequential extractions. On the figure the expression of SOLVENT (REAGENT) is abbreviated by S(R).

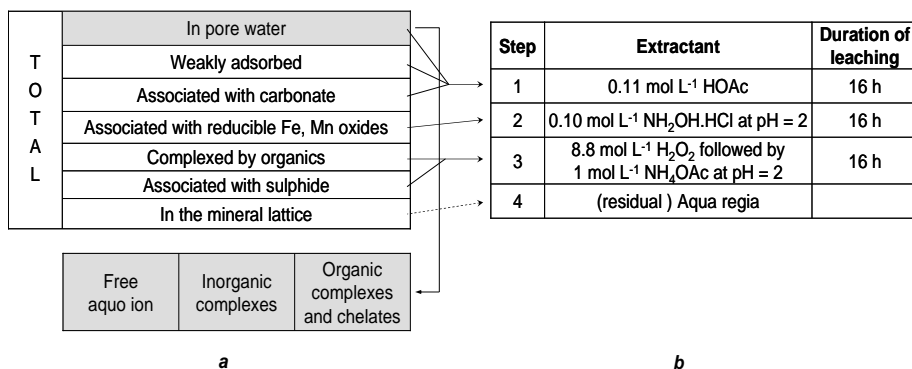


Fig. 4. a: Element fractions in aquatic sediment as classified by Tessier et al. (1979), Caroli (1996), and Kersten et Förstner (1995); **b:** Original BCR sequential extraction scheme (Ure et al. 1993, Quevauviller et al. 1997, López-Sánchez et al. 1998).

Based on this essential work several 5-8-step sequential extraction schemes were developed by which the original chemical structures (binding forms) are gradually dissolved and/or decomposed (Gleyzes et al. 2002). The total time demand of these procedures is 5-6 days, while duration of the applied batch leaching steps is determined by long setting up time of partition and other heterogeneous equilibria. The proposal for a 3(+1)-step sequential extraction procedure issued by EU Community Bureau of Reference (BCR) in 1993 is a simplified version of the above mentioned schemes (Ure et al. 1993), but it is still time consuming and labour intensive. As it can be seen in the scheme, in this method the water soluble, the weakly adsorbed and the carbonate-associated metal fractions are extracted simultaneously by acetic acid in the first leaching step, so it does not model the primary natural pathways of mobilization with water dissolution and bicarbonate formation. Due to the numerous operations and reagents applied, there is a risk of contamination during the whole procedure. Readsorption of extracted metals during the phase separation can lead to analyte losses. In the extracts the high reagent's concentrations may cause strong matrix effects during the spectrochemical element detection. The CRM 601 sediment reference material issued in 1997 was certified only for five elements (Cd, Cr, Ni, Pb, Zn) to this procedure (López-Sánchez et al. 1998, Quevauviller et al. 1997). During the elaboration of the next (BCR 701) sediment materials, the original BCR procedure was modified to avoid the analyte losses during the second leaching step due to the pH instability (Rauret et al. 1999, Sahuquillo et al. 1999, Pueyo et al. 2001, Rauret et al. 2001). This change, however, can cause difficulties when the consistency with former data is required. The BCR procedure in its original and modified versions was successfully applied to aquatic sediments and soils, however, soil CRM to this procedure was not issued up to now (Sahuquillo et al. 2003). Žemberyová (2006) successfully utilized the modified BCR three-step sequential extraction procedure for fractionation of Cd, Cr, Cu, Ni, Pb and Zn in different soil reference materials of Slovak origin. Kubová et al. (2004, 2008) successfully applied the BCR three-step sequential extraction and the dilute HCl single-step extraction procedure for soil-plant metal transfer predictions in contaminated lands.

Konradi et al. (2005) applied sequential extraction of metals from soil, based on the Tessier's procedure to evaluate soil contamination in the Bozanta Mare area nearby to the 2000 cyanide spill accident. This study has shown that soil composition and physicochemical characteristics strongly influence metal partitioning in soil. Elements are mainly bound to Fe/Mn oxides and are present in the exchangeable fraction in only small amounts. The amount in this oxide fraction increases with the increase of Fe and Mn contents in soil. The organic matter has an important influence on the mobility of several species like Al, As, Cd, Cu and Zn. Metals immobilized on the organic matter in soil become are less mobile and less bioavailable which resulted in a decrease of the exchangeable fraction.

Recently Okoro et al. (2012) reviewed the sequential extraction procedures for heavy metals speciation in soil and sediments with particular attention of strongly loaded marine systems. Jena et al. (2013) applied the five step Tessier's procedure for evaluation of heavy metal mobility in contaminated industrial soils and it was established that the soil organic matter has an important influence on immobilization.

Zimmermann et al. (2010) in a comparative study evaluated the possibilities of sequential extraction procedures (SEP) in heavy metal and trace metal analysis in soil. They concluded that there are many SEPs that can be utilized in the process of understanding the behavior of metals in various soils. The researcher trying to determine which procedure is most appropriate for their samples must take into consideration many factors including soil type, contamination level, and result comparison methods, as well as the potential problems or limitations associated with a specific SEP and it is necessary to be complemented with either RD analysis or some other kind of analytical technique to positively identify the solid components involved.

METHODOLOGICAL DEVELOPMENT OF FRACTIONATION PROCEDURES

From the above survey can be concluded that the Tessier's based fractionation procedures are widely used for heavy metal contamination assesment in soils and aquatic sediments, but the realistic interpretation of these data should require some kind of standardization which is, however, hindered due to methodological problems up to now. The main problems which limitate the mostly used BCR sequential extraction procedure have initiated a cooperativ research between Szent István University Gödöllő and the Technical University Kosice summarized as it follows (Table 1).

With extension of BCR usage to soils and aerial gravitation dust samples one has to calculate with further difficulties (Rusnák et al. 2010, Flórián et al. 2003a, Flórián et al. 2003b, Remeteiová et al. 2006). For example, in the modification of the BCR procedure the high carbonate content samples were not considered, in spite of the fact that the amount of acetic acid may not be enough to dissolve the total calcium carbonate content of 1 g sample weighed in according to the BCR protocol. Gravitation dust can significantly contribute to heavy metal contamination of soils and sediments, particularly at sites affected by industrial activity or traffic. However, the application of the BCR procedure to such kind of samples is not free from further difficulties. The available sample quantity is mostly less than that is required

to BCR procedure, therefore miniaturized BCR procedure by Dabek-Zlotorzynska et al. (2005) and realization of BCR procedure in continuous flow system by Shiowatana et al. (2001) was proposed, too. Another problem may arise from the high organic matter content of such samples (dusts, sediments) which can form separate phases during the leaching.

Table 1. *Problems of the BCR sequential extraction procedure and appropriate development*

Problems with BCR scheme		Development and Progress by SZIE and TU Košice research groups
Does not fit to chemistry of natural mobilization pathways	→	Better modelling of environmental mobility by application of H ₂ O and H ₂ O/CO ₂ solvents (Heltai et al. 2002)
Does not preserve the intact species of the sample	→	Application of biological tests for aqueous extract (Heltai et al. 2005)
Time consuming (4-5 days)	→	Acceleration of batch leaching by means of sonification (Rusnák et al. 2010)
	→	Development of continuous flow operation in supercritical fluid extractor for CO ₂ , H ₂ O and H ₂ O/CO ₂ solvents (Heltai et al. 2002)
Risk of contamination due to many operations and reagents applied	↗ →	Reduction of operations and reagents
Limited applicability and validation:		
Validation and CRM only for sediments	→	Extension of the application to other matrices: soils, gravitation dusts, biofilms, red mud, composts (Horváth et al. 2013)
Validation of the procedure only for six elements (Cd, Cr, Cu, Ni, Pb, Zn)	→	Multielemental calibration and validation for different matrices and extracts (Horváth et al. 2013)
High reagent and organic content of the extracts	↗	

To overcome the above mentioned problems the elaboration of a quite different continuous flow sequential extraction procedure was started at our laboratory ten years ago (Heltai et al. 2002, Heltai 2005). The samples were mixed in appropriate rate with pure quartz sand (SiO₂) weighed into a column of a supercritical fluid extractor (SFE). Firstly supercritical CO₂, then under the same conditions subcritical H₂O and finally their 1:10 ratio (V/V) mixture were applied as extractants, as can be seen on the following scheme (Table 2, Fig. 5):

Validation with high carbonate containing soil reference samples has proved that the complete dissolution of carbonates during the 3rd step requires longer time (90-180 min) (Halász et al. 2008, Horváth et al. 2010) (Fig. 6). Firstly an apolar organic fraction which can contain organic pollutants too can be obtained by this procedure. In the 2nd step the water-soluble and in the 3rd step the carbonate-bound fraction can be collected.

Table 2. Sequential extraction in a supercritical fluid extractor (SFE)

Step	Extractant	Chemical information	Duration
1	Supercritical CO ₂ (in SFE)	CO ₂ -soluble mobile organic bound fraction	60 min
2	Subcritical H ₂ O (in SFE)	Water soluble fraction	60 min
3	H ₂ O/CO ₂ (10:1) (in SFE)	Carbonate bound fraction	60 min
4	HNO ₃ /H ₂ O ₂ (out of SFE)	Residual	

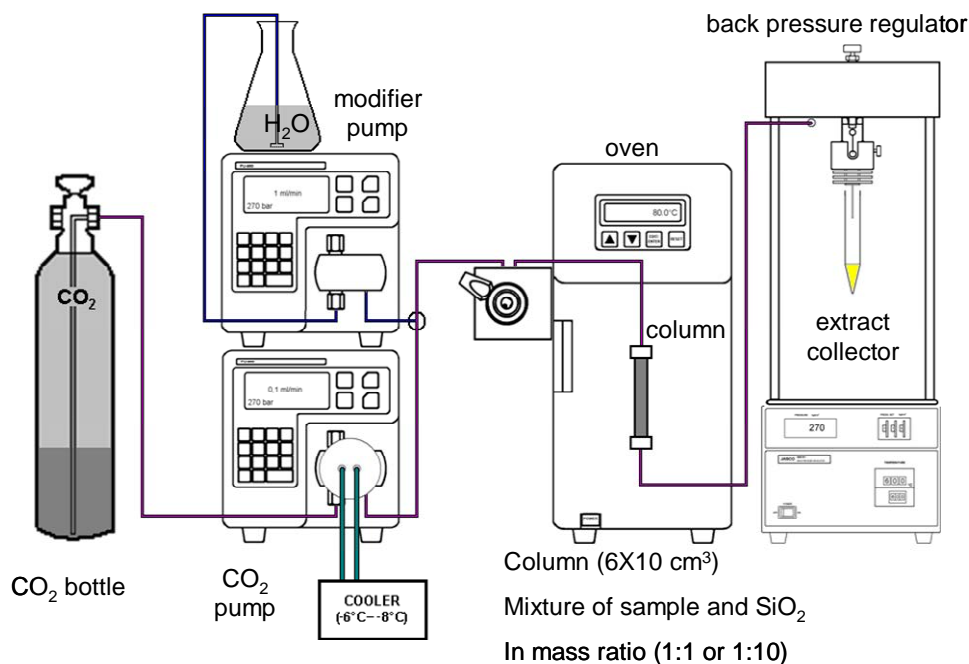


Fig. 5. The supercritical fluid extractor applied for continuous flow extraction of heavy metals by CO₂ and H₂O solvents.

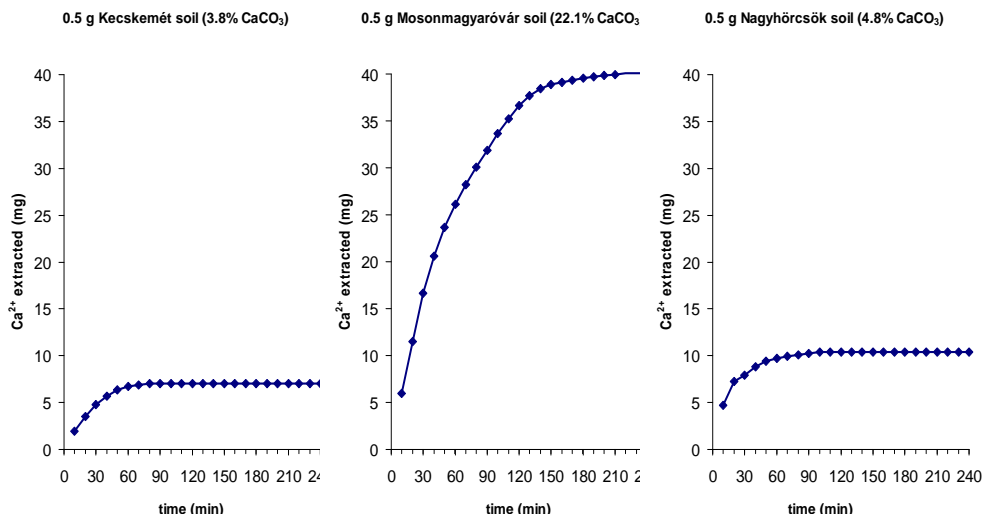


Fig. 6. SFE procedure optimization of extraction time of $\text{CO}_2/\text{H}_2\text{O}$ dissolution by measuring of dissolved Ca.

APPLICATIONS AT HEAVY METAL CONTAMINATED AREAS

Evaluation of heavy metal contamination at the Gödöllő – Isaszeg lake system

Our research group performed an intensive monitoring on the Gödöllő-Isaszeg lake system and the Rákos creek since 1993. This fishpond system along the Rákos creek from the eighties was used as a receiver of cleaned industrial and communal waste water of Gödöllő. In the sediment we could detect the heavy metal accumulations arising from these sources and the traces of the radioactive aerosol deposition arising from Chernobyl nuclear accident. The mobility of heavy metals in the sediment layers was evaluated by BCR sequential extraction and for several elements (e.g. Cd, Pb, Cr) significantly higher concentration values were detected in the most mobile BCR-fractions as the allowed threshold (Heltai et al. 1998). Later to these sediment samples was applied the SFE sequential extraction procedure elaborated by our laboratory (Heltai et al. 2002, Heltai et al. 2000) and ecotoxicological tests were performed using these extracts (Heltai et al. 2005). Following this the mobility of the contamination was investigated using biofilm samples (Horváth et al. 2013).

Evaluation of heavy metal contamination at the Upper Tisza Region in 2000

The Hungarian part of the Tisza River is highly contaminated by metals from mining activities in Romania. Mining in Maramures County (former Máramaros) traditionally exploits host ores of base metals (Cu, Zn and Pb) and precious metals (Au and Ag). Besides mining, metal pollution in upper Tisza catchment has also a long history (Nguyen et al. 2009). Nowadays the processing of old tailings pond material by using cyanide in the recovery of Ag and Au is spread over this region. Mining activities use dangerous and toxic chemicals like cyanide which is prime source of contamination. Besides this, wastewater may contain some other heavy metals associated with fine-grained sediments. However, metal concentration of river water is not remarkable 30 km downstream from the point sources (Macklin et al. 2003), sediment-associated metals are dispersed at much greater distances. In January and March 2000 two tailings dam failed in Baia Mare (Nagybánya) and Baia Borsa (Borsabánya) and resulted cyanide and metal pollution in the Lăpos - Szamos - Tisza and metal pollution in the Visó - Tisza river systems, respectively (UNEP/OCHA 2000).

The short term effects of the pollution events were studied by many of researchers, and the Lăpos-Szamos-Tisza and Visó-Tisza river systems were found to be contaminated by Cu, Zn, Pb and Cd (Macklin et al. 2003, Bird et al. 2003, Brewer et al. 2003, Győri et al. 2003a, Osán et al. 2002, Wehland et al. 2002). The mining accidents were followed by floods; therefore the metal pollution of the floodplains were also observed (Győri et al. 2003b, Kraft et al. 2006). Deposition of contaminated sediment on floodplains during flood events and the mobilization of the pollutants may increase the plant available metal content of the upper soil layer. The mobility and phyto-availability of metals depend on their chemical compositions (Kabata-Pendias et al. 2001). Hence, the floodplain soils and river sediment were measured by sequential extraction procedures (SEP) (Bird et al. 2003, Szabó et al. 2008, Farsang et al. 2009) and Lakanen-Erviö extraction (Lakanen et al. 1971, Sharma et al. 2009). A remarkable rate of Cd and Zn contents of polluted sediments (Lăpos, Szamos and Tisza Rivers) were in exchangeable form (Bird et al. 2003). Besides this, the Cd and Zn contents of the floodplain soils (Szamos and upper Tisza) could be found in an easily available form (Győri et al. 2003b). Recently the evaluation of longterm changes of mobility of heavy metal contamination by BCR sequential extraction was started in our laboratory.

EXAMPLES FOR APPLICATIONS OF METHODOLOGICAL DEVELOPMENTS

Comparative studies of BCR and SFE fractionation

These studies were performed parallel with the above contamination studies with appropriately selected samples, the details were published elsewhere (Heltai et al. 2011). Here only the most important results are presented on Fig. 7 and on Fig. 8.

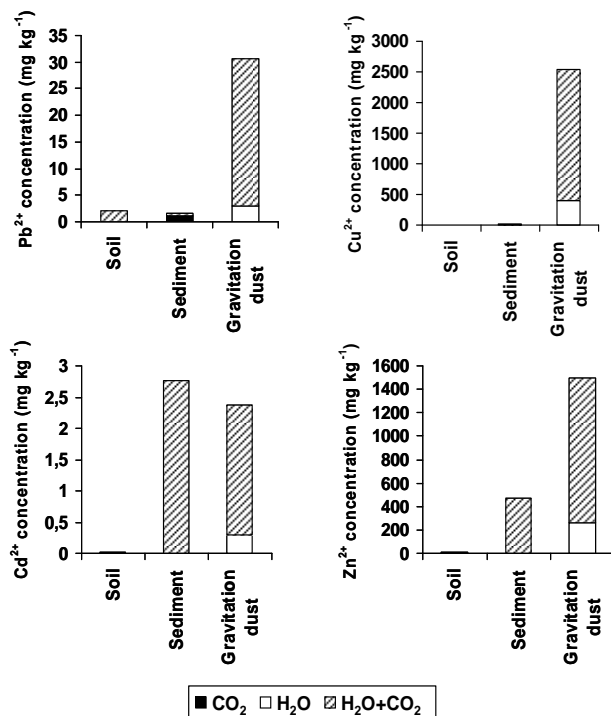


Fig. 7. SFE fractionation of the element content of the selected samples by the CO₂, H₂O and (CO₂+H₂O) solvents.

Selected samples:

- CRM 601, CRM 701 Sediment Reference Materials (BCR)
 - Lake sediment Gödöllő (contaminated), CaCO₃ = 7,6% TOC = 11%
 - Soil Nagyhörcsök (heavy metal loading experiment) CaCO₃ = 4,8% TOC = 3,5%
 - Gravitation dust Košice (Urbanic-industrial area), CaCO₃ = 2,9 TOC = 33%
- Element analytical methods applied for evaluation:
- FAAS, ICP-OES (BCR extracts)
 - ICP-OES, ICP-MS (SFE extracts)

The results show that the easily mobilizable lead, copper and zinc contents in the gravitation dust sample are much higher than in the sediment and soil sample, whereas cadmium content is similar in case of the gravitation dust and the sediment. The ratio of the fraction extracted by subcritical water-carbon dioxide mixture (i.e. the carbonate-bound fraction) is the highest for all four elements in the gravitation dust sample. Significant zinc and cadmium content can be detected from the sediment sample, mostly in the carbonate-bound fraction, whereas lead was found mainly in the fraction extracted by supercritical carbon dioxide (i.e. bound to organic material).

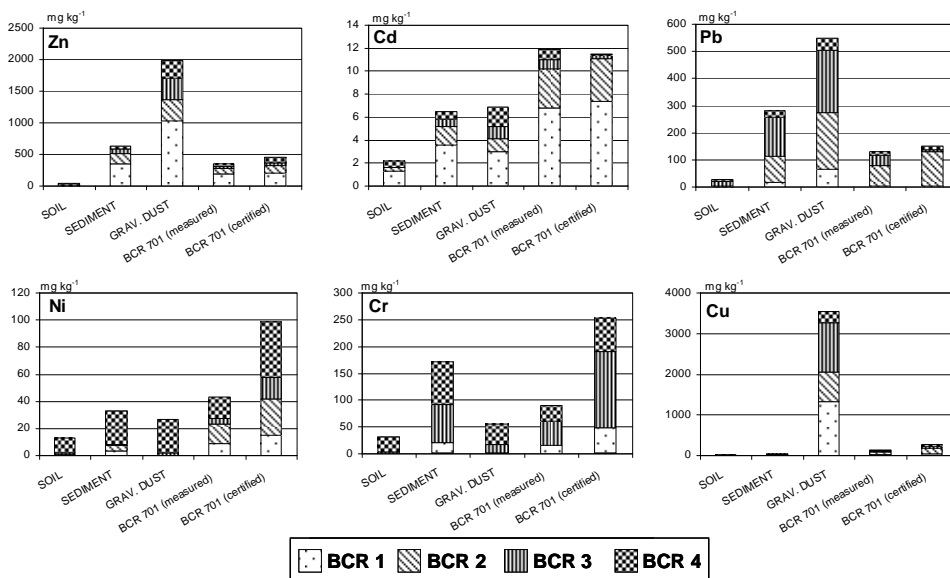


Fig. 8. Results of BCR-fractionation of element content of soil (Nagyhörcsök), sediment (Gödöllő), gravitation dust (Košice) and BCR 701 reference sample.

Detailed comparison of extracted amounts by BCR 1st step [BCR(1)] and SFE 2nd + 3rd step [SFE(2+3)] has shown that the sum of water soluble (SFE 2nd step) and carbonate bound (SFE 3rd step) fractions is mostly higher than the amount gained by BCR 1st step.

On the basis of these results it can be established that the total heavy metal contamination level of different kind of solid environmental samples increases in the order of soil < sediment < gravitation dust. By means of the BCR procedure the water soluble and carbonate bound fractions cannot be distinguished, while they are measured together, however, it characterizes more detailly the heavy metal mobilization by redox reactions in aquatic environment. The SFE procedure offers supplementary information to the BCR procedure by characterization of the above mobilization pathways for different kind of samples and offer a possibility to gain aqueous extracts keeping the original elemental species suitable for speciation and ecotoxicological studies.

Ecotoxicological test of heavy metal polluted sediment samples using aqueous SFE extracts

The possibility of application of aqueous SFE extracts for ecotoxicological testing of heavy metal polluted sediments was studied by Pollen Tube Growth Test (PTG) (Heltai et al. 2005). In these studies the following samples were used:

- Tisza-Szamos (2002) mixed samples (0-10 cm)

1. Tiszaadony
2. Olcsva
3. Tiszavalk
4. Kisköre

- Gödöllő-lake-system (2002)

5. Mixed sample (0-10 cm)

The ecotoxicological effect of sediment pore water was compared with effect of extracts gained from dried sediment samples by cold water leaching and by H₂O in SFE-procedure as it follows:

1. Separation of pore water by centrifugation (8600 rpm, 30 min)
2. Cold water leaching (24 hours in a rotating shaker, 10 rpm)
3. Three-step sequential extraction in SFE (27 MPa, 80°C)
 - (1.) Supercritical (CO₂)
 - (2.) Subcritical (H₂O)
 - (3.) Subcritical mixture of 90% (H₂O) and 10% (CO₂)

The Pollen Tube Growth Test (PTG) with two pollen charges of *Nicotiana sylvestris* was performed according the scheme presented on Fig. 9.

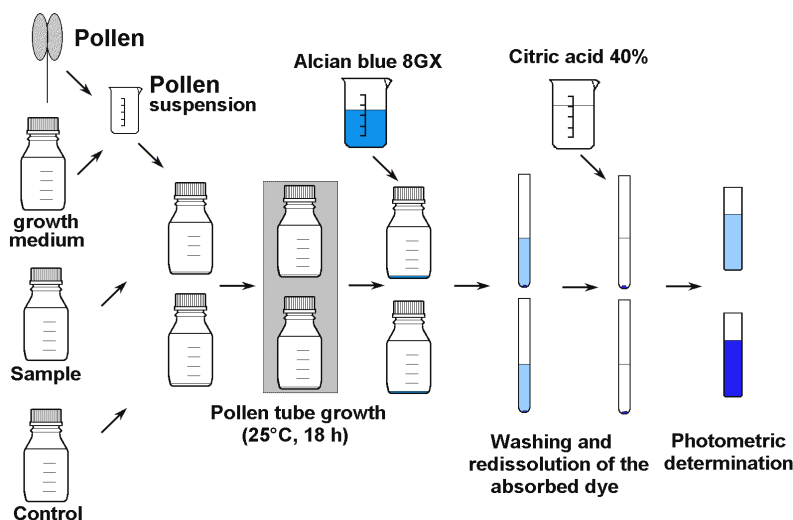


Fig. 9. The scheme of PTG test of aqueous extracts.

On the basis of results presented on Fig. 10 it could be concluded the followings:

1. By PTG test a general ecotoxicological potential can be detected.
2. The standardization is problematic due to the different response of different pollen charges.
3. Pore water extract shows higher ecotoxicological potential than the 24 h cold water extract of dried sediment.

4. Ecotoxicity of subcritical water extract is higher than that of the pore water extract.
5. One day is enough for testing.

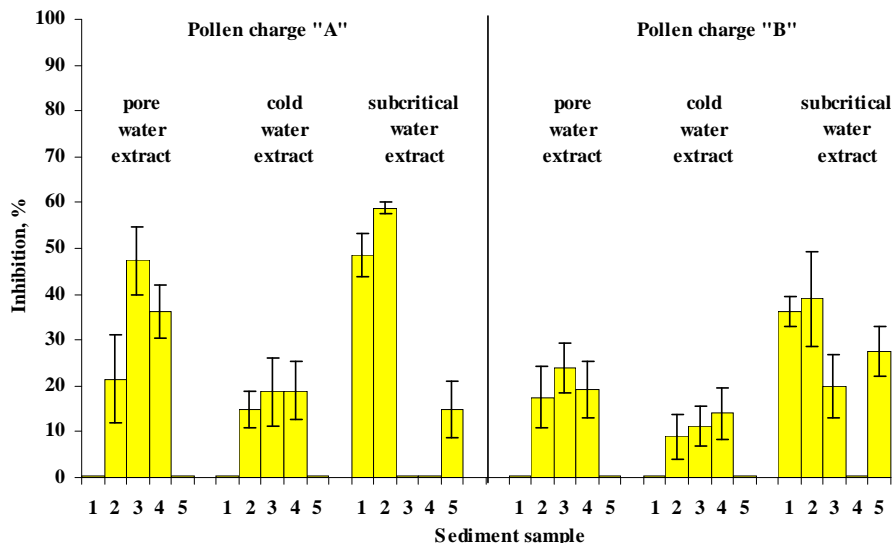


Fig. 10. The results of PTG tests.

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