

2.2. Posters

P01 CHROMATOGRAPHIC ENANTIOMER SEPARATION OF CHIRAL POLLUTANTS

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Introduction

Chirality is a typical property of many persistent organic pollutants (POPs). Chiral pollutants are often released as racemates into the environment, where undergo alterations in enantiomeric composition especially due to microbial interactions. Therefore, development and optimization of enantiospecific separation of chiral POPs is important since enantiomers often exhibit differences in biological activity, and most biochemical processes in nature are stereospecific¹. The most intensively studied chiral POPs are α -HCH, chlordanes, p,p'-DDT, toxaphene and atropisomeric PCBs. Many of these compounds have environmentally stable metabolites, which may also be chiral².

Native and derivatized cyclodextrins (CDs) are used as chiral selectors in many enantioselective separations. Cyclodextrins (CDs) are cyclic oligomers of α -D-glucose bonded through α -(1,4) linkages; the shape of a CD molecule is similar to a truncated cone with a cavity. In order to obtain cyclodextrins with the desired properties, many modified CDs have been prepared³. Permethyated β -cyclodextrin derivatized silica was used as the chiral stationary phase for HPLC (high performance liquid chromatography) separation of PCB atropoisomers and chlordane.

The purpose of the present study was to investigate if permethylated β -cyclodextrin derivatized silica can be used as a chiral selector for HPLC separation of enantiomeric pairs of atropisomeric PCBs and other chiral persistent organic pollutants. Progress in this area should prove the possibility of the isolation of enantiomerically pure PCBs for careful toxicological investigation.

Experimental

A Hewlett-Packard 1100 liquid chromatographic system, consisting of a quaternary pump, an autosampler, and a variable-wavelength UV detector, was employed. HPLC enantioselective separation was studied on the 200×4.0 mm Nucleodex β -PM column, 5 μ m (Macherey-Nagel, Dueren, Germany), based on Nucleosil 100 silica modified with permethylated β -cyclodextrin (PMCD).

Results and discussion

The retention behavior of PCB atropoisomers and chlordane was studied under different chromatographic conditions. The mobile phase composition (TEAA/methanol),

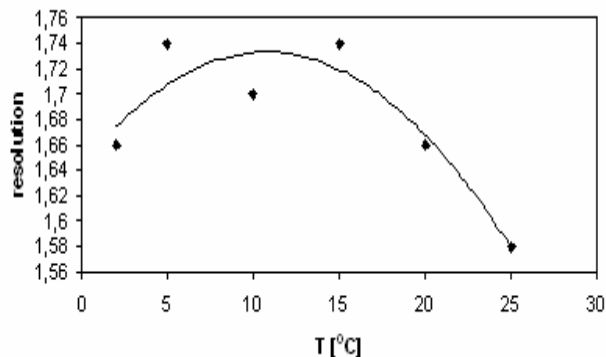


Fig. 1. Effect of column temperature on PCB 132 enantiomer resolution.

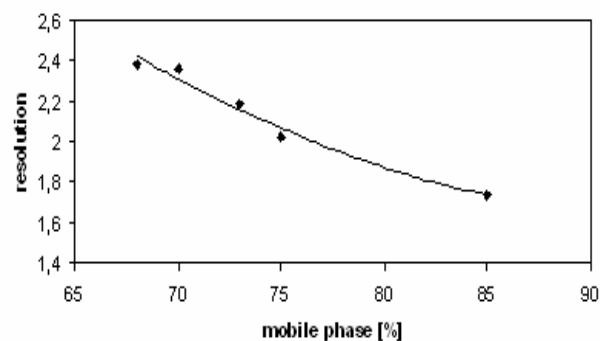


Fig. 2. Effect of methanol content in mobile phase on PCB 132 enantiomer resolution.

column temperature, and mobile phase flow rate were used as variable parameters. The optimum mobile phase flow 0.7 ml min⁻¹ was chosen from measurements of efficiency dependences.

PCB 132 was chosen as a model sample. PCB 149, PCB 136 and PCB 95 were not separated in the same conditions as PCB 132.

Changes in the column temperature in the range from 5 °C to 25 °C have had substantial effect on the enantiomeric

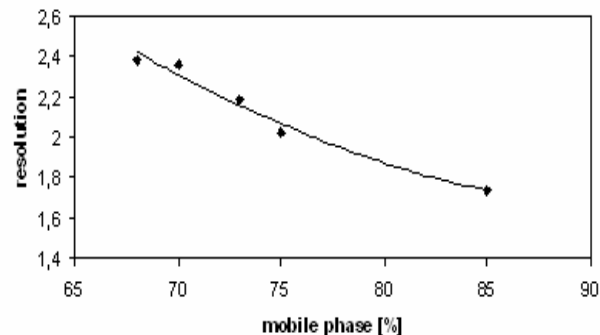


Fig. 3. Separation of PCB 132 enantiomers on permethylated β -cyclodextrin stationary phase. Mobile phase: methanol / 0.1 % TEAA pH 4.0, 85:15 (v/v), flow: 0.7 ml min⁻¹, detection: UV 230 nm, a) 25 °C, b) 5 °C

resolution (Fig. 1.). If the baseline resolution is required, for example for higher preparative recovery of enantiomers, the separation under the ambient temperature would be recommended. The increasing amount of MeOH in mobile phase decreases the resolution of PCB enantiomers (Fig. 2.).

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P02 DETERMINATION OF TRINITROTOLUENES AND PRODUCTS OF THEIR BIOTRANSFORMATIONS USING LIQUID CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS

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Introduction

Contamination of the environment by the explosives such as TNTs (2,4,6-trinitrotoluene and its constitutional isomers 2,3,5-trinitrotoluene and 2,3,4-trinitrotoluene) are often associated with munitions manufacturing facilities, storage depots and former sites of explosives use¹. TNTs are widely distributed in the environment. They can be persistent for very long times or may slowly degraded in water and soils, influenced by light, microorganisms, oxygen and reducing chemicals. TNTs and products of their degradation, aminodinitrotoluenes (ADNTs), diaminonitrotoluenes (DANTs) and triaminotoluenes (TATs) have been found to be cytotoxic presumably due to induced oxidative stress and demonstrate mutagenic capability. US Environmental protection agency (EPA) classifies 2,4,6-trinitrotoluene as possible human carcinogens^{2,3}.

Complete separation of these structurally similar compounds is necessary in order to identify and quantify individual compounds in environmental samples and during (bio)transformation studies. High performance liquid chromatography (HPLC) has remained the major analytical tool for the detection and quantification of nitroaromatic compounds⁴. At present, capillary electromigration methods, capillary electrochromatography (CEC) and micellar electrokinetic chromatography (MEKC) are studied as an alternative with higher efficiency and selectivity potential. CEC

separation of explosives without their degradation products have been used in several studies reported in the literature^{5,6}. The possibility to use above electromigration method for TNTs and metabolite separation in comparison with common HPLC method is the aim of our studies.

Experimental

Fourteen standards of TNTs and its metabolites aminonitrotoluenes (ANTs) (Fig. 1.) were used and selected in groups containing structurally similar compounds with comparable retention behaviour.

The capillary electromigration studies were performed with Capillary Electrophoretic System Agilent G 1600A Series with DAD equipped with CE Standard Bare Fused Silica Capillary, 50 mm I.D. (Agilent Technologies, US) and CEC Hypersil C18 column, 3 µm 100 mm I.D. (Agilent Technologies, US).

HPLC analyses were carried out with modular liquid chromatograph HP 1050 with diode array detection and equipped with 150 × 4 mm i.d. Zorbax Extend column C18, 5 µm. (Agilent Technologies, US).

Results and discussion

Separation of TNTs and their reduced metabolites requires detailed study of retention behaviour of individual compounds due to the potentially co-elution of the structurally similar compounds (isomers). Various parameters have to be used for these purposes, e. g. pH, ionic strength, temperature, organic solvent and other mobile phase modifiers.

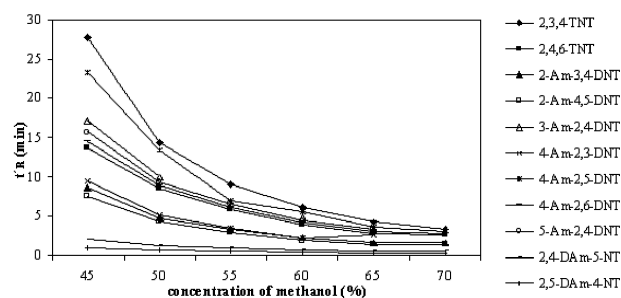


Fig. 1. Effect of organic modifier (methanol) mobile phase on HPLC analysis of TNTs and degradation products

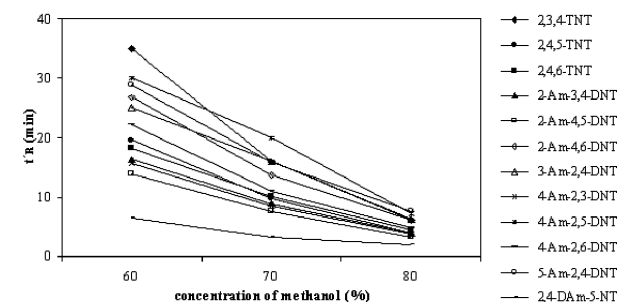


Fig. 2. Effect of organic modifier (methanol) mobile phase on CEC separation of TNTs and degradation products

Table I
Structure and serial number of TNTs and their degradation products

 1 2-amino-4,6-dinitrotoluene	 5 2,4,6-trinitrotoluene	 9 2-amino-4,5-dinitrotoluene	 13 5-amino-2,4-dinitrotoluene
 2 4-amino-2,6-dinitrotoluene	 6 2,4-diamino-5-nitrotoluene	 10 3-amino-2,4-dinitrotoluene	 14 2,4,6-trinitrotoluene
 3 2,6-diamino-4-nitrotoluene	 7 2,5-diamino-4-nitrotoluene	 11 4-amino-2,5-dinitrotoluene	
 4 2,3,4-trinitrotoluene	 8 2-amino-3,4-dinitrotoluene	 12 4-amino-2,3-dinitrotoluene	

HPLC separations were based on LC method 8330 drafted by US EPA. Mixture of methanol-water was used as a mobile phase. Effect of methanol content in mobile phase on retention and separation selectivity has been in-

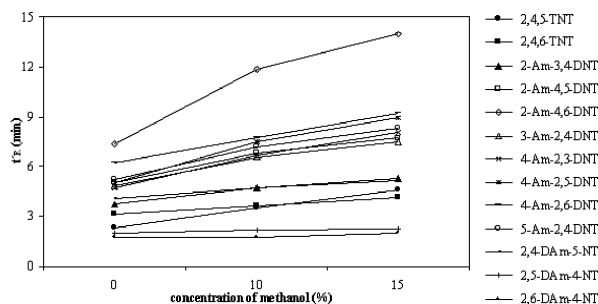


Fig. 3. Effect of organic modifier (methanol) mobile phase on MEKC separation of TNTs and degradation products

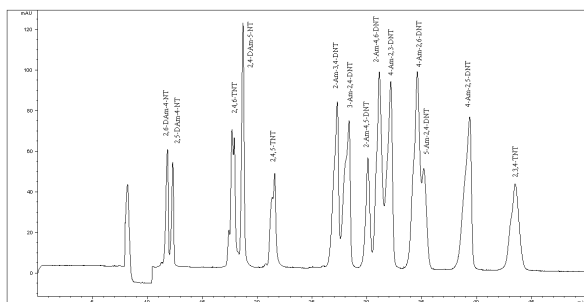


Fig. 4. MEKC separation of TNTs and ANTs in mobile phase containing SDS (100 mM), borate buffer (25 mM) and organic modifier (20 v/v MeOH)

vestigated. Significant changes in retention were observed at more retained ADNTs. DANTs are retained least on octadecyl stationary phase (Fig. 1.).

Effect of increasing methanol content in electrolyte was significant in all separation modes. In case of CEC and HPLC separation increasing concentration of methanol decreases retention times of all analytes, due to influences viscosity and dielectric constant of eluent. In case of MEKC separation content of methanol was limited by its capability to disruption of micelle formation. Increasing concentration of methanol increases retention times and separation selectivity of all analytes.

Effect of the organic modifier (Fig. 2.) mobile phase pH and buffer concentration on the solute retention during CEC separation was investigated.

Changes of MES and SDS concentration in ratio MES:SDS 2:1 has significant effect on retention of selected NTs and ANTs. Increasing concentration of MES has effect on EOF and increases retention times of all analytes. Changes of pH (4.5–6) have no significant effect on selectivity of selected NTs and ANTs.

The effect of surfactant concentration, ionic strength and organic modifier of mobile phase on the solute retention during MEKC separation was investigated. (Fig. 3.)

Increasing concentration of SDS increases retention, efficiency and selectivity. Increasing buffer concentration can decrease retention by salt-out and also increases EOF. Increasing borate buffer concentration (12.5–50 mM) increases retention times up to concentration 25 mM. Good resolution of all TNTs and metabolites was obtained especially in MEKC mode (Fig. 4.).

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P03 THERMAL ANALYSIS OF HUMIC SUBSTANCES EXTRACTED FROM CONTRASTING PEDOENVIRONMENTS

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Introduction

Thermal analysis is a simple and powerful tool to investigate some molecular aspects of humic substances. Thermogravimetry permits us to understand the resistance of these molecules to thermal degradation. Calorimetry is useful especially to identify heat effects and to its evaluation. For humic substances it can provide information on heat effects of single steps, and applied together with TG it can indicate the calorimetric types of degraded molecules. Moreover, it can also be used to compare the stability of humic substances, since peak onset temperatures are weight independent.

In this work, humic acids (HA) extracted from contrasting soils, including extreme examples for aliphaticity and aromaticity, were evaluated by the thermal analysis. Results show, that on one extreme, the HA of soils that are rich in black carbon feature a stable polycyclic aromatic nuclei, and on the other extreme, the HA of ornithogenic soils from the Antarctic are very aliphatic and easily thermodegradable structures.

Material and methods

Six HA samples, extracted from soils representing very contrasting pedoenvironments, and a humic acid like substance, synthesized from Eucalyptus charcoal, were selected (Table I). The A1 sample was extracted from an ornithogenic soil profile from Antarctica. Samples SB5 and SV5 are from high altitude Atlantic Rain Forest soils, and IC7 from a High Altitude Rocky Black soil from Brazil¹. Samples T6 and T7 were extracted from Amazonian anthropogenic dark earth soils.

Humic acids were extracted and purified as indicated by the International Humic Substances Society². The precipita-

ted HA samples (except the Antarctic HA) were treated with 0.5% HF+HCl solution twice, dialyzed, and lyophilized. Standard sample of peat HA from IHSS (n° IS 103H) was used as reference in TG analysis.

Thermodecomposition curves of HA were obtained by a TGA-50 SHIMADZU thermogravimetric analyzer using 3.3±0.1 mg samples over static air. The initial weight was stabilized at 30°C and heating curve was obtained, by 5°C min⁻¹ increments, to 105°C, with a holding time of 10 min, followed by heating at 5°C min⁻¹ rate up to 650°C. The first derivative curves (DTG) were obtained from the thermodecomposition curves. The weight loss at 105°C was considered as sample moisture. At the end of burning, the residue was considered as the ash content. The weight loss between 105 and 350°C and between 350 and 650°C was determined. The ratio of these two thermo-decomposition events was calculated and was defined as a thermogravimetric index (TGI).

Differential Scanning Calorimetry (DSC) measurements were performed at the same temperature program described for TG by means of Shimadzu DSC 60 using 1±0.1 mg of HA sample. Measurements were realized in an open aluminum crucible with a flow rate of oxygen of 20 ml min⁻¹, and an empty pan was used as reference.

Results and discussion

The DTG curves of HAs showed two well-defined burning events. The first one was associated with degradation of aliphatic, alicyclic, and partly of polar functional groups (i.e. biodegradable structures). The second degradation step could be contributed to the degradation of aromatic structures³. The relationship between the two peaks represents the resistance of humic substances to thermal degradation, which was described by a thermogravimetric index (TGI)¹.

The TGIs for the different HA are presented in Table II. The IC7 HA sample, extracted from HARC Black soil, showed very high thermal resistance. The IC7 thermogravimetric curve was formed basically by a single peak between 350 and 650°C, resulting in high TGI (Figure 1). This indicates the occurrence of polycyclic aromatic structures that have greater thermal resistance. On the other extreme, the thermogravimetric curve of the Antarctic Ornithogenic HA showed two bands almost with the same area between 105 and 350°C and 350 and 650°C (Fig. 1.) due the presence of easily thermodegradable structures, possible aliphatic.

The DSC record showed similar behavior as described for DTG (Figure 1). In fact, two well-defined degradation steps were shown (Fig. 2.). In Table II are given the basic DSC parameters: onset temperatures (extrapolated temperatures of the beginning of HA decomposition), and total heat evolution during HA samples analysis. Thermal analysis of HA reflects the dynamics of soil organic matter development. The amount of evolved heat measured by DSC and calculated as the integration of total area under DSC curve is influenced by the degree of humification, and by the amount of oxygen in humic molecules. Higher aromaticity degree

Table I
Elemental composition of HA extracted from soils of different origin

Code	Sample description	C	H	N	O	Atomic ratios			Moisture [%]	Ash
						H/C	C/N	O/C		
		Dry ash free basis								
SV5	Forest Black Soil HA	63.12	2.70	3.54	30.64	0.51	20.8	0.36	9.4	2.1
SB5	Forest Soil HA	55.50	4.17	4.37	35.96	0.90	14.8	0.49	4.5	1.1
IC7	HARC Black Soil HA	66.16	2.65	1.91	29.27	0.48	40.3	0.33	6.6	1.3
P5	Charcoal synthetic HA	62.10	1.93	2.56	33.41	0.37	28.2	0.40	9.9	0.3
T7	Anthropogenic Black Soil HA	50.84	4.54	5.99	38.63	1.07	9.9	0.57	3.8	9.2
T6	Anthropogenic Black Soil HA	59.49	4.46	6.18	29.87	0.90	11.2	0.38	5.1	8.6
A1	Antarctic Soil HA	50.32	7.18	7.30	35.19	1.71	8.0	0.52	12.8	3.8
IHSS	Peat HA (IS103H)	56.37	3.82	3.69	37.34	0.81	17.8	0.50	8.1	1.9

Table II

Results of thermogravimetric and calorimetric analysis of the analyzed HA samples. (LBI – percent of loss by ignition in the specified temperature range in relation with the total mass loss, TGI – Thermogravimetric Index) and calorimetric data of the analyzed HA samples

Code	Sample description	LBI		TGI	Onset [°C]	Total heat [kJ g ⁻¹]
		105–350 °C	350–650 °C			
SV5	Forest Black Soil HA	23	77	3.38	205	7.11
SB5	Forest Soil HA	28	72	2.52	215	5.32
IC7	HARC Black Soil HA	15	85	5.62	228	7.60
P5	Charcoal Synthetic HA	22	78	3.63	238	6.59
T7	Anthropogenic Black Soil HA	24	70	3.36	213	6.39
T6	Anthropogenic Black Soil HA	23	63	2.74	217	7.82
A1	Antarctic Soil HA	46	54	1.17	195	6.19
IHSS	Peat HA (IS103H)	27	73	2.74	–	–

implies in higher heat evolution. It is noteworthy that sample A1 (Antarctic soil) generated total amount of heat comparable with the other HA samples. Moreover, the contribution of the first decomposition step was significantly higher in

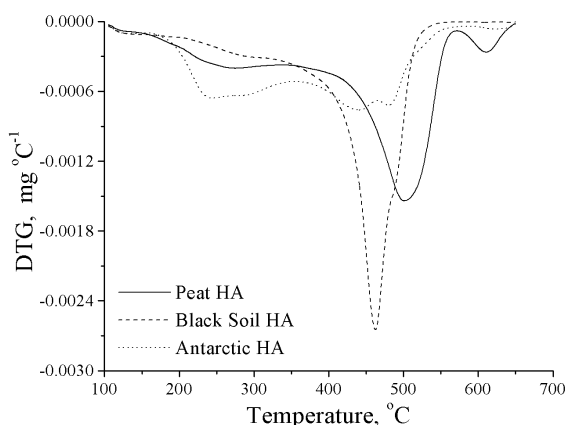


Fig. 1. First derivative of thermogravimetric (DTG) curves of Black Soil HA (IC7), Antarctic HA (A1) and Peat HA (IHSS, IS103H)

comparison with the other HA samples. This HA also had lower onset temperature, that suggests lower stability. Such results reflect biological soil formation processes that are exceptionally low in comparison with more moderate climates,

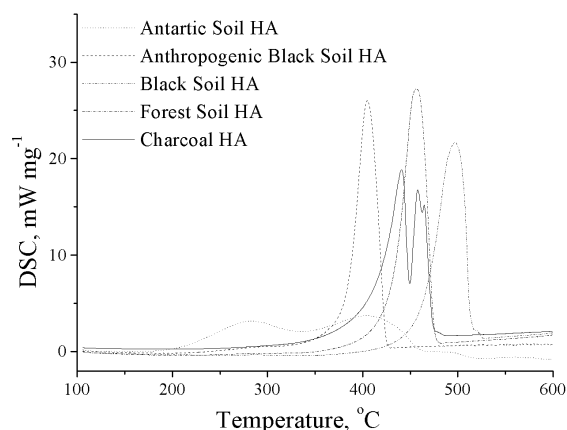


Fig. 2. Differential scanning Calorimetry (DSC) of Black Soil HA (IC7), Antarctic Soil HA (A1), Anthropogenic Black Soil (T7) and Atlantic Rain Forest Soil (SV5)

and suggest the presence of greater amount of biodegradable molecular structures in relation to the aromatic ones in HA. In contrast, Forest Soil HA (SB5), which developed under conditions that are more favorable for humus synthesis, had higher onset temperature. The sample IC7 (HARC Black Soil) had a great contribution of charred plant residues to the synthesis of its HA, that is reflected in the high humification degree and stability (i.e. high degree of aromaticity) of this sample¹. The HA of Amazonian Anthropogenic Dark Earth soils (T6, T7) gave weak heat evolution in the first stage, and narrow peak in the second stage around 400 °C. This is in concordance with climatic and other factors influencing organic matter formation in these soils, such as higher temperatures and high precipitation that contribute to the loss of the more biodegradable structures. The great amount of heat evolution, especially in case of sample T6, that is similar to that of sample IC7 (HARC Black Soil), and the fact that this soil contains high amounts of pyrogenic carbon accumulated by anthropogenic activity, suggests, that the pyrogenic material had influence on the humification process and finally on the molecular composition of the HA fraction. The Charcoal Synthetic HA (P5) showed the highest stability (onset temperature) and the most complex DSC curve. The cleavage of its DSC curve suggests that aromatic part consist of highly heterogeneous mixture of cyclic and polycyclic molecules.

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P04 IMPORTANCE OF DETERMINATION OF MICROORGANISMS IN WASTEWATERS

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Introduction

The wastewater treatment is carried out by various processes, which can be divided in three basic parts: physical, chemical and biological techniques. The aim of biological wastewater treatment are aerobic biochemical processes. During these processes we can observe particularly the growth of heterotrophic bacterial species but also other microorganisms. Microorganisms decompose organic sub-

stances to get energy, which can use for their living processes and for building of new cells. Two types of active cultures could be recognized, suspended culture (activated sludge) and scum culture (biofilm)¹.

Activated sludge is composed of bacterial species, e.g. filamentous microorganisms (*Thiothrix ssp.*, *Microthrix parvicella*, *Sphaerotilus natans*, *Leucothrix etc.*). Excessive amount of filamentous microorganisms in activated sludge can cause technological problems e.g. deteriorate sedimentation and separation properties of the sludge (sludge bulking). Because of the above mentioned sludge features monitoring and determination of microorganisms is necessary.

By monitoring the microorganisms, the course of activation process and, its eventual problems can be put under control. Besides many various types of bacteria many species of fungi, yeast and moulds can be identified in the activated sludge biocenosis. For identification of microorganisms present at activated sludge can be used a lot of methods. The common method used is microscopic analysis of basic morphological characteristics of microorganisms discussed, especially determination to types. Lately molecular-biological methods have been used widely, e.g. the FISH method (fluorescence *in situ* hybridization) represents one of them².

Discussion

The part of regular analytical monitoring of biological treatment process is microscopic technique. The base points, which must be observed during microscopic analysis are:

- presence or absence of filamentous microorganisms, their quantification and identification of dominant species,
- diversity and abundance of microorganisms in compared with “standard” microscopic image,
- characterization of flocs as base elements of activated sludge with a view to their separation,
- analysis of possible bad separation of activated sludge and causes of this process.

The identification of flocs, as the base elements of activated sludge, are important as well for monitoring of the whole active process. Lopez et al observed flocs structure by CLSM (confocal laser scanning microscopy) and TPE-LSM (photon excitation laser scanning microscopy)³.

Because of difficult determination of microorganisms to individual taxons is used to identify with types (Eikelboom, 1975). Types are signified by Latin name or number. They describe microorganisms, which have the same or similar morphological properties (present of filaments, their form, location, mobility, size, quantity, etc.) and positive or negative reaction to Gram and Neisser staining. The principle of the method is microscopic analysis of base morphologic characters of filamentous microorganisms in wet preparation. Monitoring is aimed at presence. Jenkins observed in his work⁴ classification of individual types of filamentous microorganisms in compared with working conditions.

In Czech waste treatment plants were identified these important types⁵: *Microthrix parvicella*, *Thiothrix ssp.*, *Sphaerotilus natans*, *Nostocoida limicola*, *Haliscomenobacter hydrossis*, *Nocardioform actinomycetes*, Typ 0041/0675, Typ 0092, Typ 021N, Typ 0803, Typ 0961.

In common use methods depend on cultivation of microorganisms⁶ and classification can be suffer from bias. There is absence of real conditions for organism in suspension culture and it is not possible to detachedly observe population dynamics of activated sludge.

Lately molecular-biological methods have been used widely; they are based on analysis of RNA and DNA. One of advance methods is method FISH (fluorescence in situ hybridization), it is modern technique of molecular biology, which can carry out reliable, fast method and that doesn't employ cultivation of microorganisms. By fluorescence *in situ* hybridization is analyzed molecule 16S rRNA⁷ (with 1500 nucleotides). It is possible to find short segments of 16S rRNA that is unique segment only for some typical species. Samples are detected by epifluorescence microscopy, laser microscopy or flow cytometry⁸.

Conclusion

The presence of microorganisms in wastewaters is essential for their biological treatment. Excessive amount of microorganisms can cause technological problems during the activated sludge process (influence of sedimentation and separation properties of the sludge, etc.). Because of this fact the determination of microorganisms is necessary. The eventual problems of the activated sludge process by monitoring the microorganisms can be put under control. Besides a common microscopic technique, lately are used molecular-biological methods (e.g. FISH method).

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P05 OCCURRENCE OF AROMA PRECURSOR IN ALLIUM SP.

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Introduction

Since ancient time, *Allium* vegetables (garlic, onion, leek and chive) have been used as foods, spices and herbal remedies¹. Sulfur containing flavour compounds are responsible for a characteristic smell and taste of plants representing *Alliaceae* family. Formation of these substances is catalysed by the action of alliinase (EC 4.4.1.4) on cysteine derivates (see Figure 1) when disruption of plant material occurs². The structure of dominating cysteine derivates classified as S-alk(en)-yl-L-cysteine sulfoxides (ACSOs) is shown in Figure 2. Since ACSOs and products of their enzymic degradation exhibit canceroprotective, antimicrobial, antiatherosclerotic, and antioxidative potential¹, determination of these compounds levels in *Allium* vegetables is of concern.

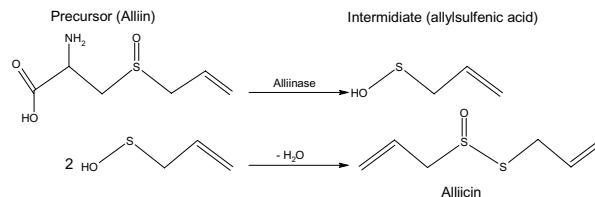


Fig. 1. Formation of volatile flavour compounds from alliin³

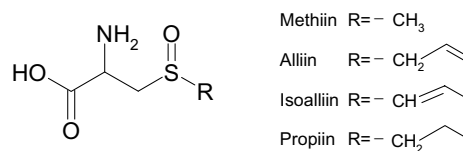


Fig. 2. Structure of most common S-alk(en)-yl-L-cysteine sulfoxides

Currently, there is a lot of information in scientific literature on ACSOs content in garlic and/or onion (see Table I), however as regards other edible crop of this family the data are not so comprehensive; for instance the distribution of ACSOs in such vegetable as leek has not been studied in detail yet. The aim of presented study was to provide more information on ACSOs in leek and similar vegetable.

Experimental

Materials

Samples – leeks were delivered to ICT laboratory by Swedish project partner – National Food Administration, Uppsala. Garlic and onion were purchased at Czech retail market.

Table I
Common levels of ACSOs in *Allium* vegetables^{2,4-9}

<i>Allium</i> vegetable	Methiin [g kg ⁻¹]	Alliin [g kg ⁻¹]	Isoalliin [g kg ⁻¹]	Propiin [g kg ⁻¹]
Garlic	0.33–1.26	1.73–11.79	tr*–1.35	nd-tr*
Onion	0.14–1.00	nd-tr*	1.31–1.56	0.61
Leek	0.04–0.28	tr*	0.18–2.28	tr*
Chive	0.32–0.68	0.02–0.40	0.31–0.65	nd*–0.07

*tr – trace, nd – not detected

Table II
Method performance characteristic

S-alk(en)yl- -L-cysteine sulfoxide	Limit of detection (LOD) [g kg ⁻¹]	Relative standard deviation [RSD, %]
Methiin	0.01	4.0
Alliin	0.02	3.5
Isoalliin	0.02	3.5
Propiin	0.04	3.6

Standards – methiin, alliin and propiin were obtained from Prof. Velišek (ICT, Prague, Czech Republic) who synthesised standards⁴. Isoalliin was quantified as an alliin.

Methods

Chopped sample was homogenized with O-(carboxymethyl) hydroxylamine hemihydrochloride (alliinase inhibitor; 1.1 g l⁻¹) and norleucine (internal standard; 5 g l⁻¹). The volume of extract was made up to 100 ml by distilled water. Samples were filtered through micro filter (5 µm) prior to injection into LC-MS/MS system which consisted from Agilent 1100 HPLC apparatus coupled to Finnigan LCQ Deca mass spectrometric detector. The chromatographic separations were accomplished on Hypurity Aquastar column (250 × 4 mm; 5 µm). Flow rate of mobile phase was 0.8 ml min⁻¹, column temperature 20 °C. Injection volume 1 µl (sample equivalent 0.1 mg) was used. Ionization was

made by atmospheric pressure ionization (APCI) in positive mode.

Results and discussion

New simple LC-MS/MS method for determination of ACSOs in *Allium* vegetables was developed within this study. Method performance characteristics are summarized in Table II.

Using the developed method ACSOs were determined in leek, garlic and onion. The highest content of these compounds was found in garlic, the levels of dominating alliin (86 % of total content) followed by isoalliin and methiin were 9.51, 1.23 and 0.20 g kg⁻¹, respectively. Markedly different ACSOs pattern was determined in onion. The major component of this group was isoalliin (84 % of total content), the content of methiin was lower – 0.15 g kg⁻¹. Regarding the leek, ACSOs pattern was quite similar to onion with higher isoalliin level (2.54 g kg⁻¹ vs. 1.18 g kg⁻¹).

In a more detailed study focused on this vegetable, altogether 72 leek samples were analysed, see Table I. The first batch of 20 samples was harvested after 8 weeks after planting, the second one 8 weeks later. Some decrease of isoalliin (approx. 28 %) was observed, there was no difference in methiin content.

To evaluate the distribution of ACSOs within the leek (see Figure 3), various parts (A – F) of selected leek samples were analysed. The highest levels of target analytes were found out in internal parts layers of stem (part C – cross cut); the lowest ACSOs content was in part D (longitudinal cut). In general, higher levels of ACSOs were determined in

Table III
ACSOs in leeks after 8 and 16 weeks after seeding

Leek	No. of samples	Methiin [g kg ⁻¹]	Alliin [g kg ⁻¹]	Isoalliin [g kg ⁻¹]	Propiin [g kg ⁻¹]	Total ACSOs [g kg ⁻¹]
8 weeks old average (range)	20	0.14 (0.05–0.28)	(1.40–4.53)	3.07 (<LOD–<LOQ)	(1.47–4.86)	3.31 (<LOD–<LOQ)
16 weeks old average (range)	52	0.14 (<LOQ–0.60)	(<LOD–0.09)	2.22 (1.43–3.98)	(<LOD–<LOQ)	2.39 (1.44–4.22)

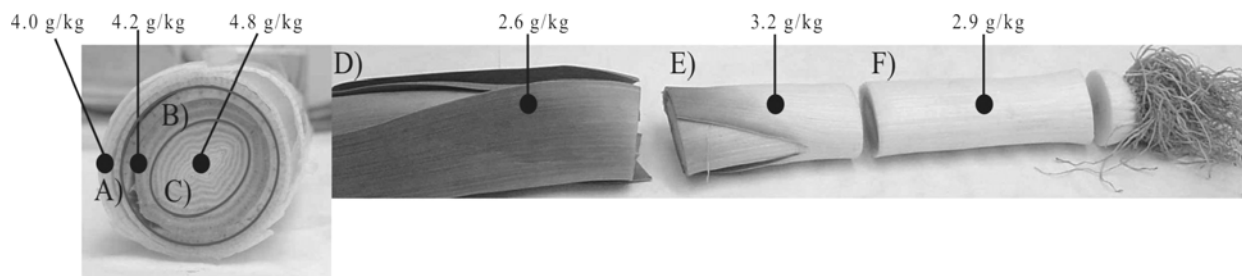


Fig. 3 Distribution of ACSOs in leek (average value obtained by analysis of 3 leeks) A–C) cross-section, D–F) longitudinal section

edible part of this vegetable (outer leaves and green parts are typically removed).

Conclusions

The ACSOs profile in determined vegetables obtained from Czech retail market was comparable with results of foreign scientific group. ACSOs content depends on maturity of crop; in younger leeks we found higher ACSOs content. The study focused on ACSOs distribution within the leek documented the relative increase of these compounds concentration when expressed on edible part of plant. In other words relative dietary intake of health promoting components (ACSOs and their breakdown products) when calculated on the weight basis might be increased by 17 % during culinary procedure.

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P06 PREDICTION OF RETENTION TIMES OF POLYCHLORINATED NAPHTHALENES

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Introduction

Polychlorinated naphthalenes (PCNs) form a complex mixture of up to 75 congeners containing from one to eight chlorine atoms per naphthalene molecule. PCNs were among the first synthetic chemicals and have been used continually for nearly 70 years in the United States and Europe. Although PCNs are no longer produced commercially, they continue to be released to the environment through leakage of PCN-containing equipment and by volatilisation from background compartments, such as soils, that act as reservoirs of previously released chemical¹. Although PCNs have been studied for decades, only recently have they come to the forefront of environmental analytical chemistry. Recent investigations have shown that they are environmentally mobile and bioaccumulative and they are ubiquitous in environmental compartments including air, soil, sediment and biota². They are also capable of long-range transport to arctic environments and have been detected in arctic air and biota. The tetrachlorodibenzodioxin (TCDD)-toxic equivalency factors (TEFs) for PCNs have been determined and results for several congeners are similar in magnitude to the coplanar PCBs¹.

For the analysis of PCNs capillary gas chromatography is the most suitable method. Although the number of CN congeners is relatively low (75) it seems to be difficult to separate all PCNs on a single capillary column. The use of multidimensional-GC techniques is necessary for a more complete separation of CN congeners³. To sum up, isomer specific analysis of PCNs is challenging due to the presence of the 75 theoretically possible isomers, lack of individual purified congeners for characterising the Halowax mixtures, and the similarities in chemical properties among different congeners. Resolving complex mixtures might require additional tools involving the knowledge of molecular interacti-

Table I

Observed retention times of PCNs on columns HT-8 and DB-17MS and sub-cooled liquid vapour pressures of PCNs, calculated RTs and the difference between observed and calculated RTs

PCN	RT_{obs} [min]		Vapour pressure at 25°C [Pa]	HT8		DB-17MS	
	HT8	DB-17MS		RT_{cal} [min]	$ RT_{\text{obs}} - RT_{\text{cal}} $	RT_{cal} [min]	$ RT_{\text{obs}} - RT_{\text{cal}} $
2	9.058	9.376	2.53	8.159	0.90	8.316	1.06
5	10.967	11.255	0.35	10.952	0.01	10.993	0.26
6	11.016	11.344	0.35	10.952	0.06	10.993	0.35
17	13.461	13.498	$9.01 \cdot 10^{-2}$	13.404	0.06	13.312	0.19
42	14.432	14.052	$4.15 \cdot 10^{-2}$	15.044	0.61	14.850	0.80
48	16.513	16.436	$1.77 \cdot 10^{-2}$	17.079	0.57	16.747	0.31
54	19.660	19.160	$4.28 \cdot 10^{-3}$	21.099	1.44	20.460	1.30
53	20.236	19.973	$4.75 \cdot 10^{-3}$	20.774	0.54	20.161	0.19
67	23.028	21.825	$1.57 \cdot 10^{-3}$	24.496	1.47	23.569	1.74
68	23.858	22.918	$1.34 \cdot 10^{-3}$	25.081	1.22	24.102	1.18
70	27.084	26.493	$7.34 \cdot 10^{-4}$	27.432	0.35	26.237	0.26
73	32.381	30.422	$2.78 \cdot 10^{-4}$	31.698	0.68	30.088	0.33
74	32.505	31.855	$2.46 \cdot 10^{-4}$	32.280	0.22	30.612	1.24
75	45.493	44.231	$6.84 \cdot 10^{-5}$	39.057	6.44	36.669	7.56
			Σ [min]	14.57	Σ [min]	16.78	

ons of PCNs with a stationary phase. This can be achieved using quantitative structure–retention relationship (QSRRs) which provides equations that relate molecular structure with the retention phenomena⁴. The objective of this paper is to provide a model to predict the chromatographic retention of individual PCN congeners based on their sub-cooled liquid vapour pressures and their molecular properties in HT-8 and DB-17 MS columns.

Experimental

Analytical standards of 14 PCNs (nos. 2, 5, 6, 17, 42, 48, 53, 54, 67, 68, 70, 73, 74 and 75) were analyzed by gas chromatograph HP6890 (Agilent) with two electron capture detectors (2D GC-ECD) and with HP7683 autosampler with a PTV injector. For analysis was used two columns with different polarity HT8 (SGE, 50 m \times 0.22 mm \times 0.25 μ m) and DB-17MS (Agilent, 60 m \times 0.25 mm \times 0.25 μ m). All samples were injected in splitless mode (1 min). The PCNs were separated using a temperature programme starting at 80°C raised to 180°C at 20°C min⁻¹ and raised to 250°C at 10°C min⁻¹ a finally raised to 270°C at 2°C min⁻¹. The PTV injector started at 90°C (0.1 min) and raised to 350°C at 720°C min⁻¹ and held there for 5 min. As a carrier gas was used hydrogen (generated by Domnick Hunter generator) at constant flow 1 ml min⁻¹. As a make-up gas was used nitrogen at 10 ml min⁻¹.

The sub-cooled liquid vapour pressures of the 14 PCNs were related to their gas chromatographic retention times (RTs) by linear regression analysis. Then the RTs of the 14 PCNs were calculated from the liquid vapour pressures using the regression equation. The observed RTs were compared with the calculated RTs. The RTs of another 57 PCNs were computed using the prediction model.

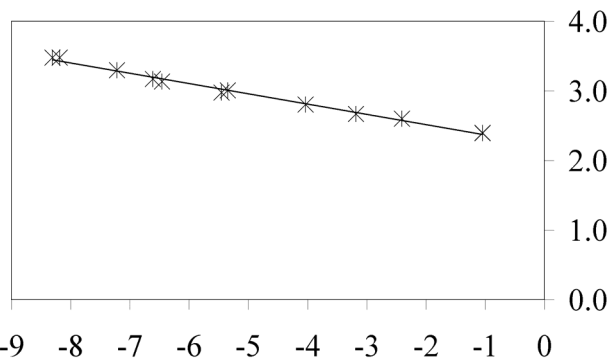


Fig 1. Plot of logarithm vapour pressures of PCNs versus logarithm their RTs on HT8. PCN 2 and 75 were omitted. An equation of the model was $\ln RT = -0.1476(\ln p) + 2.2223$ with a correlation coefficient $R^2 = 0.9938$

In order to improve the prediction power of the model, 7 molecular descriptors of PCN molecules were calculated and related to their RTs by a multiple linear regression analysis (analytical tool Regression in Excel). Retention times were then calculated from equation: $RT_i = a_1 D_1 + a_2 D_2 + \dots + a_n D_n$, (1), where D_1 , D_2 and D_n are molecular descriptors and a_1 , a_2 and a_n regression coefficients obtained by the multiple linear regression. The comparison between observed and calculated RTs was made.

Results and discussion

For column DB-17MS was the equation of the model $\ln RT = -0.1386(\ln p) + 2.2491$ and the correlation coefficient $R^2 = 0.9877$. For column HT-8 is depicted the dependence logarithm RTs of PCNs on logarithm their vapour

Table II
Molecular descriptors for 14 PCNs

PCN	E total [a. u.]	E HOMO [eV]	E LUMO [eV]	Dipole [D]	Surface [Å ²]	Volume [Å ³]	log <i>P</i> [ACD]
2	845.500	6.05	1.29	2.12	177.83	164.40	4.04
5	1305.090	6.12	1.59	0.73	190.69	177.84	4.57
6	1305.090	6.14	1.59	0.00	190.69	177.87	4.52
17	1764.681	6.37	1.78	3.08	206.79	191.49	5.27
42	2224.275	6.48	2.10	0.00	221.81	205.10	6.01
48	2224.270	6.57	2.02	0.00	223.33	205.25	5.36
53	2683.840	6.42	2.22	2.41	252.25	248.94	5.83
54	2683.855	6.63	2.20	1.15	236.28	218.61	5.95
67	3143.440	6.69	2.38	0.00	248.96	231.89	6.42
68	3143.427	6.58	2.39	0.93	247.26	231.84	6.13
70	3143.423	6.58	2.34	1.97	247.51	231.84	6.16
73	3603.006	6.65	2.55	0.85	260.37	245.22	6.56
74	3602.991	6.57	2.62	0.94	259.97	245.42	6.22
75	4062.563	6.59	2.64	0.00	269.72	258.21	6.58

pressures and the equation of the model in Fig. 1. On the whole the prediction was better on column HT8 as it can be seen in Table I. The sum of differences was 14.57 min compared with 16.78 min on DB-17MS. On both columns was the biggest difference at PCN 75: 6.44 resp. 7.56 min. From equations of the model was calculated elution order of another 57 PCNs and compared with chromatogram of Halowax 1014. Many discrepancies in elution order were found especially at tetra- and penta-CN congeners.

To conclude, it was found out that the prediction power of this simple model was insufficient to identify individual PCN congeners. However, the model may be useful for the

rough estimation of the elution order of PCNs on columns HT8 and DB-17MS.

Another and probably smarter way how to create model, which would enable us to predict *RT*s of PCNs is using molecular descriptors. Calculated molecular descriptors, which were used for the model, are given in Table II. Parameters of the model are in Table III, as well as results of comparison between observed and calculated *RT*s. The sum of differences observed and calculated *RT*s was similar for both columns 7.18 (HT-8) and 7.21 min (DB-17MS), which means improvement by 0.96 a 2.01 min, respectively in comparison with prediction only from vapour pressures (after subtraction PCN

Table III
Intercept and coefficients calculated by analytical tool Regression for columns HT-8 and DB-17MS and a comparison between observed and calculated *RT*s of PCNs. PCN 75 was omitted

	HT8	DB-17MS	PCN	HT8 [min]			DB-17MS [min]		
				<i>RT</i>	<i>RT</i> _{cal}	<i>RT</i> _{cal} - <i>RT</i>	<i>RT</i>	<i>RT</i> _{cal}	<i>RT</i> _{cal} - <i>RT</i>
<i>b</i>	121.378	117.801	2	9.058	9.208	0.15	9.376	9.672	0.30
<i>a</i> ₁	0.016	0.013	5	10.967	11.213	0.25	11.255	11.460	0.21
<i>a</i> ₂	-21.219	-22.145	6	11.016	10.681	0.33	11.344	10.818	0.53
<i>a</i> ₃	-10.421	-3.786	17	13.461	13.185	0.28	13.498	13.318	0.18
<i>a</i> ₄	0.328	0.534	42	14.432	14.222	0.21	14.052	13.916	0.14
<i>a</i> ₅	0.624	0.766	48	16.513	15.929	0.58	16.436	16.015	0.42
<i>a</i> ₆	-0.510	-0.611	54	19.660	19.664	0.00	19.160	19.164	0.00
<i>a</i> ₇	-2.941	-4.179	53	20.236	20.150	0.09	19.973	19.701	0.27
<i>R</i> ²	0.9932	0.9928	67	23.028	23.842	0.81	21.825	22.504	0.68
			68	23.858	26.195	2.34	22.918	25.339	2.42
			70	27.084	27.125	0.04	26.493	26.149	0.34
			73	32.381	30.569	1.81	30.422	29.002	1.42
			74	32.505	32.215	0.29	31.855	31.549	0.31
					Σ [min]	7.18		Σ [min]	7.21

75). Nevertheless, according computed *RTs*, PCN 6 would eluate on both columns before PCN 5, which is not in accord with observed reality. The best prediction of *RTs* was for PCN 54 on both columns, *RTs* differed in thousandths. In contrast, PCN 68 has the biggest difference on both columns 2.34 and 2.42 min, respectively.

To summarize, the comparison of the calculated and observed *RTs* showed that the prediction power of the model was better, but was still unable to determine individual PCN congeners positively.

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P07 DETECTION OF POLYCYCLIC AROMATIC HYDROCARBONS BY TANDEM “IN-TIME” MASS SPECTROMETRY

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Introduction

Polycyclic aromatic hydrocarbons (PAHs), many of which are genotoxic that may cause mutations and certain types of cancer, have been detected widely in environment¹.

The main problem with the determination of polycyclic aromatic compounds is the complexity of environmental matrices and the presence along with the PAHs of many interfering substances that cannot be removed by repeated extraction and purification². Contemporary mass spectrometric techniques were mainly used in scan and/or selected ion monitoring modes to identification and quantification of analytes in the environmental samples. Nowadays ion trap equipments can use multiple ions processing, mainly tandem “in-time” mass spectrometry, sometimes called as MS/MS or MSⁿ method³. This capability enables the ion trap to isolate an ion of interest and then produce characteristic product ions by collision induced dissociation and to distinguish the compounds from other compounds that have parent ions of the same mass-to-charge ratio. Previous MS/MS experiments on the ion trap mass spectrometers have been performed for example on analysis of pesticide residues in food⁴, polyaromatic quinones in a complex environmental matrix⁵, hydroxylated polychlorinated biphenyls⁶ and nonylphenol polyethoxylates in river water and sewage effluents⁷.

There are eleven condensed polycyclic aromatic hydrocarbons⁸ with combination of one five-numbered ring and six-numbered rings with molecular weight of 216. From these compounds are sometimes analyzed benzo[a]fluorene and benzo[b]fluorene. Methylated derivatives of PAHs with

molecular weight of 202 (fluoranthene and pyrene) have the same molecular weight. From occurrence and toxicology point of view, possibility of identification and quantification of these different substances with the same molecular weight is essential.

This study pointed to use of low resolution MS/MS experiment for distinguishing of parent compounds (benzo[a]fluorene and benzo[b]fluorene) from methylated derivatives of pyrene and fluoranthene, all with the same molecular weight of 226 in the real environmental matrices.

Experimental

Parent and substituted PAHs were supplied by Dr. Ehrenstorfer (Augsburg, Germany). The organic solvents used were of purity for organic trace analysis.

GC/MS system with ion trap mass spectrometer (Saturn 2100T, Varian, USA) was used for MS/MS experiments, identification and quantification of PAHs with molecular weight of 216. GC separation was performed in a DB 5 ms fused silica capillary column (25 m × 0.25 mm I.D., 0.25 μm stationary phase film thickness – J. & W. Scientific, USA).

The contaminated sediment samples were extracted for 3 hours with of 80 ml dichloromethane using automatic Soxhlet extractor (SOXTEC, Tecator, Sweden) operated at 70 °C. One half of solvent extract was fractionated using column chromatography on silica gel, as described elsewhere⁹.

Results and discussion

The resonant decomposition curves for the molecular ions (data not shown) provide maximum information concerning characteristic fragmentation of selected compounds.

It was found intensity of [M-15]⁺ ion of monomethyl derivatives is lower than for substances with more than one methyl group or longer alkyl side chains, due to the favourable loss of a proton followed by ring expansion to form the tropylium ion. MS/MS experiment confirms stability of this ion [M-1]⁺, followed with fragmentation to [M-1-C₂H₂]⁺ ion. On the contrary this fragmentation has a small yield in the case of parent compounds. This difference between parent and methylated compounds was used to identification and quantification of these contaminants in the real samples. Analyzed river sediment samples contained only methyl pyrenes, benzofluorenes and other parent PAHs with molecular weight of 216.

MS/MS technique moreover dramatically increases sensitivity of analyte identification and quantification by reduction of chemical noise. This reduction of chemical noise of instrument analysis causes an increase of signal-to-noise ratio (*s/n*). Increasing of these values is demonstrated in Table I for selected substances analysed in the river sediment sample.

Signal-to-noise ratio increase from full-scan (scan), selected ion storage (SIS), MS/MS to MS/MS/MS mode of ion analysis many times.

Table I
Signal-to-noise ratio of selected analytes in different mode of MS analysis

	Scan	SIS	MS/MS	MS/MS/MS
Benzo[<i>a</i>]fluorene	61	249	1855	2598
Benzo[<i>b</i>]fluorene	58	162	1207	1523
1-Methylpyrene	15	79	185	256
4-Methylpyrene	36	81	191	291

The data obtained in this study demonstrate the usefulness of ion trap GC-MS/MS experiments for detecting and quantifying trace amounts of benzofluorenes and methyl-derivatives of pyrene and fluoranthene in complex environmental matrix (river sediment samples).

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P08 IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS IN AMBIENT AIR BY SOLID-PHASE MICROEXTRACTION

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Introduction

Volatile organic compounds (VOCs) have been determined to be human risk factors in urban environment, as

well as primary contributors to the formation of photochemical oxidants. Some of these important air contaminants even exhibited carcinogenic potential to human.

These indoor and outdoor air contaminants were detected in urban and rural locations¹⁻⁴. Atmospheric volatile aromatic hydrocarbons, mainly benzene, toluene, ethylbenzene and xylenes (BTEX) are ones of the commonly observed traffic-related air pollutants found in urban centres, and their origin is from oil and petrol⁵ containing these substances up to 1–5 %.

Method used to sample collection and analysis, solid-phase microextraction – SPME, is considered to be a simple and cost-effective alternative to the conventional air sampling methods for the analysis of volatile organic contaminants emitted to the atmosphere from different sources. The main advantage of this method is the combination of process of sampling, sample handling and detection of analytes to the one step. Analytes are extracted from the air matrixes to the polymeric phase immobilized on quartz fibre, and then are directly thermal desorbed to gas chromatographic column.

The proposed study was focused on the device developed for dynamic sampling of these risk substances by solid-phase microextraction of urban air from roadside sampling sites collected in the city of Brno. Three types of SPME fibres coated with different sorbent polarity (non-polar Polydimethylsiloxane, Carboxen and polar Carbowax/Divinylbenzene) were used to air sample collection.

Experimental

All standards were purchased from Promochem (Wesel, Germany), SPME fibers were from SUPELCO (Bellefonte, PA, USA).

All air samples were collected by dynamic method using home-made sampling device appropriate for simultaneous sampling to three SPME fibres with different sorbent polarity at selected sampling sites.

The GC/MS separation of BTEX was performed in a DB VRX fused silica capillary column (60 m × 0.25 mm I.D., 1.4 μm – J. and W. Scientific, USA). An ion trap mass spectrometer (Saturn 2100, Varian, USA) was used for the detection and identification of the analytes. The mass spectrometer was operated in EI mode at electron ionisation energy of 70 eV.

Table I
Relative efficiency of BTEX sorption on the three types of SPME fibres

Compound Name	PDMS [%]	CW+DVB [%]	Carboxen [%]
Benzene	0.02	0.10	99.9
Toluene	0.28	6.35	93.4
Ethylbenzene	0.48	21.1	78.4
m,p-Xylenes	1.19	26.8	72.0
o-Xylene	1.83	26.9	71.3

Table II
Mean concentrations of BTEX in selected sampling sites

	BTEX concentration [$\mu\text{g m}^{-3}$]				
	Sportovní	Kotlářská	Pionýrská	Zvonařka	FCH VUT
Benzene	4.36	2.56	6.51	6.55	1.06
Toluene	13.1	7.99	20.1	19.9	3.15
Ethylbenzene	6.06	4.59	11.0	9.79	1.59
m+p-Xylenes	12.3	6.95	15.6	16.3	2.14
o-Xylene	7.84	5.68	8.91	7.44	0.94

Results and discussions

SPME fibres with different sorbent polarity have well different selectivity to collection of compounds from the ambient air. Relative efficiency of sorption BTEX compounds on the three types of SPME fibres are summarized in Table I.

Relative efficiency of BTEX sorption was calculated as perceptual content of individual compounds related to sum of their concentrations determined by all three SPME fibres. The greatest selectivity to BTEX sorption was found for Carboxen (100 %), followed by Carbowax with Divinylbenzene (CW+DVB – 16.3 %) and Polydimethylsiloxane (PDMS – 0.7 %) of sorption efficiency in the sampling of ambient air.

Table II shows detected mean concentrations of BTEX in the roadside samples collected in the selected sampling sites. BTEX level were much higher in sites with high traffic intensity (Pionýrská and Zvonařka street) in comparison with rural site location (FCH VUT).

Expect volatile aromatic compounds, chlorinated hydrocarbons, n-alkanes and branching alkanes were among the most abundant contaminants detected in the air samples. Under occurrence and concentration profiles of analysed compounds was found that traffic was the most important sources of VOC emissions in the sampling sites.

The mean concentrations of benzene, toluene, ethylbenzene and xylenes in the urban air in the city of Brno in the day time were 4.21, 12.9, 6.6 and 8.4 $\mu\text{g m}^{-3}$, respectively. These concentrations are with great agreement with concentrations detected in other cities with moderate traffic intensities.

This work was supported by the Czech Ministry of Agriculture, grant No. MZE 0002716201.

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P09 ASSESSING AEROBIC BIODEGRADABILITY IN AQUEOUS ENVIRONMENT BY ANALYSING COMPOSITION OF EQUILIBRIUM GASEOUS PHASE

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Introduction

Increasing production and consumption of plastics and plastics packaging gives rise to the issue of their environmentally safe disposal after use. The enormous pace at which the waste is produced is alarming. In the past, a way out with particular view to economy could seem to be landfilling of these wastes. However, this mode of dealing with wastes in the long run is untenable. Fully acceptable technologies of waste disposal comprise biological procedures which, however, apply to wastes based on biodegradable materials.

There are several standardized methods for observing biodegradation course of various substances in different conditions of the environment¹. Technique frequently employed involves methods consisting in observing changes in gaseous phase in reaction environment, specifically biological oxygen demand (BOD) or produced CO₂ or possibly both components. Practical performance of these tests is specified by Czech, or more frequently, foreign (ISO, ASTM) standards^{2,3}.

The objective of this study was to verify potential use of gas-chromatographic analysis of gaseous phase composition in an enclosed two-phase gas-liquid system to observe course of biodegradation in an aerobic environment. This analytical end-point would enable to determine in parallel quantity of produced CO₂ as well as of consumed O₂ in gaseous phase.

The first part of the contribution has a mathematical description of acidobasic CO₂ equilibrium in a real biological system for conditions of investigating biodegradations. In the next part of this contribution, this mathematical balance was experimentally verified by testing biodegradability of easily degradable substrate (glucose) by analyzing gaseous phase composition.

Acidobasic equilibrium of CO₂ in gas-liquid system

At a pH level of liquid phase below 4, originating CO₂ was dissolved in liquid merely in free form, quantity of ionic forms under these conditions was practically negligible and total CO₂ quantity could be easily calculated from Henry's law and partition coefficient K'_{lg} . At higher pH levels (approaching pK'_1 of H₂CO₃ and higher), concentration of ion-bonded CO₂, i.e. $c_{\text{HCO}_3^-}$ and $c_{\text{CO}_3^{2-}}$, becomes comparable with or greater than concentration of "free" dissolved CO₂. It holds that $c_{\text{CO}_2\text{l}} = c_{\text{CO}_2\text{T}} - c_{\text{HCO}_3^-} - c_{\text{CO}_3^{2-}}$ where $c_{\text{CO}_2\text{T}}$ is total concentration of all forms of CO₂ in solution.

In a system with dissolved carbon dioxide, conditions of phase equilibrium, i.e. Henry's law or an equivalent expression by partition coefficient, must be valid at the same time as both relations for dissociation constants of carbonic acid. In a usual biodegradation regime, system pH is not too high and dissociation of carbonic acid to second degree does not practically occur, i.e. CO₂ presence in the form of CO_{3²⁻} is insignificant whereas, on the contrary, its presence as HCO_{3⁻} is significant.

During biological tests, pH values may range within approx. pH limits 6.8–7.2, i.e. in a region where quantity of CO₂ in the form of HCO_{3⁻} is dominant in the solution and should also be taken into account in total CO₂ balance. The ratio of CO₂ concentrations in liquid and gaseous phase, i.e. $c_{\text{CO}_2\text{T}}/c_{\text{CO}_2\text{g}}$ will now be designated

$$K'_{lg} = \frac{c_{\text{CO}_2\text{T}}}{c_{\text{CO}_2\text{g}}} = \frac{c_{\text{CO}_2\text{l}} + c_{\text{HCO}_3^-}}{c_{\text{CO}_2\text{g}}}$$

which, under these conditions, is the effective formal partition coefficient (K'_{lg}) of CO₂ between liquid and gaseous phase in a real system.

Knowing pH of liquid phase at the moment of GC analysis and volumes of liquid and gaseous phase, it is possible to calculate total produced quantity of CO₂ in real test conditions according to equation (1)

$$\begin{aligned} n_{\text{CO}_2\text{g+l}} &= (V_g + V_l \cdot K'_{lg}) \cdot c_{\text{CO}_2\text{g}} = \\ &= \left(V_g + V_l \cdot K'_{lg} \cdot \left(1 + 10^{-pK'_1} \cdot 10^{\text{pH}} \right) \right) \cdot c_{\text{CO}_2\text{g}} \end{aligned} \quad (1)$$

Important parameters in the balance equation (1), apart from pH, are volumes of liquid and gaseous phase designated V_l and V_g . By decreasing ratio V_l/V_g , we are capable of increasing the so-called "degree of CO₂ transfer to gaseous phase" expressing the portion of carbon dioxide found at a given pH level in the gaseous phase (capable of being determined through GC analysis) of the total produced quantity of CO₂.

It is experimentally advantageous to work at a low V_l/V_g ratio. With a lower V_l/V_g ratio the degree of CO₂ transfer to gaseous phase increases, and changes in concentration of CO₂ in gaseous phase produced by possible changes of pH in liquid phase during test decrease. On the other hand, CO₂ concentration in the gaseous phase will decrease with a falling V_l/V_g ratio thus making greater demands for sensitivity and accuracy of GC analysis. Taking into account facts above, suitable conditions for balancing CO₂ by means of GC

analysis may be expected at a ratio of $V_l/V_g < 0.1$, with pH of liquid phase stabilized with phosphate buffer system (part of standard biomedium, pH approx. 7.1).

Testing biodegradability of model sample by analyzing gaseous phase

Testing apparatus

All biodegradability tests were performed in apparatus depicted in Fig. 1. This apparatus is designed on basis of ISO 14852 standard² defining conditions for testing biodegradability of plastics but duly adapted to enable withdrawal of gaseous samples during test for GC analysis (through gastight septum in cap of each flask).

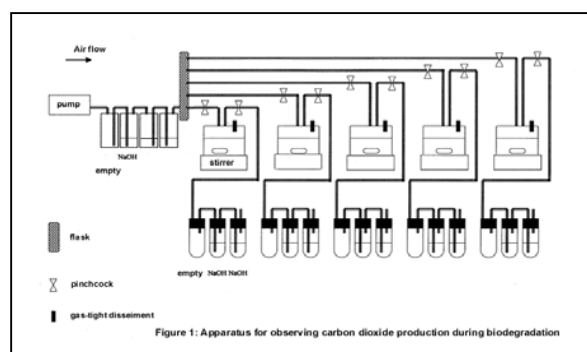


Fig. 1. Diagram of employed apparatus for testing biodegradability by analyzing gaseous phase

Employed inoculum

Microbial inoculum was prepared by mixing 20 g commercially supplied soil substrate (approx. 50% moisture content) with 100 ml mineral medium solution (in accordance with ISO 14852), the mixture was shaken 2 hours on shaker and then filtered through previously washed filter paper. Prior to application in test, the inoculum was aerated 24 hours in suitable vessel. Dosage of inoculum was selected so as to achieve 10⁶ cells per 1 ml reaction suspension (calculated for total amount of cells from microbiological analysis)⁴.

Tested samples and experimental conditions

In these "verifying" tests glucose was employed, as a readily degradable substrate for substantially shortening length

Table I
Volumes in testing flask of apparatus

	variant A	variant B
Total volume of measuring flask [ml]	1 140	1 140
Volume of liquid phase [ml]	85	285
Volume of gaseous phase [ml]	1 055	855
Ratio V_l/V_g	0.08	0.33

Table II
Table of experimentally measured data

sample	$V_l/V_g = 0.08$				$V_l/V_g = 0.33$			
	pH _{start} [–]	pH _{end} [–]	(D_{CO_2}) ₂₁₀ [%]	(D_{TOC}) ₂₁₀ [%]	pH _{start} [–]	pH _{end} [–]	(D_{CO_2}) ₂₁₀ [%]	(D_{TOC}) ₂₁₀ [%]
blank	7.10	7.12	–	–	7.12	7.14	–	–
glucose 100	7.10	7.10	90.8	95.6	7.12	7.05	82.8	91.1
glucose 100	7.11	7.10	94.6	95.8	7.12	7.07	81.8	96.0
glucose 150	7.11	7.11	91.4	97.4	7.11	7.05	89.8	96.1
glucose 150	7.11	7.11	94.5	96.0	7.11	7.06	83.9	96.2

glucose 100 glucose solution of starting concentration approx. 100 mg carbon/L

glucose 150 glucose solution of starting concentration approx. 150 mg carbon/L

pH_{start} pH of liquid phase at start of test

pH_{end} pH of liquid phase at end of test

(D_{CO_2})₂₁₀ disposal percentage related to produced CO₂ at 210th hour of test

(D_{TOC})₂₁₀ disposal percentage related to decrease in DOC at 210th hour of test

of experiment. It will be subsequently applied for investigating biodegradation of plastics for which the technique was proposed. The test was executed at two different volume ratios of liquid and gaseous phase (Table I) anticipated in accordance with mathematical considerations mentioned above.

Starting concentration of organic carbon was 100 and 150 mg l⁻¹. At start and end of test, pH values of reaction suspension were measured owing to content of dissolved organic carbon (DOC).

Evaluation of results

Produced quantity of CO₂ was determined in all test flasks by GC analysis of gaseous phase. The identified quantity was corrected according to conditions of acidobasic equilibrium of CO₂ in a gas-liquid system in accordance with equation (1), and related to theoretical CO₂ quantity calculated for a theoretical case of complete breakdown of tested substrate (D_{CO_2} – disposal percentage related to produced CO₂). DOC values measured at start and end of test were used to calculate D_{TOC} – disposal percentage according to decrease in dissolved organic carbon. Values of D_{CO_2} and D_{TOC} should be mutually comparable assuming validity of mathematical model of acidobasic equilibrium. Results obtained are presented overall in Table II including relevant pH values at start and end of test.

At ratio $V_l/V_g = 0.08$, results of D_{CO_2} and D_{TOC} were in good agreement. Potential deviations were caused by erroneous analysis of investigated parameter. Small changes in pH during tests were very positive. With ratio $V_l/V_g = 0.33$ there appeared a problem with greater retention of CO₂ in liquid

phase, whereby greater changes in pH appeared (compared to lower ratio) together with related changes in content of CO₂ in gaseous phase.

Conclusions

- A mathematical model of acidobasic CO₂ equilibrium in a gas-liquid system was proposed for conditions of observing biodegradations expressed by balance equation (1).
- Validity of the model was experimentally verified by observing degradation of glucose by means of analyzing CO₂ in gaseous phase at two different volume ratios V_l/V_g .
- It was found that ratio $V_l/V_g \cong 0.1$ is suitable for observing course of biodegradations by this analytical method. Value of pH is sufficiently stabilized throughout the test and, thereby, also degree of CO₂ transfer to gaseous phase, while quantity of produced CO₂ in gaseous phase is still capable of being determined by GC analysis.
- At present, applicability of this analytical mode to testing of plastics biodegradability is being verified.

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P10 DETERMINATION OF THE VOLATILE ORGANIC COMPOUNDS IN PAINTS AND VARNISHES

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Introduction

Some of volatile organic compounds (VOC) are used as paint additives. VOC can react to sunlight, changing to ozone and other pollutants that produce photochemical smog in the lower atmosphere¹. One of the way of VOC minimizing is increasing usage of water-based emulsion paints. Emulsion paints and varnishes consists of emulsion resin incorporated with hydrophilic polymer, ion group or emulsifier. Major agents are emulsified organic or inorganic binders. Water-based emulsion paints do not contain high VOC as solvent paints do. However, these paints include low VOC (e. g. glycols), which are long-lasting released into air environment in small concentrations, what is potentially dangerous to human beings. Thus, it is necessary to monitor composition of paints as prevention from health-damage. The aim of this work is to determine VOC in water-based varnishes using gas-chromatography system.

Experimental

The model emulsion paint for testing of in-can VOC analysis was prepared by dilution of the water-dispersion of copolymer of styrene with acrylic acid esters, 78.2 % (Mowilith 7460), with 16.6 % of water and than by mixing of the diluted dispersion with coalescents, as shown in Table I.

The VOC in a very small amount (15 ± 3 mg) were heated at 150 °C for 4 min. in a septum-sealed vial, after full evaporation, vapour phase was transferred to non-polar capillary column, then determined by gas-chromatographic analysis. Standard volumes of stock mixture were added at four concentration levels (10, 20, 30, 40 μ l) to obtain the

VOC content. Stock mixture consisted of 1 g of each reference compounds (each coalescent and tetradecane as final marker). Each standard addition was added to 10 g of original sample and shaken well. At least five analyses were made of each concentration level.

GC-MS measurement of VOC from paints were made with Hewlett-Packard GC-MS apparatus GC-MSD 5890A/5790B, equipped with gas chromatograph (model HP 5890) and mass spectrometer (model HP 5970B). Supelco fused-silica capillary GC column PTE-5 (30 m \times 0.25 mm i. d.) with a SE-54-type phase coating (0.25 μ m) was used. The injector temperature was 250 °C and the temperature of transfer line was 275 °C. The GC oven temperature program: 3 min. at 35 °C, increased at 10 °C min^{-1} to 100 °C, then increased at 15 °C min^{-1} to 250 °C. Analyses were performed in splitless mode. Helium was used as the carrier gas. Mass spectrometer detector temperature was set to 275 °C, scan range 42–250 amu, threshold 1500, the electron energy 70 eV, scan 2.06 scan sec^{-1} , solvent delay was 3 min. The identification of separated components – mass spectra were interpreted with the help of mass spectra library databases.

Results and discussion

Although the water-based paints and varnishes are proclaimed as non-harmful for human health, it is necessary to analyse the constitution declared by producers from the point of view of VOC. The accurate volatilization mechanism of glycols used as coalescents and released from EPs for many years in small concentrations is not well-known up to present.

Fig. 1. shows typical GC-chromatogram, recorded under above-described experimental conditions. The method of standard additions was used to correlate and quantify the content of individual components, present in samples. The quantitative analysis was carried out as follows: the area of chromatogram peak was related to 1 mg of the sample weight. The average values for identical components were calculated for each volume of sample. Linear regression analysis was carried out, as depicted in Fig. 2., where the dependence of average area on the volume of the stock reference compound mixture is shown. The so-obtained calibra-

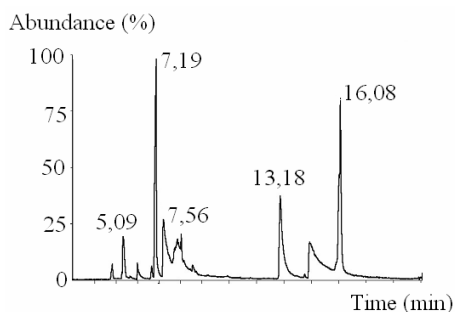


Fig. 1. Representative gas-chromatogram of the sample with standard addition of 40 μ l of the stock reference compound mixture

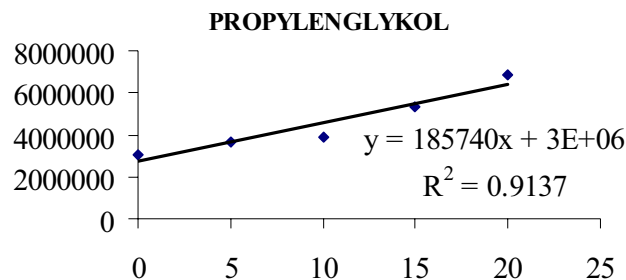


Fig. 2. Linear regression analysis shows dependence of average area on the volume of the stock reference compound mixture for 1,2-propanediol

Table I

Sample characterization	Chemical term	Expected [% by mass]	Experimental [% by mass]
Propyleneglycol	1,2-propanediol	1.04	0.34
Methoxybutanol	3-methoxy-1-butanol	2.08	0.02
Dowanol DPnB	Di(propyleneglycol) butyl ether	2.08	0.02

tion curve could be used for the determination of the VOC content in real sample.

The results, obtained from our variant of the GC-MS analyses should confirm its suitability as a method reliable for the VOC determination. However, the theoretically expected quantity of analyzed components differ from that determined from the calibration curve. This phenomenon could be explained in two ways. The first choice is that the chromatographic conditions (used column) were not the most proper for this purpose, and it was impossible to keep totally identical conditions during sampling. The second choice could follow from the mathematical evaluation – mainly the integration procedure of chromatograph peak, which is subsequently used for future processing

Conclusion

The aim of this work was to analyze declared content of VOC in emulsion varnishes. It was found, that the used method of GC-MS analysis of the vapour phase of sample obtained by 4-minutes heating of the samples at 150 °C gives, 34 % of propyleneglycol and 1 % of both methoxybutanol and dipropyleneglycolbutylether from the original content which are sufficiently lower values than their total concentrations used.

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P11 THE APPLICABILITY OF THE SOLID PHASE MICROEXTRACTION TECHNIQUE FOR THE ANALYSIS OF THE GLIDING ARC DISCHARGE PRODUCTS

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Introduction

Gliding discharges combine both equilibrium and non-equilibrium plasma and offers high energy efficiency and

selectivity for chemical reactions. The excitation energy can be transported to specific molecules in the reacting gas mixture. Recent studies showed that the reaction stimulation efficiency by the gliding arc discharge plasma is very high¹. This plasma can be applied in many industrial branches, especially in the surface treatment². The work is focused on the studies of gliding discharge with respect to its use in the decomposition of volatile organic compounds (VOC). The VOCs decomposition products were sampled by Solid Phase Microextraction (SPME) and analyzed by Gas Chromatography linked to Mass spectroscopy. We tried to check up the SPME technique suitability for the discharge exhausts gas analyses and if this technique can be used efficiently in the field to extract VOCs.

Experiment

The Gliding Arc (Glidarc) is a well-known example of a gliding discharge. Most of the Glidarc plasma exists in the non-equilibrium condition³ (80 %). The reactor consists of a single pair of diverging knife-shaped copper electrodes (thickness 1 mm, size 60 mm × 40 mm each), whose minimal distance is 0.6 mm. Gliding arc discharge was created in various gas mixtures of nitrogen and oxygen at the atmospheric pressure. Just before entering the reactor, carrier gas was enriched by toluene or cyclohexane which were chosen as the model VOCs. The total gas mixture flow rate was typically 1000–1300 ml min⁻¹. The applied DC voltage was from 3.5 kV up to 4 kV with the discharge current of 35 mA. The solid-phase microextraction is a solvent-free equilibrium extraction method which, with the due calibration, can allow also the quantitative determinations of organic compounds. The fiber coating removes the compounds from the gas or liquid sample by absorption or adsorption. SPME has gained a wide spread acceptance as the technique of preference for many applications including flavors and fragrances, forensics and toxicology, environmental and biological matrices, and product testing, to name a few⁴. After the extraction, the SPME fiber is inserted directly into the Gas Chromatograph for desorption and analysis. For quantitative analysis, it is very important to explore the dependence of the analyte mass in the fiber as a function of the extraction time.

Results

Tree types of solid-phase microextraction fibers were used for the experiments, namely polydimethylsiloxane (PDMS), Carboxen/polydimethylsiloxane/divinylbenzene

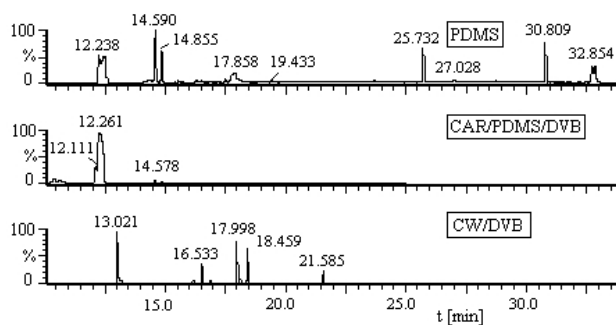


Fig. 1. Chromatographic spectra of toluene destruction products by different fibers

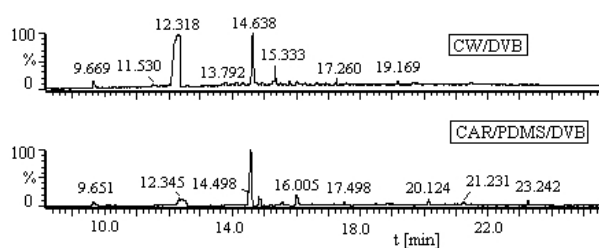


Fig. 2. Chromatographic spectra of cyclohexane destruction products by different fibers

Table I

Analysis of toluene destruction products

CW/DVB	PDMS	CAR/PDMS/DVB
toluene	toluene	toluene
methylbenzene	trimethylsilanol ^m	2,3-dihydrofuranone
phenol	2,3-dihydrofuranone	benzaldehyde
benzaldehyde	Octamethylcyclotetrasiloxane ^m	benzenemethanol
benzenemethanol	Benzaldehyde	—
4-methylphenol	2,4-dimethylimidazol	—
4-(phenylmethoxy)benzaldehyde	—	—
2-methylphenol	—	—

Table II

Identified cyclohexane destruction products.

CW/DVB	CW/DVB	CAR/PDMS/DVB
cyclohexane	2,4-dimethylpentane-3-one	cyclohexane
cyclopenta-1,2-diol	3-methylbutan-2-ol	2,3-dihydrofuranone
benzaldehyde	4-methylcyclohexa-2-en-1-ol	2-ethylhexan-1-ol
heptanale	3-undecene-1-yne	4-phenoxybutanoic acid
2,4-dihydrofuran-2-one	4-decen	2-phenoxyethanol
2-methylcyclohexanol	—	phenol acetate
2,3-dihydrofuranone	—	benzenemethanol
3,5-dimethylhexan-3-ol	—	tetradecane

(CAR/PDMS/DVB), Carbowax/divinylbenzene (CW/DVB). Fig. 1 shows the chromatographic spectra of toluene destruction products extracted using all three tested SPME fibers. The most abundant higher molecular products of the toluene destruction in the nitrogen – oxygen gas mixture are shown in Table I. Besides the traces of various compounds generated by the discharge some compounds from the fiber material (marked by ^m in the Table I) were identified.

Fig. 2 presents the chromatographic spectra of cyclohexane destruction products extracted by the CAR/PDMS and CW/DVB SPME fibers. In the case of PDMS fiber, only

cyclohexane and compounds released from fiber material or chromatographic column were observed and thus this spectrum is not presented. The main high molecular products of cyclohexane decomposition are presented in Table II.

Conclusion

The presented work gives the results about applicability of the Solid Phase Microextraction in the analysis of Volatile Organic Compounds destruction in the non-thermal plasma. Using this technique, better results can be obtained in comparison with the classical Solid Phase Extraction (sorption tubes

with an active coal). The results show that the fiber coated by CW/DVB can extract the highest number of destruction products, the CAR/PDMS coating can be used for the extraction of some other compounds such as phenol acetate or benzenemethanol (C_2 – C_{12} carbons), and the PDMS coating could not be applied for the discharge exhaust gas analysis. The following experiments will be focused on the estimation of concentration dependencies for more or less all discharge products on the discharge conditions.

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P12 SAMPLING OF POLAR POLLUTANTS OF AQUATIC ECOSYSTEM

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Introduction

Passive sampling techniques have been increasingly used to enable effective evaluation of pollution of various environmental compartments. Until recently fish and other aquatic biota were employed to monitor the bioavailable fraction of pollutants, however, they might not represent all pollutants of interest. Moreover, some analytes might be biotransformed. On this account other passive sampling strategies are preferred. One of the recent approaches is based on the utilization of a polyethersulphone membrane which allows selective diffusion and accumulation of specific target polar organic compounds in a solid-phase material. This technique provides a novel and robust solution to the problem of monitoring in situations where large temporal fluctuations in pollutant levels may occur and the repetitive water (spot) sampling is not appropriate due to the cost and laborious work. From this point of view passive sampling is an interesting alternative for the determination of pollutants in aquatic ecosystem.

“STAMPS” (Standardised Aquatic Monitoring of Priority Pollutants by Passive Sampling) coordinated by University of Portsmouth is one of the EU-funded projects

concerned with optimization of integrative sampling strategy applied to control of water pollution. ICT Prague is one of the projects participants and its role consists of three main tasks: (i) implementation and validation of methods for determination of organic pollutants in passive samplers, (ii) proposal and construction of device prototype for sampling and (iii) validation of passive samplers performance through experimental sampling of the Elbe river. Two field trials focused on sampling of the Elbe river water were performed in the Czech republic during spring and summer 2004. Pollutants of interest were both non-polar substances (PAHs, organochlorine pesticides) and some persistent polar compounds (simazine, atrazine, isoproturon, diuron, lindane, and pentachlorophenol). The results of two alternative sampling approaches, i. e. levels of selected polar compounds accumulated in passive samplers and those determined in water obtained by spot sampling were compared.

Experimental

Passive samplers for polar compounds used in our study (prototypes developed by University of Portsmouth) consisted of a high-affinity receiving phase (C_{18} -Empore disc, 47 mm i. d.) separated from the external environment by a diffusion barrier (polyethersulphone membrane). The sampler body is made of polytetrafluorethylene (PTFE). The C_{18} -Empore disc was loaded with calibration internal standards (CIS: D_{10} -biphenyl, D_6 -dimethylphthalate, D_6 -simazine, D_5 -atrazine) and analytical internal standards (D_{10} -anthracene, D_{12} -benzo[*a*]anthracene) prior to use. D_{10} -Anthracene was employed as GC syringe internal standard. It was added to the sample prior to GC/MS analysis for correction of volumetric inaccuracies during sample preparation. D_{12} -Benzo[*a*]anthracene was used as surrogate standard applied to the C_{18} -Empore disc together with the calibration internal standards. The testing set contained 6 samplers loaded with a nominal amount 5 μg /sampler of CIS and D_{12} -benzo[*a*]anthracene, 3 fabrication sampler blanks containing only CIS and D_{12} -benzo[*a*]anthracene at level 5 μg /sampler and 1 field sampler blank containing only CIS and D_{12} -benzo[*a*]anthracene at level 5 μg /sampler. Spot water samples (collected approximately each 3rd day) were analysed to characterise water quality during 28 days of the passive samplers deployment. Passive samplers were taken out after 14 and 28 days. Simazine, atrazine and lindane were analysed in all the samplers consisting the test kit.

After careful disassembling of the sampler the disc extracted with ethyl acetate-isooctane mixture (50:50, v/v). The extract was evaporated and the residue was dissolved in n-hexane. Water samples obtained by spot sampling were processed as follows: 1 liter of water was filtered through 0.45 μ membrane filter and subsequently passed through the ENVI-Carb column. The dichloromethane-methanol mixture (8:2, v/v) was used to elute the analytes. After evaporation of the eluate the residue was dissolved in isooctane. All samples were analysed by gas chromatography coupled to mass spectrometric detector (GC/MS) using electron impact ioni-

Table I
Concentrations of polar compounds in polar disc samplers (ng/disc)

Sampler	D ₆ -diMe-phthalate	D ₅ -atrazine	D ₆ -simazine	D ₁₀ -biphenyl	Simazine	Lindane	Atrazine
Field blank	814	1274	1532	281	<5	<1	<5
Fabrication blank	755	1241	1480	257	<5	<1	<5
RSD (%)	17	25	21	15	–	–	–
Sampling period 14 days	351	913	1138	197	<5	<1	50
RSD (%)	30	5	8	14	–	–	10
Sampling period 28 days	314	775	943	300	<5	<1	87
RSD (%)	17	24	17	24	–	–	19

zation. A DB-5MS (30 m × 0.25 mm i. d., 0.1 μm film thickness) capillary column was used for the separation of target analytes. The mass spectrometer was operated in SIM mode. Monitored *m/z* were: **201**, 173, 186 (simazine), **200**, 173, 215 (atrazine), **219**, 181, 183 (lindane) **162**, 164 (D₁₀-biphenyl), 166 (D₆-dimethylphthalate), **206**, 178, 191 (D₆-simazine), **205**, 178, 220 (D₅-atrazine), **240** (D₁₂-benzo[*a*]anthracene) and **188** (D₁₀-anthracene).

Results and discussion

Table I shows the results obtained by analysis of polar samplers. With the exception of field blank, all results presented are average of three parallel experiments. No data were corrected for blank sample.

Determined concentrations of calibration internal standards (CIS) in fabrication blank and field blank were quite comparable. This fact supports the assumption that the transportation and handling of samplers in field does not result in loss of CIS. During deployment of samplers, concentrations of CIS in disc decrease due to the diffusion into the external aquatic phase. Correlation between offload of CIS and uptake of target analytes can be used to correct the sampling rates “in situ” for biofouling and temperature fluctuations. In our experiments atrazine – widely used herbicide was the only pollutant occurrence of which was proved in polar sampler. The other two target analytes – simazine and lindane were below detection limits. Table I illustrates the accumulation of atrazine during the sampling period.

Results of spot samples analyses corrected for recoveries are summarized in Table II. Again, only atrazine was present. Relatively large variation of its concentration could be observed during the sampling period.

To characterize sampler performance, linear uptake rate (sampling rate) is expressed as the daily volume of water cleared of chemical by a sampler (e. g. ml/day), and its value is independent of concentration in water. Uptake rates for individual compounds are measured during calibration of passive samplers (calibration process should approximate sampling process under controlled conditions). However, the use of calibration data for estimating the concentration of analyte in various aquatic environments (i.e. under different conditions) is not straightforward because the relevant sampling rate is affected by water temperature, turbulence and biofouling of samplers.

Table II
Concentrations of polar compounds in spot samples [ng l⁻¹]

Sampling exday	Simazine	Lindane	Atrazine	Atrazine pected ^{1,2}
0	<3.5	<0.5	24	—
1	<3.5	<0.5	23	—
3	<3.5	<0.5	<2.5	—
7	<3.5	<0.5	64	—
10	<3.5	<0.5	37	—
14	<3.5	<0.5	72	—
Time weighted average ³ (0–14days)	<3.5	<0.5	49	62
17	<3.5	<0.5	61	—
21	<3.5	<0.5	59	—
25	<3.5	<0.5	10	—
28	<3.5	<0.5	160	—
Time weighted average ³ (0–28days)	<3.5	<0.5	58	54

1) value calculated on the basis of results obtained by passive sampling, see Table I

2) not corrected for temperature and biofouling

3) $T_{WA} = [\sum(c_i \times \Delta t_i)] / \Delta t$

Supposing sampling rate about 58 ml/day (determined in previous phase of STAMPS project by University of Portsmouth for atrazine), the estimation of average concentration of atrazine in water in current experiment (see calculated value in Table II, last column) is relatively close to the time weighted average of atrazine concentrations measured in spot samples – even without the correction for temperature and biofouling calculated from CIS offload.

Conclusion

Passive sampling is undoubtedly a challenging approach applicable to monitoring of water pollution. Nevertheless, more data are needed to demonstrate fully its potential. Cali-

bration of passive samplers with respect to different sampling conditions as well as the use of suitable CIS is still under investigation. Also the original sampler body design will have to be changed to increase the active sampling area and – consequently – the capacity and of samplers and, consequently the sensitivity of measurements. Relatively cheap, disposable passive samplers will be undoubtedly available in future for routine monitoring of water pollution without repetitive sampling and analysing of high volumes of water.

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P13 HEAVY METALS IN SOLID IMMISSIONS IN THE AREA OF JELŠAVA

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Introduction

The Jelsava area has had more than a centennial tradition in the magnesite-based refractory material production. The thermal decomposition of magnesite is a dusty technology producing polydisperse magnesian and gaseous emissions. Besides the main magnesian components, the dust outlet from individual production centres of magnesite processing contains a wide range of other elements including heavy metals. Currently, there are two magnesite ore processing plants operating in this area, namely Jelsava and Lubeník. A specific feature is the location of the plants in a relatively narrow valley of the Muráň stream with NW-SE orientation, wherein a frequent dead calm occurs and prevailing wind flow is in

the direction of the valley orientation. The yearly emissions of solid polluting substances decreased in Jelsava from maximum values of more than 5000 t in 1970 to about 120 t and in Lubeník from almost 3000 t to currently about 60 t. The main sources of dust particles outlet in the Jelsava plant are rotary furnaces. These relatively fine-grained dust particles contains a 35.9 % share of grain size fraction under 5 µm emitted by a 120 m high chimney. As to observed heavy metals they contain a 2700 ppm of Mn, a 400 ppm of Zn, a 90 ppm of Cr, a 75 ppm of Cu, a 3.7 ppm of As and less than a 1 ppm of Pb and Cd (Ref.¹). The solids from magnesite processing technologies by their special composition also significantly influence the composition of dust fallout, especially in the vicinity of such technological centres. Since 1996 the dust fallout has been continually monitored in the Jelsava area by the Institute of Geotechnics in co-operation with SMZ Jelsava, Inc. using informative sedimentation method. This paper presents the results of monitoring of heavy metal contents in the dust fallout carried out in the period of 1996–2003. The samples have been taken from eight sampling points.

Methodics

The determination of dustfall was performed in terms of Annex to the Methodological Instructions of the Bulletin of the Ministry of Health of the Slovak Republic No. 13/1982, making some special modifications. For the purpose of analytical processing, the samples of monthly dustfalls from individual sampling points have been analysed using gravimetric method and cumulated into one half-year sample and analysed using AAS method. The average yearly dustfalls of individual elements have been calculated on the basis of chemical analyses and total dustfall. The sampling points were distributed in the distance of 1.6–4.7 km from main source. Two points were placed towards the NE of source, the others in the direction of prevailing NW-SE wind flow.

Results and discussion

The average values of observed heavy metals contents during all monitoring period from 8 sampling points are introduced in Table I.

The Slovak standard stipulates the hygienically admissible limit for the overall dust deposition, however the content of individual elements is not normatively determined. The evaluation can be based on the comparison of levels of

Table I

Average content of observed elements in dustfall, minimal and maximal values from 8 sampling points in the years of 1996–2003.

Element	Mn	Zn	Pb	Cu [mg m ⁻² year ⁻¹]	Cr	Cd	As
Average	227	62	8.3	17.4	10.6	0.68	4.7
Min.	147	40	3.3	15.2	8.2	0.51	1.0
Max.	406	78	17.5	20.8	12.5	1.12	7.7

individual elements measured in the dust deposition in other localities. Generally, the lowest values were registered in the sampling points located outside of main wind flow direction. The strongest influence of distance increasing from main source on the value of element content was recorded in the case of Mn. As to other elements, a reduction of their content with distance increasing is not so strong. The detected values of Mn in dustfall of the Jelšava area approach maximal value $660 \text{ mg m}^{-2} \text{ year}^{-1}$ being recorded in the Dolný Kubín area in late 1980s, which was loading by metallurgical plant Kovohty Istebné². The contents of other elements are comparable with the ones in mid-loaded municipal agglomerations, so they do not attain values in strongly loaded localities^{2,3,4}. But on the other side they significantly exceed the values recorded in relatively clean environment.

The content of heavy metals has been also analysed in the soil^{5,6}. The medium and high concentrations of Zn, Cu, Cd, but mainly Mn have been identified, what corresponds to their content in the dustfall. In the past the effect on the heavy metals deposition was even stronger due to the multiply higher emissions. In addition to magnesite, heavy fuel oil used in rotary furnaces is probably another source of some heavy metals in the dustfall. The local municipal sphere also contributes to the content of some heavy metals, especially in the area of Jelšava.

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P14 THE DIFFUSIVE GRADIENT IN THIN FILMS TECHNIQUE (DGT) FOR THE MERCURY DETERMINATION IN AQUATIC SYSTEMS

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The Diffusive Gradient in Thin films technique (DGT) represents very simple and modern method for in situ determination of labile metal species both in natural aquatic systems¹ and also sediment pore water².

In case of natural waters analyzed metal species are accumulated in a simple DGT unit deployed in a studied aquatic system for a certain period. The fundamental of this accumulation is metal diffusion through a defined diffusive layer (polyacrylamide gel) of known thickness and area and subsequent absorption in a binding agent formed by a layer of gel with embedded resin (for example Chelex-100). Protection against the mechanical or biological damage of the outer diffusive layer is provided by a membrane filter.

After the exposition in studied system, units are transported to the laboratory and the mass of the analyte is determined by common spectral techniques after elution from the resin gel. The concentration of analyzed metal in the solution is then calculated from the Fick's law of diffusion (Eq. 1).

$$c = \frac{M \cdot \Delta g}{D \cdot A \cdot t} \quad (1)$$

where M is mass of metal accumulated in resin, Δg thickness of a diffusive gel, D diffusive coefficient of metal, A exposure area and t time of exposure.

Mercury determination by DGT differs a bit from other metals determination. Polyacrylamide gel is employed as a diffusive layer and successfully used for the determination of many different metal species³. Contrary to these metals mercury can not be determined by the DGT unit in this common arrangement with polyacrylamide gel because of the reactivity of amide group with mercury⁴, which causes accumulation of mercury already in diffusive layer. From this reason the agarose gel having another structure and not binding mercury has been tested and proved to be suitable and effective alternative to the polyacrylamide diffusive layer in case of determination of mercury concentration in natural aquatic systems⁵.

Because of its toxicity mercury belongs to the most monitored metal pollutants in the environment. The determination of mercury by DGT is very useful because of DGT ability to measure labile species. It means species, which can be easily transported through the biological membranes and possibly accumulate in living organisms. Concerning the water environment, certain mercury compounds are able to accumulate in fish so that also human might be endangered through a food chain.

Significant role in speciation, transport, faith and bioavailability of metals in water environment plays dissolved organic matter, particularly humic and fulvic compounds.

Because of their complexation ability they bind various metals and form large and stabile complexes. In this way they also considerably influence the determination of metals in natural aquatic systems by DGT.

Their influence on the mercury determination in water by DGT has been studied⁶. Two basic recommended² DGT tests have been performed in a solution of mercury with humic acids and results compared with the theory. It was found out that both in time-dependence and diffusive layer thickness experiments the measured mass of mercury found

in resin deployed in the solution containing humic acids was lower than that calculated from the Eq. 1.

The possibility that it could be caused by the lower diffusion coefficient of large complex with bonded mercury in gel can be excluded, because the change of this value in case of diffusion through the agarose diffusive gel is negligible⁷. It is assumed that the main decrease in measured mass of mercury is caused by the high stability of these complexes that significantly compete with the resin gel, so that smaller amount of mercury is absorbed in the resin, which bonds only free Hg(II) ions.

Concerning the obtained results^{5,6} it is apparent that DGT with agarose diffusive gel proves to be a suitable tool for the mercury determination in water. The measured mass of mercury in natural aquatic systems is significantly influenced by a type and amount of dissolved organic matter affecting the bioavailability of mercury.

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P15 INVESTIGATION OF TRAPPING INTERFERENCE EFFECTS OF ARSENIC, ANTIMONY AND BISMUTH IN COLLECTION OF SELENIUM HYDRIDE WITHIN AN IRIIDIUM-MODIFIED THGA

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Introduction

The technique of in situ trapping of hydride forming elements within electrothermal atomisers in atomic absorption spectrometry (ETAAS) becomes a mature method. It enables to enhance sensitivity of determination due to pre-concentration effect and eliminate matrix effects in atomisation step. Usually, it is performed manually or automatically in commercial longitudinally or transversally (THGA) heated atomisers, bare or equipped with platforms.

Chemical modification is a common approach applied in most methods in which a graphite furnace is used¹. If volatile

elements are to be determined, the most important aim of modification is to stabilise the analyte to higher temperature. To enhance the trapping efficiency, the graphite surface is very often modified by noble metals (platinum group metals – Pd, Pt, Rh, Ir...), among those thermally reduced iridium (1000 °C) can only be used as a permanent modifier².

Relatively serious trapping interference effects of other co-volatilised hydride forming elements present in the sample were recently observed^{3–5}. This appears to be the main drawback of the in situ trapping technique. Therefore, a systematic investigation was undertaken to shed light on the processes causing interference effects.

In this work, the influence of interfering elements (As, Sb, Bi) on trapping efficiency of selenium (analyte) hydride is studied by using twin-channel hydride generation system. The twin-channel continuous flow-system is designed to separate generation, introduction and trapping of analyte and interferent hydrides, so enabling to distinguish interference effects occurring in gaseous phase from interference effects occurring in liquid phase. Hydrides of analyte and interferent are generated independently in two separate channels, one for generation of analyte hydride and another one for generation of interferent hydride. Both types of hydrides are mixed and introduced into the atomiser during the trapping step by means of a thermally insulated wide bore quartz GC-capillary inserted through the sampling hole.

Hydrides are formed when mixing the acidic analyte solution and an alkaline solution of sodium tetrahydroborate (NaBH₄). As the NaBH₄ is immediately decomposed with acidic solution, hydrogen is evolved simultaneously. The volatile hydride is stripped from the aqueous solution of reaction products with the aid of the hydrogen produced and argon admixed upstream of the reaction loop. Finally, the gaseous phase is separated from the liquid phase in G/L-separator and the hydride is carried by argon into the atomiser.

Conclusions

The mechanism of interference effects in hydride trapping is still not fully known. Therefore, gaseous phase interference effects caused by a volatile form of the potential interfering generated simultaneously hydride forming elements (As, Sb, Bi) were studied. Based on experimental results, following conclusions were drawn.

- Se-stabilisation and Se-trapping capacity are enhanced when increasing Ir-modifier mass,
- 50–200 µg Ir is the optimum amount of permanent modifier,
- optimum trapping temperature is 400 °C (higher temperatures are also applicable, however with reduced sensitivity),
- simple gas phase modification by oxygen can eliminate interference of As, Sb and Bi up to a concentration level 3–4 orders of magnitude higher than analyte.

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P16 APPLICATION OF SPME FOR DETERMINATION OF TRICHLOROANISOLE (TCA) IN CORK STOPPERS

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Introduction

Traces of chloro-anisoles were identified as being responsible for musty/mouldy taints in a number of foods and beverages, including wine. The most potent of these compounds is 2,4,6 tri-chloro-anisole, usually referred to as TCA. The aroma detection threshold of TCA in wine has been determined as ranging from 4 to 50 ng l⁻¹ depending on wine type. Other members of the group the mono-, di-, tetra-, and penta-chloro-anisoles are neither so potent nor frequent.

Although TCA contamination of wine could arise from oak barrels or other contact materials, it was found out, that cork is the major vehicle for the transmission of TCA to bottled wine. It was clarified, that TCA formation starts during bleaching of cork stoppers, when originating tri-chloro-phenol is than converted to TCA by the action of certain moulds¹.

The purpose of our study was to develop a method sufficiently sensitive, which would enable to control of very low TCA levels in wine and cork material. With respect to this requirement, the solid-phase microextraction (SPME), known as a very powerful sensitive technique, was tested.

Experimental

Analysed samples

To determine 2,4,6 TCA in cork material with a cork taint off-flavour, 50 commercial cork closures representing

single lot produced by the Czech company were analysed.

Materials

Standard of 2,4,6-trichloroanisole (99.9%) was supplied from Sigma-Aldrich (CR). Sodium chloride (analysis grade) was supplied by Penta Chrudim (CR) and ethanol (96.5%) was supplied by Bioferm-Lihovar Kolin (CR).

SPME fibre: the divinylbenzene/carboxene/polydimethylsiloxane (30/50 µm DVB/CAR/PDMS) fibre (Supelco, USA) was used for TCA headspace sampling. Short conditioning (30 min/250 °C) and blank run of the fibre were carried out daily before the use of fibre for sampling.

Instrumentation

Gas Chromatograph Hewlett Packard 5890 Serie II equipped with electron capture detector (GC/ECD).

Preparation of cork leachate and isolation of 2,4,6-TCA using manual SPME

Five litres Erlenmayer flask containing 50 pieces of cork stoppers was filled in with wine simulant (10% (v/v) ethanol in water). Bottle was than sealed with glass cap lined with parafilm agitated and held at room temperature (23 °C) for 24 hours prior to analysis. 10 ml aliquote of leachate was then put into a 20 ml glass headspace vial containing 2 g of natrium chloride. Afterwards the vial was sealed with hole cap and teflon-faced silicone septa (Supelco). The incubation of the sample was carried out at 30 °C with magnetic stirring for 5 min. Extraction was carried out with divinylbenzene /carboxene/ polydimethylsiloxane (DVB/CAR/PDMS) fibre for 20 min at 30 °C. After extraction the fiber was withdrawn and introduced to the injection port of the gas chromatograph, where thermal desorption of analytes took place (4 min at 250 °C) . The fiber was left inside the GC injection port during a whole GC run (30 min).

Chromatographic conditions

DB – 5 (60 m × 0.25 mm × 0.25 µm) and DB – 17 (60 m × 0.25 mm × 0.25 µm) fused – silica capillary columns and helium as a carrier gas (1 ml min⁻¹) were used. The columns were initially held at 60 °C for 4 min, the temperature was then programmed up to 240 °C at a rate 10 °C min⁻¹ (GC run time was 30 min). The injector was operated in splitless mode with 4 min splitless period. The temperature of injection port was set at 250 °C.

Linearity

For linearity testing standards in wine simulant were prepared with TCA levels of 5, 10, 50 and 130 ng l⁻¹.

Repeatability

The method repeatability was studied by five replicate analyses of wine simulant spiked to the level 5 ng l⁻¹.

Results

According to common strategy², the extent of cork stoppers contamination is assessed on the basis of TCA content in leachate obtained under standardized conditions. This approach is in agreement with observation of good correlation between the amount of TCA in wine and concentration of this contaminant transferred into simulant. Determination of total TCA is not suitable for prediction of wine contamination probably due to variations in cork quantity (namely texture) since changing extent of leaching.

As demonstrated in our study optimized SPME headspace sampling coupled with GC/ECD provides very low detection limits (LOD 1 ng l⁻¹) with limit of quantification 2 ng l⁻¹, which meets the requirement to control these chemical at levels close to its (very low) odour threshold. Also other performance characteristics of newly developed method were very good: using manual SPME relative standard deviation (% RSD) 7 % could be attained for repeated measurements (n = 6). The linearity range was 2–130 ng l⁻¹ with correlation coefficient 0.9973. The method allows the analysis for chloroanisoles at very low concentration with good repeatability. Fig. 1 shows example of cork leachate analysis.

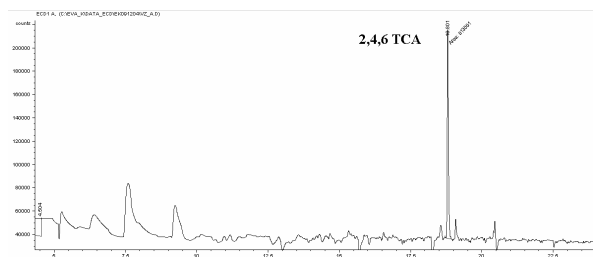


Fig. 1. Chromatogram of cork leachate. TCA level of 47 ng l⁻¹

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P17 ASSESSMENT OF METAL BIOAVAILABILITY IN SOILS BY THE DGT TECHNIQUE

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Introduction

Risks associated with heavy metal contamination in soils are difficult to assess. In most ecotoxicological studies,

the toxic effect of heavy metals have been related to total soil concentrations or some operationally defined extractable fractions. The leaching procedure¹ are often used for determination of metal concentrations in soils. But these processes do not usually provide information about fractions really available to plants, microorganisms or soil fauna. Therefore new approaches, which could characterize concentrations and transport of bioavailable forms of metals in soils much better, have still been found.

The DGT (Diffusive Gradients in Thin films) technique has proved to be a very promising tool to assess both kinetics of resupply and the bioavailability of metals to plants. Like plant roots, DGT locally decreases the metal concentration of the soil solution and triggers resupply by diffusion from the soil solution and solid phase. The kinetics of this resupply determines the availability of metals to plant roots^{2,3}.

In this study the DGT technique was used for determination of the bioavailable part of selected metals in soils. Then for assessment of the degree of metal resupply from the soil solid phase to the soil solution and proportional representation of organic and inorganic (metal ions and hydrated ions) forms of metals in selected South Moravia arable soils fertilized by sewage sludge in 1980s.

The DGT technique principle

This technique uses two polyacrylamide gel layers placed in a plastic unit. Metal cations diffuse across the membrane filter and diffusive layer and they are immobilized and concentrated by a sorption in the resin. Trapped metal ions are eluted from the resin by nitric acid and trace metals are determined in eluate by ET AAS, ICP AES or ICP MS. When a linear concentration gradient is established across the diffusive layer, the flux of trace metals from the solution to the resin is governed by I. Fick's law of diffusion⁴

$$F = D \cdot \frac{dC}{dx} \Rightarrow C_{DGT} = \frac{M \Delta g}{DtA}$$

where F is flux, M is mass of trace metal accumulated in resin gel, A is exposure area, Δg is thickness of diffusion gel layer, D is diffusion coefficient, t is deployment time.

Experimental

Polyacrylamide hydrogels used in this experiment contained 15 % by volume acrylamide (Sigma-Aldrich, Germany) and 0.3 % by volume agarose cross-linker (DGT Research Ltd., UK). N,N,N',N'-Tetramethylethylene-diamine was used as a catalyst and ammonium persulfate (8 %) was used as an initiator for preparation of gels. Resin gels contained specific ion-exchange resin Chelex-100 (Na-form, 200-400 wet mesh, Bio-Rad Laboratories, CA, USA) besides.

Soil samples were represented by South Moravia arable soils, neutral, middle type, fertilized by sewage sludge in 1980s, examined within the bounds of the project INCO Copernicus: Cycling trace metals in sustainable management

of agricultural soils. Fertility requires the inventory of input metals (FERTILIA), IC15-CT98-0124, collected from three sampling sites Tuřany, Zlín and Chrlice from three depth profiles of 0–10, 40–60 a 90–110 cm (ref.⁵).

Soil slurries were prepared into plastic vessels. After 24 hours equilibration DGT units were deployment for 48 hours by temperature 25 ± 2 °C. Exposure windows of DGT units were in an intimate contact with the mixture. Water/soil phase ratio was for Tuřany sample 0.5 ml g^{-1} , Zlín sample 0.6 ml g^{-1} and Chrlice sample 0.375 ml g^{-1} . The constant moisture of the soil suspense was kept during the experiment.

Two types of diffusive gels with a different pore size⁶ were used in this study. APA gels (polyacrylamide gel crossed by agarose cross-linker) with pore size $> 5 \text{ nm}$ were used for determination of the sum of labile inorganic and organic forms of metals. RG gels (polyacrylamide gel crossed by Bis-acrylamide) with pore size $< 1 \text{ nm}$ (DGT Research Ltd., UK) were used for determination of labile inorganic forms of metals it means metal ions and hydrated ions.

In soil samples cadmium, copper, nickel, lead and chromium were determined by electrothermal atomic absorption spectrometry (SpectrAA-40, Varian, Australia) after elution of the resin gel in 1 ml of 1 mol dm^{-3} nitric acid.

Results and discussion

Determination of the degree of the metal ion resupply from the soil solid phase by ratio R

A degree of the metal ion resupply from the solid phase is possible to find out by a ratio R (see Table I) which is expressed by the ratio between the concentration C_{DGT} and the total concentration C in the soil solution (C is measured by an independent analytical method)

$$R = \frac{C_{\text{DGT}}}{C}, \quad 0 < R < 1$$

Cadmium and chromium are the most mobile metals among the five ones mentioned above ($R \rightarrow 1$) and thus the

most available ones, particularly in 0–10 and 40–60 cm of soil samples depth profiles where they occur in labile forms. In this case the soil resupplies the metal sufficiently and quickly from the solid phase to the soil solution and metal concentration is relatively constant throughout the deployment. However, the cadmium and chromium mobility evidently changes with depth and metal releasing from the solid phase in 90–110 cm depth profile is much slower with the exception of chromium in Tuřany soil sample. The copper mobility and thus bioavailability is in all depth profile of all soil samples relatively constant but the metal resupply from the solid phase is slower than in case of cadmium and chromium. The nickel mobility and thus bioavailability is the highest in case of Zlín sample and the lead mobility is evidently limited ($R \rightarrow 0$) in all depth profile and the metal is not practically available because of no metal resupply from the solid phase with the exception of Zlín sample.

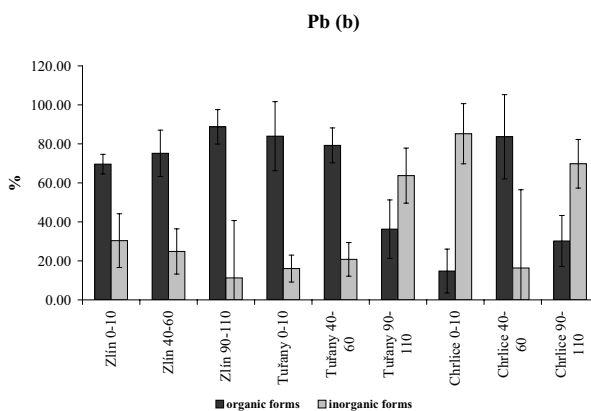
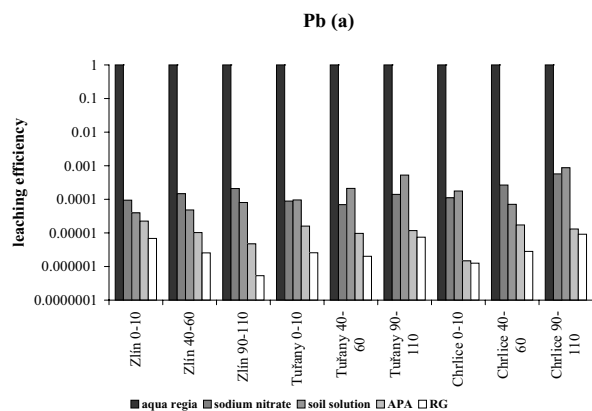
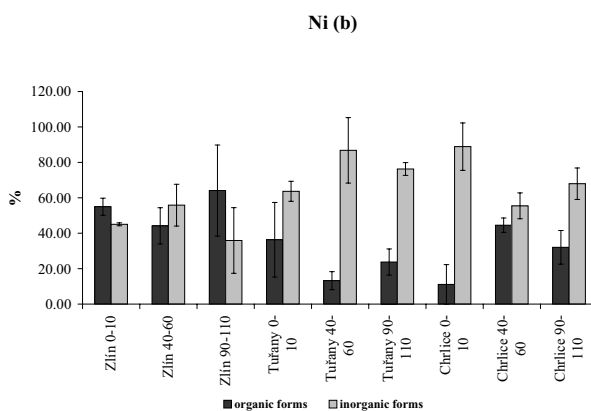
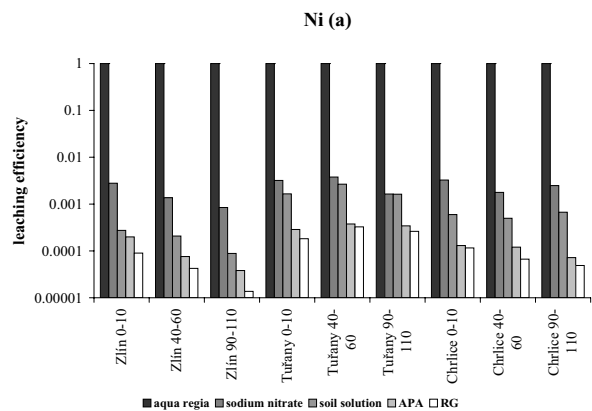
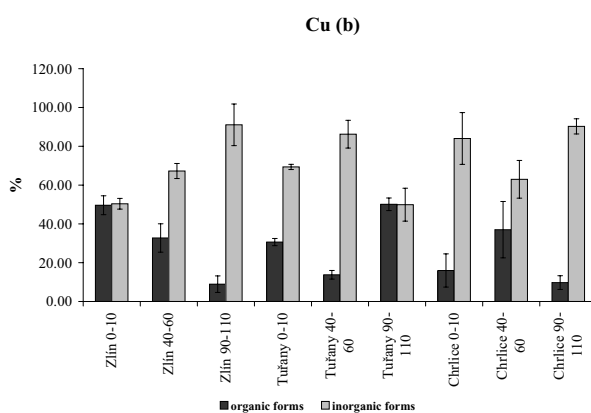
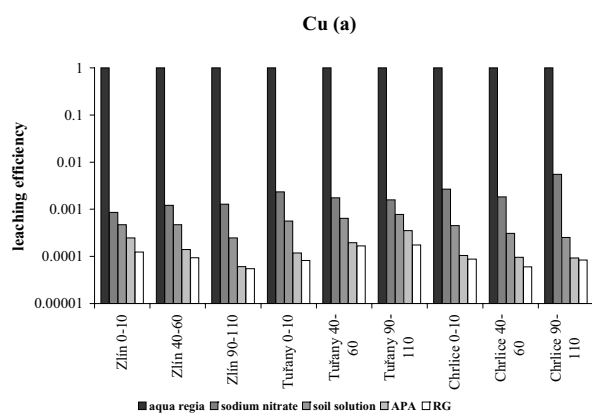
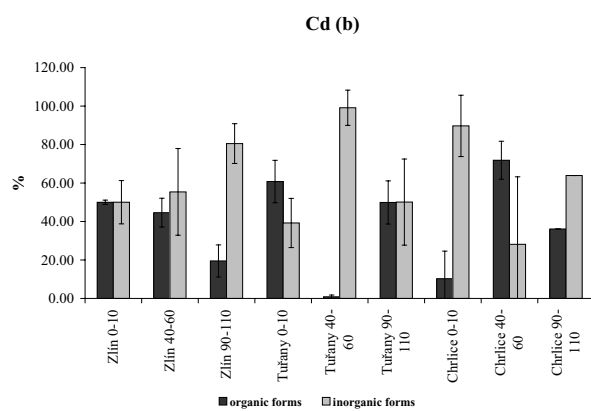
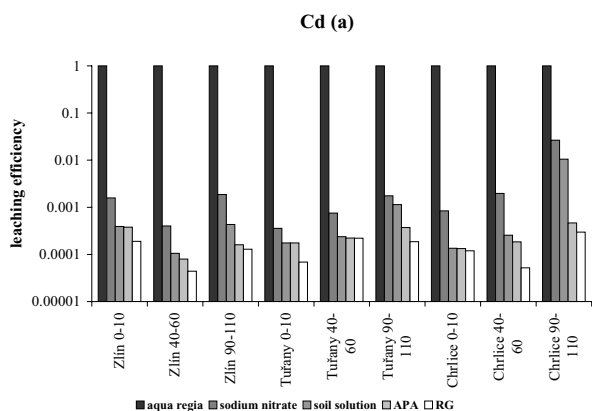
Comparison of the DGT technique and leaching procedures

Metal concentrations determined in soil samples by the DGT technique are presented in figures in logarithmic scale and they are related to the total metal contents determined by leaching with aqua regia. The comparison of results obtained by the DGT technique and those obtained by leaching with aqua regia and sodium nitrate (results were adopted from project FERTILIA) is shown in Figure 1a and the proportional representation (%) of organic and inorganic forms of metals in soil samples is shown in Fig. 1b.

The leaching procedure employing sodium nitrate is usually used for determination of the bioavailable part of metal in soils⁷. However, the concentration of constituent metals determined by the DGT technique were lower in comparison with concentrations determined by the leaching with sodium nitrate approximately by one to two orders. It means the leaching with sodium nitrate does not provide exact information about the metal fraction really available to the plant root system. Whereas, it has been shown that the DGT units are able to imitate this system very well². Also the metal concen-

Table I
Ratio R values

	depth profile [cm]	Cd	Cu	Ni	Pb	Cr
Tuřany	0–10	1.00	0.21	0.17	0.17	0.97
	40–60	0.94	0.30	0.14	0.05	0.75
	90–110	0.33	0.45	0.21	0.02	0.64
Zlín	0–10	0.96	0.53	0.73	0.57	0.98
	40–60	0.75	0.30	0.36	0.21	0.45
	90–110	0.37	0.24	0.43	0.06	0.33
Chrlice	0–10	0.98	0.23	0.22	0.01	0.18
	40–60	0.72	0.31	0.24	0.24	0.90
	90–110	0.04	0.37	0.11	0.02	0.69



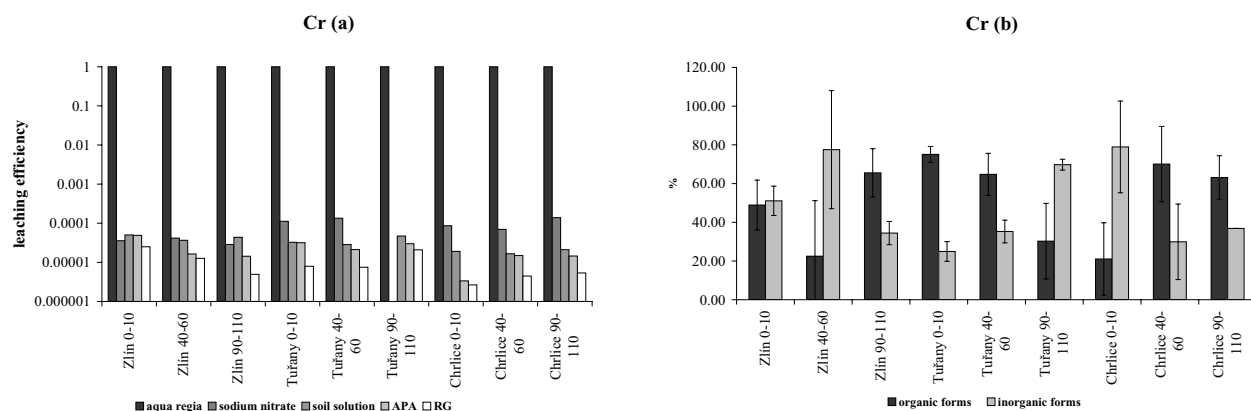


Fig. 1. The comparison of the DGT technique and leaching efficiency of aqua regia and sodium nitrate (a), the proportional representation of organic and inorganic forms of metals in soil samples (b)

trations measured by the DGT technique were much lower than in the soil solution with the exception of cadmium and chromium. Inorganic forms of metals formed a predominant part from the total content of these metals in examined soils (see Figure 1b) with the exception of lead and chromium. However, the mobility, compounds and forms of these metals especially depend on the pH value, content of organic matter, redox potential and many other factors.

Conclusion

The DGT technique provides the possibility of determination of labile metal forms in soils which are available to the plant root system. This technique reflects actual available metal ion concentrations in the soil solution. By the choice of the pore size of diffusive gel the proportional representation of organic and inorganic (metal ions and hydrated ions) forms of metals can be determined.

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P18 TRAPPING OF ANTIMONY AND BISMUTH HYDRIDES ON A MOLYBDENUM-FOIL STRIP

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Introduction

In situ collection of hydride forming elements (As, Bi, Ge, In, Pb, Sb, Se, Sn and Te) with subsequent electrothermal atomic absorption spectrometry (ETAAS) detection is typically performed within a commercially available graphite (GF) or tungsten (WETA) heated atomizers¹. Nowadays, it is a mature and one of the most sensitive techniques for trace element determination. Nevertheless, this technique can also be applied in other atomic spectrometry methods². For this purpose, conventional electrothermal atomizers are modified and/or special devices are designed.

Studies of the sequestration of arsenic and selenium within transversely heated graphite and tungsten tubes, including the radiography experiments with selenium radionuclide were performed^{3,4}. The results showed that the main part of the analyte is trapped on the very small area of the tube, opposite the hole of the introduction capillary. This observation suggests to design special miniature collection/vaporization devices useful for trapping and subsequent introduction of these analytes. These devices can be coupled to various types of atomizers, i.e. quartz tube atomizers, various type of flames and plasmas, typically used in atomic spectrometry methods (AAS, AFS and AES). Thus very sensitive and specific detection of elements of interest can be performed.

A prototype of a miniature collection device was designed for collection of hydride forming elements. It is based on a strip of the molybdenum foil (which is typically used in production of “halogen” bulbs) and combined with miniature

hydrogen diffusion flame for specific analyte detection in atomic absorption spectrometry. Capability of trapping of hydrides in this device was studied employing antimony and bismuth hydrides as volatile species of analytes. Influence of trapping temperature, modification of the foil surface with platinum group metals – Ir, Pt and Rh, argon carrier gas flow rate, distance between the orifice of the injection capillary and the strip, sample volume and composition of the gaseous phase (argon-hydrogen-oxygen) was investigated. Analytical figures of merit were also estimated for both analytes at optimum collection conditions. Capability of trapping of antimony and bismuth hydrides is compared with that of arsenic and selenium ones⁵.

Conclusions

Bare molybdenum surface treated in hydrogen atmosphere is capable of trapping antimony, arsenic, bismuth and selenium. Antimony and bismuth exhibit different behavior. Maximum trapping efficiencies were found at temperatures of approximately 300–500 °C lower than for arsenic and selenium (1100–1200 °C). In contrast to arsenic and selenium, modification of the surface reduces trapping efficiency and presence or absence of hydrogen in the gaseous phase during collection step does not play significant role in analyte trapping. The useful analytical lifetime of the strip vaporizer was above 400 firings at the optimum vaporization temperature of 2300 °C.

This work was supported by The Grant Agency of the Academy of Sciences of the Czech Republic (Project No. A400310507).

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P19 COATED FERTILIZERS WITH GRADUAL EFFECT

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Introduction

Tendency of our work is to find substances with convenient parameters for surface treatment of fertilizers. It

must guarantee good shelf life of fertilizers modified after this manner and provide gradual loosening of nutrients into the soil. Next important aspect is a view of decomposability of solids in the nature. In a practical application we will reach more effective supply farmlands with needy nutrients. If the demand of fertilizers will lower on an unit of farmland, burden on environment will lower too (mainly watercourses), so complete costs on fertilizers will lower too.

Our research is specialized in a monitoring and testing influence of some coating substance cellulose-based on physico-chemical properties of an industrial fertilizers. We evaluate mainly solution rate, strength and last but not least shelf life fertilizers with such surface treatment.

Experimental part – testing of coating substances

We used a type of fertilizer NPK as a base. It is a classic granulated fertilizer with the ratio of nutrients 15% N: 15% P₂O₅: 15% K₂O. Analyte was 15 minuts sifted on an automatic classifier with screens. From this mass of pellets with diameter 2–4 mm was for every partial coating weighted 200 g. This mass was put into a laboratory disc-type granulator, where was distributed coating dilution.

Coating pellets of fertilizer with oxycellulose

Into glass jet was apart weighted water alkaline solution of oxycellulose. (below „OC“). Jet was attached to the compressed air and a coating was done on the pellets of fertilizer. Analytes were always let leisurely run dry for 24 h.

Coating pellets of fertilizer with ethylcellulose and cellulose acetate

Same method as in previous point, we weighted a dilution of ethyl cellulose (below „EC“) in ethanol. Jet was attached to the compressed air and a coating was done on the pellets of fertilizer. In the second case was weighted a solution of medium viscous cellulose acetate (below AC) in acetone.



Fig. 1. Laboratory disc-type granulator



Fig. 2. Apparatus TMZ-3U for measuring strength

This way was created a range of analytes with several percentage by weight of the coating. Analytes were always let to run dry for 24 h.

Measuring of solution rate of analytes

Measurement proceeded in an environment of distilled water (500 ml, 25 °C). 5 g of analyte was put into the dosing equipment. From this equipment fertilizer came loose into the water during perpetual mixing. Conductivity was automatically measured and written down in intervals 10 s. Limit conductivity for uncoated fertilizer was measured out 11.28 mS cm^{-1} (1g of fertilizer was dissolved in 100 ml distilled water and shaken for 30 minutes).

Measuring of strength coated pellets of fertilizer in a pressure

Measuring proceeded on an automatic equipment TMZ-3U (Micro-Sensor, CZ). 20 pellets from every analyte were measured. Every pellet was exposed to pressure and strength value was marked.

Results and discussion

After the application solutions OC, pellets slowly ran dry because of high dampness of the analytes. After the application solutions EC and AC, pellets quickly ran dry thanks to easy evaporation of ethanol.

Results of measuring solution rate component analytes and comparison with uncoated fertilizer are stated in graphs on Fig. 3., 4. and 5. Results of measuring strength component analytes and comparison with uncoated fertilizer are stated in graphs on Fig. 6., 7. and 8.

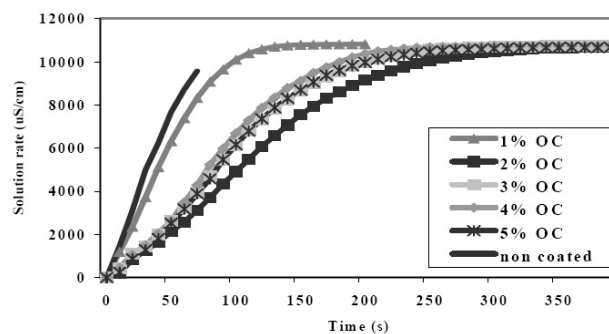


Fig. 3. Comparison solution rate analytes coated in OC

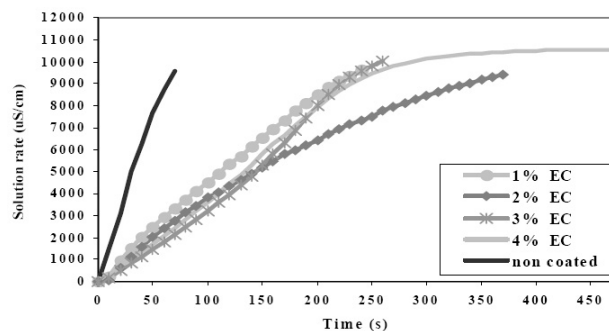


Fig. 4. Comparison solution rate analytes coated in EC

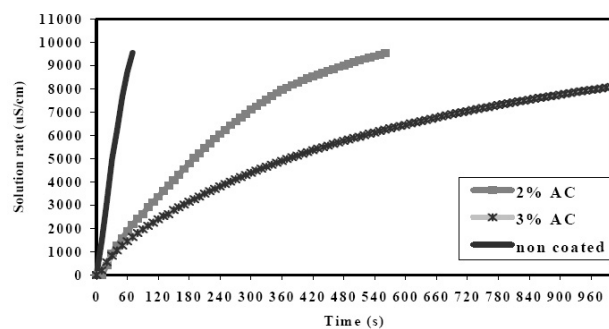


Fig. 5. Comparison solution rate analytes coated in AC

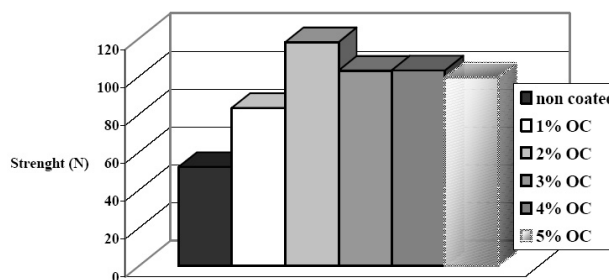


Fig. 6. Comparison strength analytes coated in OC

Conclusion

Analytes with surface treatment shows higher strength and slower dissolving then uncoated pellets. We reached higher strength in conjunction with slowing down of loose-

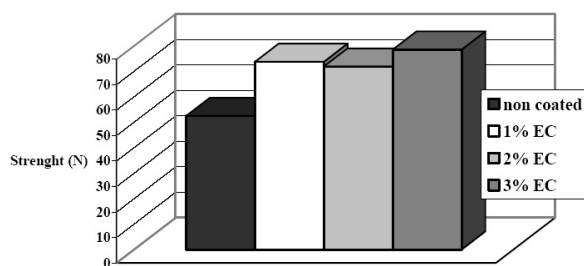


Fig. 7. Comparison strength analytes coated in EC

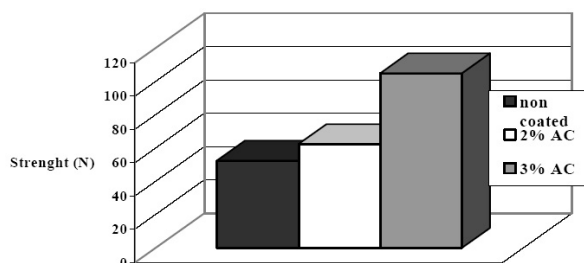


Fig. 8. Comparison strength analytes coated in AC

ning nutrients with growing percentage by weight of coating substance. Concrete values are stated in graphs on Fig. 3–8.

Another coating substances and percentage by weight will be tested further.

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P20 PRECONCENTRATION OF CHROMIUM, MOLYBDENUM AND VANADIUM ON MODIFIED SILICA ANION-EXCHAGER IN THE PRESENCE OR ABSENCE OF ORGANIC AGENT AND THEIR DETERMINATION BY ICP-AES

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Introduction

The determination of chromium, molybdenum and vanadium is of considerable interest because they are biosignificant or bifunctional elements and in excess can also cause metabolic disturbances^{1,2}. Metal's concentration in natural waters are lower than LOD of common analytical methods and so that prior separation and preconcentration is necessary.

All these elements exist in environment in variable oxidation states. Cr(VI) and Cr(III) are the most stable oxidation states of chromium in natural waters. Cr(VI) is considered much more toxic than Cr(III). Even though thermodynamic calculations show that Cr(VI) should predominate in oxidative conditions, Cr(III) coexists due to the slow kinetics of Cr(III) oxidation. Cr(III) has a strong tendency to adsorb onto surfaces while Cr(VI) has a high mobility in natural waters³. Molybdate – Mo(VI) is the most abundant species of molybdenum in oxic aquatic environments, and also has a generally high mobility. In anoxic environments, Mo(VI) is reduced to lower oxidation state being as sulfides and oxides³. Vanadium dissolves in natural waters as V(IV) and V(V)⁴. V(V) can be also reduced to V(IV) by a number of biological-reducing agents, including ascorbate, cysteine etc. and presents in reducing environments such as shale and carbonaceous sediments^{4,5}.

Mononuclear or oligonuclear forms are present during equilibria in aqueous medium in dependence on its concentration and pH^{6,7,8}.

Mononuclear species

Alkaline solutions	Acid solutions
MoO_4^{2-} , HMoO_4^- ,	H_3MoO_4^+ , $\text{H}_4\text{MoO}_4^{2+}$,
$\text{H}_2\text{Mo}_2\text{O}_4$, $\text{MoO}_2(\text{OH})_4^{2-}$	MoO_2^{2+}
HCrO_4^- , $\text{Cr}_2\text{O}_7^{2-}$	CrO_4^{2-}
VO_4^{3-} , VO_3^- , HVO_4^{2-} ,	VO_2^+
H_2VO_4^-	

Oligonuclear species

$\text{Cr}_2\text{O}_7^{2-}$, $\text{Cr}_3\text{O}_{10}^{2-}$, $\text{Cr}_4\text{O}_{13}^{2-}$, $\text{Mo}_7\text{O}_{24}^{6-}$, $\text{Mo}_8\text{O}_{26}^{4-}$,
$\text{Mo}_{36}\text{O}_{112}^{8-}$, $\text{V}_2\text{O}_7^{4-}$, $\text{V}_3\text{O}_9^{3-}$, $\text{V}_4\text{O}_{12}^{2-}$, $\text{HV}_{10}\text{O}_{28}^{5-}$

Preconcentration technique based on retention of hydroxyanions and metal complexes with 4-(2-pyridylazo) resorcinol (PAR) and 8-hydroxyquinoline (8-HQ) on modified silica anion exchanger (Separon SGX AX, Tessek) was optimised.

Experimental

Retention and elution arrangement
Solutions for the retention (50 ml) or the eluent (10 ml) were pumped through the plastic cartridges with the anion exchanger placed in the vacuum pump-operated vacuum suction device Dorcus™ (Tessek Prague). A peristaltic pump PCD82.4K (Kouřil, Czech Republic) was attached with 3 mm wide silicon tubing to the cartridges Ismatec and operated with a solution flow rate 1.1–4.5 ml min⁻¹.

The retention and elution efficiency calculated in the recovery values were always tested independently. The confidence interval of mean recovery resulted from 3 independent sorptions and elutions using the span between the lowest and highest values of the variation interval⁹.

ICP-AES Instrumentation and operating conditions

An echelle-based ICP-spectrometer with a prism predisperser IRIS AP™ (Thermo Jarrell Ash, U.S.A.) containing

a CID detector with 512×512 pixels for 195–900 nm, axial plasma discharge and echelle grating with $54.4 \text{ lines mm}^{-1}$. The plasma source was a generator with 27.12 MHz and power output of 1.15 or 1.35 kW. The signal integration time was 20 s and every results were the average of 3 measurements. The Meinhard nebulizer was fed by a peristaltic pump with a flow rate 1.85 ml min^{-1} . Spectral lines with high intensity and selectivity and low background influences were selected and optimized for simultaneous determination of concentrations Cr, Mo and V in solution. The increased power output 1.35 kW enabled to decompose the interfering organics from solution in the plasma and raises the slope of calibration plots. The instrumental detection limits based on the 3 s definition and evaluated from 10 measurements of the blank solutions are: Cr(267.716 nm) – 9 ppb, Mo(202.030 nm) – 22 ppb, V(290.882 nm) – 16 ppb.

Results

Optimization of parameters

The column filled with strongly basic anion exchanger with aliphatic quarternary groups was conditioned by 10 ml 3 M HCl prior to the retention. 50 ml of solution containing $10 \mu\text{g}$ of Cr(VI), Mo(VI) and V(V) in the presence or absence of chelating agent was pumped through the column using flow rate 1.2 ml min^{-1} . The anion-exchanger was washed

from non-retained residues and metal anions were eluted with 10 ml of elution agent.

The effect of pH (Fig. 1), effect of eluent conditions and volume (Fig. 2), effect of organic agent (PAR, 8-HQ), metal's concentration, flow rate and sample volume, effect of humic acid and the retention of Cr(III) and V(IV) besides of Cr(VI) and V(V) were tested.

Verification of optimized procedure

Optimized procedure was successfully verified on spiked river, tap and mineral waters.

Conclusion

Silica based strongly basic anion exchanger Separon™ SGX AX in the presence or absence of PAR and 8-HQ may be used for the selective preconcentration of $0.25\text{--}10 \mu\text{g}$ of Cr(VI), Mo(VI) and V(V) from $50\text{--}500 \text{ ml}$ sample volumes. The anion-exchanger used is suitable for the $10\text{--}100$ fold preconcentration of Cr(VI), Mo(VI) and V(V) prior to the final determination by ICP-AES. Ions, commonly occurred in natural waters (Na, K, Ca, Mg, Fe, Al) do not interfere retention process and this method may be used for quantitative determination of Cr(VI), Mo(VI) and V(V) concentrations in mineral, tap and river waters.

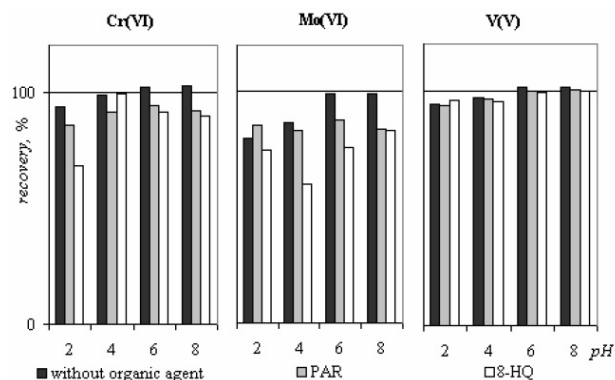


Fig. 1. Effect of pH on recovery of Cr(VI), Mo(VI) and V(V) in presence or absence of organic agent

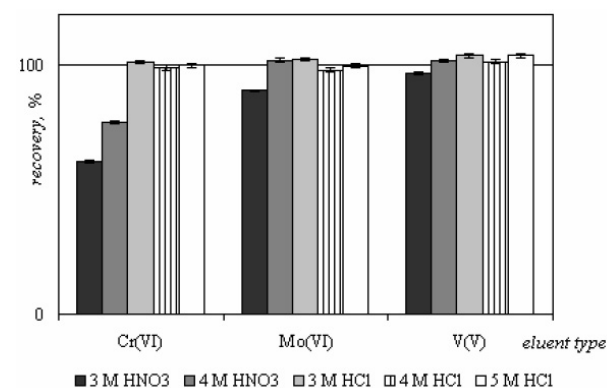


Fig. 2. Effect of eluent conditions on quantitative elution of Cr(VI), Mo(VI) and V(V) anions

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P21 APPLICATION OF SPMD FOR SAMPLING OF ORGANIC POLLUTANTS FROM POROUS MEDIA – MODEL STUDY

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Introduction

Persistent organic pollutants (POPs) are ubiquitous contaminants resistant to photochemical, biological and chemical degradation. POPs are often halogenated compounds and

characterized by low water solubility. Due to high affinity of POPs to lipids, their bioaccumulation in fatty tissue typically takes place. Regarding environmental pollutants occurring in various abiotic matrices such as soil, water sediment, the assessment of their bioavailability for biota is an important issue^{1,2}.

Currently used procedures of extraction of POPs from solid matrices are based on the use of organic solvents which in most cases enable exhaustive isolation of analytes. However, selective extraction which extract only of bioavailable fraction are needed for purpose of risk assessment^{3,4}.

Semi-Permeable Membrane Devices (SPMDs) are passive samplers consisting of a tubular lay flat low-density polyethylene (LPDE) membrane containing a thin film of high-molecular weight lipid (triolein). SPMDs can be used to detect variety lipophilic substances in water, sediment/soil and air. When placed into respective environmental compartment, SPMDs passively accumulate hydrophobic organic compounds and thus simulate biological membrane function by allowing size selective diffusion of organic compounds, such as polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs)^{5,6}. In general, the process of passive sampling of hydrophobic organic chemicals (dissolved and/or in vapor phase) is driven by membrane- and lipid-water partitioning⁵⁻⁷.

In the presented study we attempted to determine the bioavailable POPs in several environmental matrices commonly classified as porous media. We tested 3 different types of samples collected in the locality Žernoseky (Elbe River, Czech Republic): (i) river sediment, (ii) agricultural soil flooded during huge floods in August 2002 and (iii) soil from unflooded field close to the river. Soxhlet extraction by relevant organic solvents was employed to determine the total content of target analytes (PAHs, PCBs, OCPs and PBDEs) in the above porous media (collected in years 2003 and 2004). In the next part of study we prepared aqueous leachate of each medium for sampling of bioavailable fraction by SPMDs.

Methods

Porous media

Isolation of target analytes from porous media was performed by Soxhlet extraction by dichloromethane. The purification of crude extract was carried out by gel permeation chromatography employing Bio Beads SX-3. Cyclohexane-ethyl acetate mixture (1 : 1, v/v) was used as a mobile phase for clean-up of PCBs, OCPs and PBDEs extracts.

High resolution gas chromatography using two parallel electron capture detectors was used for analysis PCBs and OCPs. Final identification and quantification of PBDEs was accomplished by gas chromatography connected to mass spectrometric detector with quadrupole analyzer operated in negative chemical ionization mode.

Aqueous leachates

Aqueous leachates from tested porous media were prepared by mixing of 300 g sample dry mass) together with

600 ml of deionized water in a flask. The dynamics of POPs extraction was tested both under static and dynamic conditions. Dynamic leaching was realized by vigorous shaking of sample in Erlenmeyer flasks for 24 hours. In static leaching experiments samples were taken for analysis after for 1, 3, 7 and 14 days.

Isolation of target analytes from aqueous leachates was carried out by dichloromethane extraction in separatory funnel (3-times for 5 minutes). Quantification of analytes was conducted in the same as in case of porous media.

Results and discussion

Significantly higher levels of PCBs and PBDEs as compared to the other media were found in river sediment (Fig. 1. and 3.). Relatively more extensive contamination

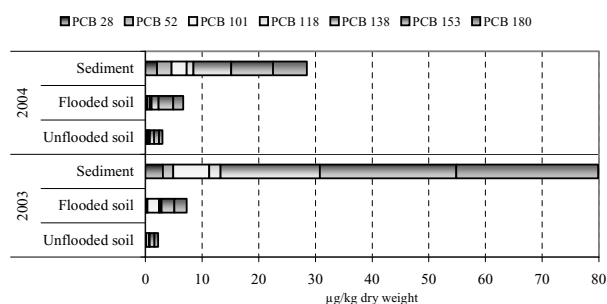


Fig. 1. PCBs in porous media (µg kg⁻¹ dry weight)

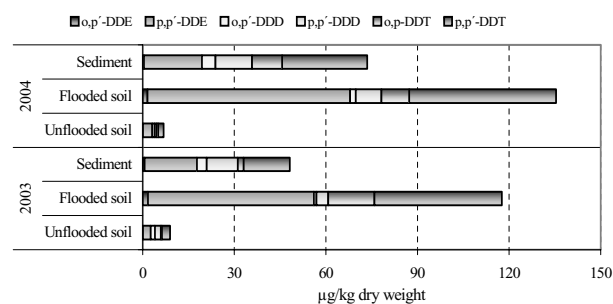


Fig. 2. DDTs in porous media (µg kg⁻¹ dry weight)

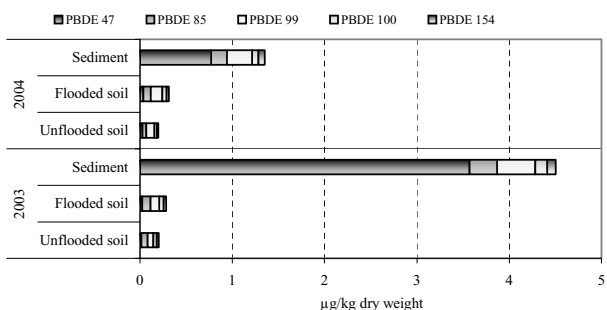


Fig. 3. PBDEs in porous media (µg kg⁻¹ dry weight)

of this sediment by POPs in 2004 can be due to different hydrometeorologic conditions in comparison with year 2003 (a municipal rain-water drain located above the sampling site can be regarded as a possible POPs contamination source). PCB levels (sum of indicator congeners) found in examined samples are shown in Fig. 1. Higher chlorinated congeners no. 138, 153 and 180, respectively, were the most abundant PCBs in all measured samples. Chemical industry plants situated upstream (e. g. in the agglomeration Neratovice) might be responsible for emissions of these POPs.

The concentrations of DDT (Fig. 2.) and its metabolites (DDD and DDE) were comparable in both year experiments. In case of substances representing DDT group, flooded soil was the most polluted porous medium. Major analytes were *p,p'*-DDE and *p,p'*-DDT. It may be the consequence of the flood (year 2002), when seriously polluted river sediments were deposited on the agricultural soil. Alternatively, former contamination because of floods together with persistence of these compounds in soil over decades is other possible reason, but this would not explain relatively low levels of DDTs found in the unflooded (adjacent) part of field.

Contamination trends observed for PBDEs were similar as above shown for PCBs again with sediments representing the matrix with the highest content of these flame retardants. (Fig. 3.). BDE 47 was the predominant congener detected together with lower level of BDEs 85, 99 and 100, respectively.

Preparation of aqueous leachates was tested in last year (2004) for each group of organic pollutants. In this study aqueous leachate was defined as a water column above the porous medium, containing solution of target analytes, hydrocolloids, suspended particles, etc.

Levels of contaminants found in aqueous leachates are illustrated for PCBs as an example. There are no significant differences between results obtained by tested leaching conditions. Concentrations of PCBs (sum of indicator congeners) fluctuated in relatively narrow concentration levels. The highest concentrations were found in leachate obtained after 1 day of static leaching.

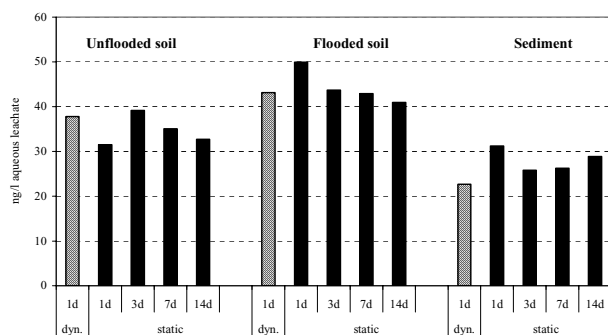


Fig. 4. PCBs (sum of indicator congeners) in aqueous leachates of porous media (ng l^{-1} leachate) – comparison of dynamic and static sampling

Conclusions

In the first part of the study we examined three types of porous media total content of PCBs, OCPs and PBDEs. Based on leaching experiments, procedure and most relevant conditions for bioavailable fraction sampling was suggested. In following experiments dynamics of SPMDs sampling process will be studied and bioavailable fraction of porous media will be determined.

This study was performed as a part of an Action COST 629 "Water pollution in natural porous media at different scales: fate, impact and indicator".

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P22 PHOTOSTABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN ORGANIC SOLVENTS

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Introduction

The organic compounds don't penetrate into the environment in most of occurrence in such quantity as anorganic compounds, but most of them has a hazard properties for the environment. They have toxic and genotoxic effects. The pointest of them is very resistance to chemical, thermic, photochemical and biochemical decay. This high persistent result in long-life cumulation of their compounds in all parts of environment.

PAHs are rampanted in environmental and they were detected in all biotic, abiotic parts and in industry areas. Retention time of PAHs in environmental depends on properties of compound.

Persistent organic contaminants are distributed among components of environmental until constituting of

dynamic balance. More of these compounds have lipophilic properties. Lipophilicity results in bio-accumulation in the fatty tissue of organisms and cumulation in the food chains.

The photochemical reactivity of PAHs in organic solvents has been studied for almost 50 years¹. It is generally accepted that the photodegradation of PAHs in solutions is an oxidative process which is highly accelerated by the presence of photo-initiators. Generally, the more polar the solvent is, the faster is the degradation process of PAH².

Experimental

PAHs with molecular weight 252 and 278 were used under study.

Objective of the study was comparison of single PAHs stability exposed to solar radiation and UV irradiation in two organic solvents: isooctane and dichloromethane. Isooctane was selected for it often applies in gas chromatography as solvent for injection of samples and dichloromethane as extraction solvent, respectively.

The liquid standards of PAHs were prepared about different concentrations as default (Table I). After the experimental solutions were prepared about uniform concentration 50 $\mu\text{l ml}^{-1}$ of solvent from them. Calculated amount of alkane C24 (concentration 10 $\mu\text{l ml}^{-1}$ of solvent) was added to prepared solvents (internal standard).

Table I

Default liquid standards

PAHs	Molecular weight [g mol ⁻¹]	Concentration [$\mu\text{g ml}^{-1}$]
Benzo[<i>b</i>]fluoranthene	252	130
Benzo[<i>j</i>]fluoranthene	252	106
Benzo[<i>k</i>]fluoranthene	252	134
Benzo[<i>a</i>]pyrene	252	116
Benzo[<i>e</i>]pyrene	252	128
Perylene	252	165
Dibenz[<i>a,c</i>]anthracene	278	122
Dibenz[<i>a,j</i>]anthracene	278	151
Dibenz[<i>a,h</i>]anthracene	278	126

The samples were analysed in GC-MS system CE 8000/TRIO 1000 and chosen samples were reanalysed in GC-MS system Saturn 2100T. Lamp HPL-N 400 W was used for experiments with UV irradiation.

Results and discussion

The great stability of aromatic systems is induced with delocalization of electron in bonding orbital. The conjugated systems have internal energy less than systems whose electrons are locating.

It was compared stability of six PAHs with molecular weight 252: benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*e*]pyrene and perylene.

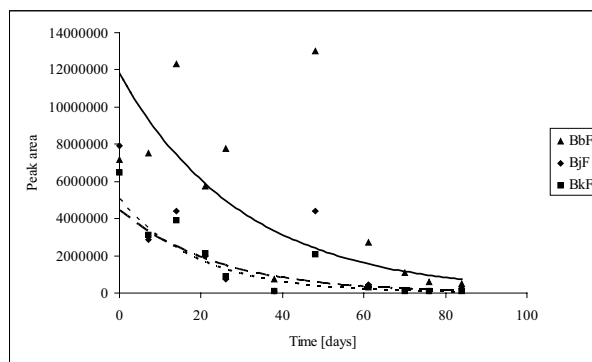


Fig 1. Degradation of PAHs in isooctane: BbF – benzo[*b*]fluoranthene, BjF – benzo[*j*]fluoranthene, BkF – benzo[*k*]fluoranthene

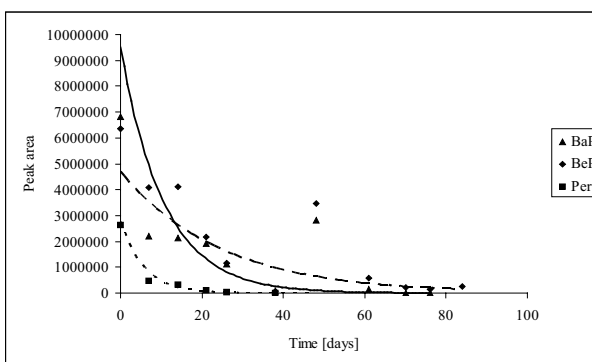


Fig 2. Degradation of PAHs in isooctane: BaP – benzo[*a*]pyrene, BeP – benzo[*e*]pyrene, Per – Perylene

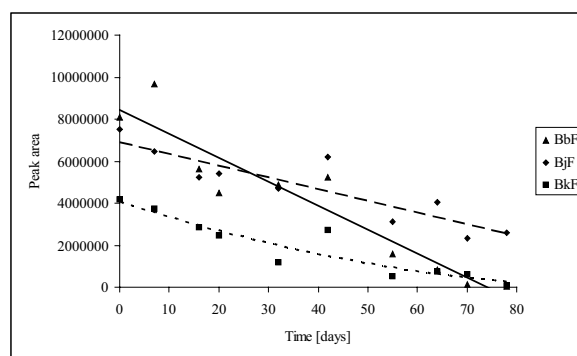


Fig 3. Degradation of PAHs in dichloromethane: BbF – benzo[*b*]fluoranthene, BjF – benzo[*j*]fluoranthene, BkF – benzo[*k*]fluoranthene

Benzo[*b*]fluoranthene, benzo[*j*]fluoranthene and benzo[*k*]fluoranthene have included a quinary ring in their structure that probably contributes to their stability. Of course, it is particularly about the location of condensation of the benzene ring on the basic structure of fluoranthene.

Benzo[*a*]pyrene and benzo[*e*]pyrene are compounds that have condensed a benzene ring on the basic structure of pyrene. Their reactivity is interesting. Besides the introduction of substituents, the first and second order of substitution into positions six and eight³. Probably, the second order of substituent (for example –COOH group) will be linked

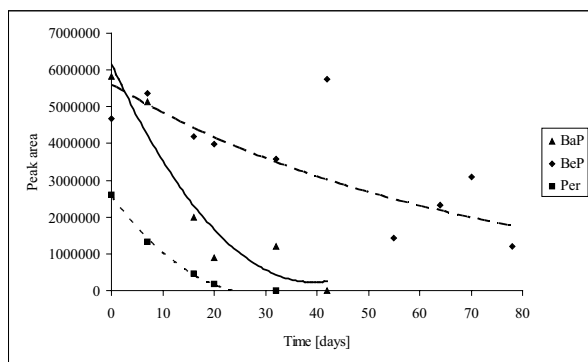


Fig 4. Degradation of PAHs in dichloromethane: BaP – benzo[a]pyrene, BeP – benzo[e]pyrene, Per – Perylene

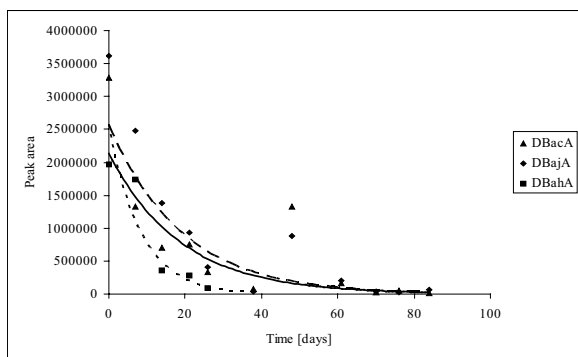


Fig 5. Degradation of PAHs in isooctane: DBaCa – dibenz[a,c]anthracene, DBaJA – dibenz[a,j]anthracene, DBaHA – dibenz[a,h]anthracene

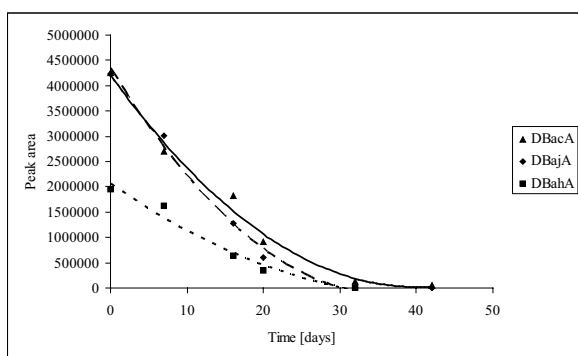


Fig 6. Degradation of PAHs in dichloromethane: DBaCa – dibenz[a,c]anthracene, DBaJA – dibenz[a,j]anthracene, DBaHA – dibenz[a,h]anthracene

to benzo[a]pyrene or benzo[e]pyrene into position six or eight. This reaction will evoke negative mesomeric effect.

The perylene has only 20 π -electrons on 24 carbon coupling. Probably aromatic character will be balanced little. Perylene will be high reactive.

In isooctane (see Fig. 1. and 2.), decay of PAH's were exponential. Decay kinetics show their high unstable. Perylene was the least stable.

As products of degradation were identified mostly anhydrides and esters of phthalic acid. Nothing chlorinated products of decay were detected despite deal instable of dichloromethane.

In dichloromethane (see Fig. 3. and 4.) degradation proceeded slowly then in isooctane. Probably high stable intermediate of degradation was arised.

These chemical intermediate were not identified unluckily. Perylene was the least stable again.

The stability of three PAHs (dibenz[a,c]anthracene, dibenz[a,j]anthracene and dibenz[a,h]anthracene) with molecular weight 278 were investigated as ones mentioned above.

These compounds have bonded another benzene rings on basic structure of anthracene. It was caused that in complete molecule of anthracene π -electrons aren't distributed steady but π -electrons are localized partly. Instability of these compounds will be evoked with localization of bonds, probably.

In isooctane (see Fig. 5.), the degradation of these substances were exponential in relation of time. Their were very instable compounds. Dibenz[a,h]anthracene was the least stable.

Decay of PAHs in polar dichloromethane (see Fig. 6.) were regulated to any order of reaction. Dibenz[a,h]anthracene was the least stable again. Degradation products were especially anhydrides, esters of phthalic acid optionally ketone.

UV irradiation

By reason of reconnaissance irradiation effects about shorter wave length following PAHs were exposed activity of UV irradiation with wave length 365 nm. Exposure with UV irradiation was performed at specific period of time in isooctane and dichloromethane into the quartz cell. For identification products of photodegradation were used system GC-MS TRIO 1000 that detected phthalic anhydride. PAHs in dichloromethane exhibited less stability then in isooctane (Table II).

For example, the degradation kinetics of benzo[a]pyrene in dichloromethane were exponential (see Fig. 7.), but benzo[k]fluoranthene in dichloromethane was linear (see Fig. 8).

Table II

Table of half times and reaction orders of PAHs stability

PAHs	Half time [min]	Reaction order
Benzo[k]fluoranthene in isooctane	64.18	1
Benzo[k]fluoranthene in DCM	76.23	0
Benzo[a]pyrene in isooctane	54.15	1
Benzo[a]pyrene in DCM	13.15	1

Interference

Photochemical reactivity of polycyclic aromatic hydrocarbons was regarded as oxidation process. Stability of PAHs

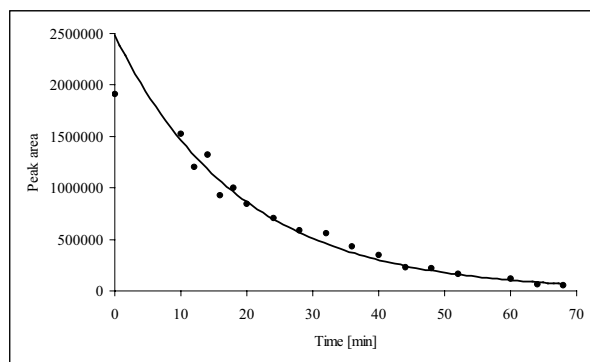


Fig 7. Degradation of benzo[a]pyrene in dichloromethane

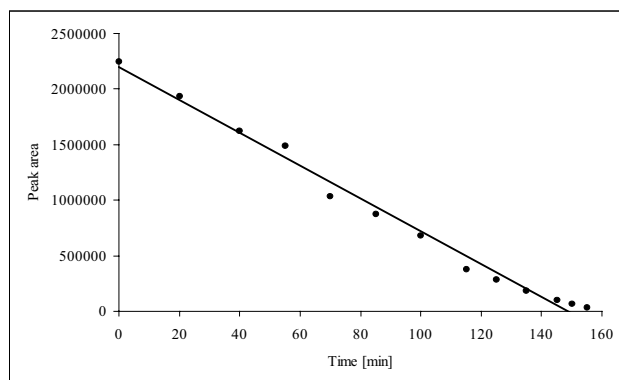


Fig 8. Degradation of benzo[k]fluoranthene in dichloromethane

is given with delocalization of electrons on bonding π -orbital. Conjugated systems have internal energy less than localization electrons systems. The most reactive PAHs have bonds localization bigger.

The stability of PAHs is depended on solvent in use. The more polar solvent causes the faster degradation of compounds. However some of the PAHs are decomposed faster in isooctane then in dichloromethane. In dichloromethane, stability of some intermediate products, evidently arised deceleration of another process of decay. The presence of those intermediate products are suppositional only, because their weren't registered by the mass spectrometer.

The stability of polycyclic aromatic hydrocarbons decreased in the raw:

benzo[b]fluoranthene > benzo[e]pyrene >
> benzo[j]fluoranthene > benzo[k]fluoranthene >
> dibenz[a,j]anthracene > dibenz[a,c]anthracene >
> benzo[a]pyrene > dibenz[a,h]anthracene > perylene

and in dichloromethane:

benzo[j]fluoranthene > benzo[e]pyrene >
> benzo[b]fluoranthene > benzo[k]fluoranthene >
> benzo[a]pyrene > dibenz[a,c]anthracene >
> dibenz[a,h]anthracene > dibenz[a,j]anthracene > perylene.

The degradation products of PAHs in solvent were anhydrides and esters of phthalic acid that probably arised with fission of single PAH and oxidation or esterification their rests.

Calculated half times and reaction order are summarized in Table III and IV.

Table III
Half times and reaction orders constants PAHs v isooctane

PAHs	Half time [day]	Reaction order
Dibenz[a,c]anthracene	12.98	1
Dibenz[a,j]anthracene	13.03	1
Dibenz[a,h]anthracene	5.75	1
Benzo[b]fluoranthene	20.88	1
Benzo[j]fluoranthene	16.54	1
Benzo[k]fluoranthene	13.33	1
Benzo[a]pyrene	7.39	1
Benzo[e]pyrene	16.91	1
Perylene	3.95	1

Table IV
Half times and reaction orders of PAHs v dichloromethane

PAHs	Half time [day]	Reaction order
Dibenz[a,c]anthracene	11.77	0.51
Dibenz[a,j]anthracene	10.25	0.12
Dibenz[a,h]anthracene	11.38	0.41
Benzo[b]fluoranthene	35.48	0
Benzo[j]fluoranthene	67.07	0
Benzo[k]fluoranthene	31.29	0.49
Benzo[a]pyrene	12.06	0.56
Benzo[e]pyrene	47.15	1
Perylene	7.96	0.49

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P23 DGT TECHNIQUE: SOLUTIONS OF METALS AND HUMIC ACIDS

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Introduction

Diffusive Gradients in Thin films (DGT) is a recently developed technique^{1,2}. The technique is based on the accumulation of solutes in a resin layer after passing through a well-defined diffusive gel layer. The mass of solutes accumulated in the resin during a period of deployment time is measured.

DGT technique is usually used for the determination of trace metals in the environment. Results of in situ DGT applications are interpreted as average concentrations (hours to weeks) in waters, or as fluxes in soils and sediments, or as bioavailable fraction of metals in soils. In situ DGT applications in sediments provide vertical (and/or horizontal) profiles at very high resolution^{3,4}.

The metals are important substances of the environment and may be either essential or toxic. The toxicity and mobility of metals in the environment depend on their concentrations, present metals species and features of the nature system. Humic acids (HA) are natural substances capable to bound the metals. They can play a very important role in the behaviour of metals.

Several laboratory experiments were performed in order to study the influence of HA on determination of metals by DGT technique. DGT units were deployed in the solutions of Cd and Cu at various concentrations of HA. The effect of deployment time on the mass of metals determined by DGT was investigated. Different types of diffusive gel were used in the experiments.

Procedure

Diffusive gel (APA-gel) and resin gel were based on an agarose polyacrylamide hydrogel. These gels were prepared according to the conventional procedures (DGT Research, Ltd., Lancaster, UK) and are described in the Ref. (5). The second type of diffusive gel, so-called restrict gel (RG-gel), was purchased from DGT Research, Ltd.

DGT unit (2.5 cm in diameter) was set by placing the resin gel, the diffusive gel (APA or RG) and the filter on the cylindric part (piston) of the unit. The plastic top with a window which had 2.0 cm in diameter sealed the unit (Fig. 1.).

DGT units were deployed in the solution containing 50 $\mu\text{g dm}^{-3}$ of Cd and Cu each of them and various concentrations of HA of 0; 0.3; 1; 3.1; 10; 31; 100 and 316 mg dm^{-3} . pH of solutions was 6.5–7.0. The DGT units were deployed

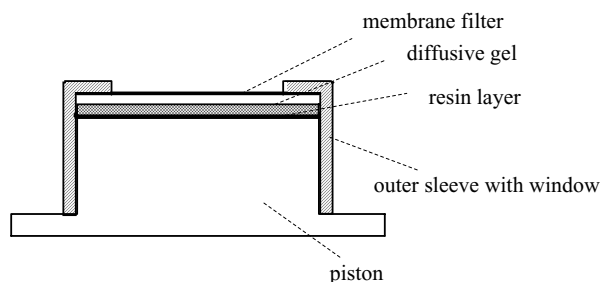


Fig. 1. Scheme of DGT unit

at 26 ± 2 °C for 8, 24 and 32 hours. After deployment, the DGT units were disassembled and the resin gels were eluted by 1 mol dm^{-3} nitric acid for 24 hours.

The metals were determined in eluate and exposition solutions by ET AAS employing Perkin-Elmer Model 4110 Zeeman atomic absorption spectrometer. Recommended conditions and the standard addition method of calibration were applied.

The mass of metals accumulated during deployment in the resin was calculated according to the 1st Fick's law of diffusion.

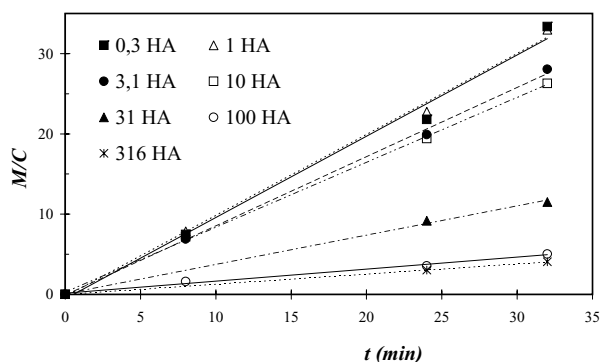


Fig. 2. Dependence of accumulated mass of cadmium on deployment time, converted to the unit concentration of cadmium in the solution (M/C) for various humic acids concentrations [mg dm^{-3}]

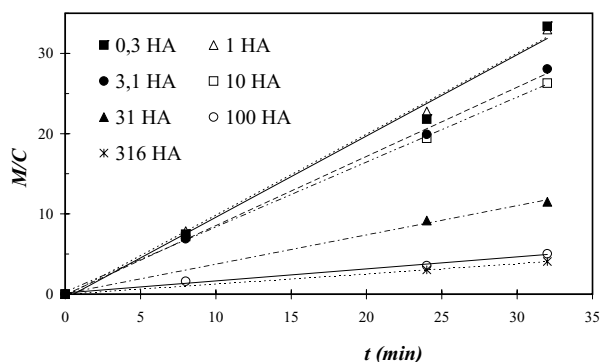


Fig. 3. Dependence of normalised values of diffusion coefficients ($D_{\text{cal}}/D_{\text{tab}}$) of cadmium and cooper on humic acids concentrations (APA gel)

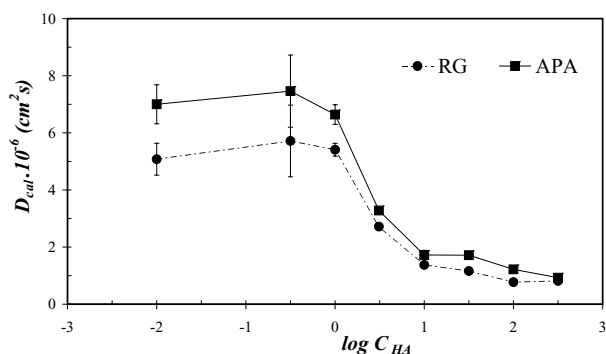


Fig. 4. Dependences of calculated values of diffusion coefficients (D_{cal}) of copper on humic acids concentrations (APA gel, RG gel)

Results and discussion

The results of deployment of DGT units with APA diffusive gels in solutions of Cd, Cu and HA are shown in Fig. 2. for example for Cd (Cu showed similar behavior). The mass of metal (M) accumulated during the deployment time (t) was converted to the unit concentration of metal in the solutions (M/C).

The mass of the accumulated metal increases linearly with time for all of the concentrations of HA in solutions. This indicates that the fluxes of DGT-attainable metal species are constant, time independent. However, the metal fluxes are reduced with increasing concentration of HA. This observation could be explained by reduced mobility of metal ion complexes related to high molecular HA species or by formation of relatively stable complexes of HA with metal ions.

Diffusion coefficients were calculated from the results (D_{cal}). Normalized values of D_{cal} (i. e. $D_{\text{cal}}/D_{\text{tab}}$) are compared in Fig. 3. for Cd, Cu and HA concentrations. The values of $D_{\text{cal}}/D_{\text{tab}}$ decrease when the concentration of HA increases. Experiments with the restrict gel (RG-gel), which excludes diffusion of high molecular species, showed similar dependencies for APA and RG-gels (see Fig. 4. – example for Cu).

Conclusion

HA species present in the permeable gel can cause a very fast restriction of the diffusion of other metal species into the resin gel, especially when a large excess of HA is applied. However, no depletion of the metal uptake was observed for a long period as can be concluded from Fig. 2. Nevertheless, element specific influence of HA can be observed as documented in Fig. 3. This suggests that HA complexes diffuse through the permeable gel, and thus affect the interaction of metal ions with specific groups of the resin.

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P24 THE USE OF SPME FOR DETERMINATION OF PESTICIDES IN WATER

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Introduction

Determination of analytes in biotic matrices generally represents a difficult procedure from isolation, purification, through concentrating to efficient separation of analyte. The main pitfall is usually the separation of the selected analyte from other substances present in the sample. The method of the solid phase micro-extraction (SPME) represents a solution of the above-mentioned problems and offers a number of advantages in the sample processing; less manipulation with the sample, elimination of use of organic solvents, possibility of a repeated use of fibres^{1,2}.

Pesticides are chemical preparations used in agriculture in order to improve the yield of the agricultural production and to protect the agricultural products against pest. By improper use in agricultural practice, imissions or a long-distant transport from distant places the pesticides may get in the hydrosphere as well. Their presence in the living environment is considered as undesirable and therefore they belong among the priority contaminants^{3,4,5}.

Experimental part

In order to determine the pesticides in water SPME was performed as follows: in a 10ml vial containing 5 ml (head-space analysis) or 7.5 ml (direct SPME) of deionised water 2 µl of mixed pesticide standard was added. The final concentration in the sample was in the case of the head-space analysis 0.06 µg ml⁻¹, in the case of direct SPME 0.04 µg ml⁻¹. During the experiment the influence of stationary phase, temperature and sorption time were monitored.

The determination itself was performed using the Trace GC 2000 gas chromatograph (Thermo Quest, USA) with a mass-spectrometric detector of the POLARIS Q ion trap type (Finnigan, USA); for separation the RTX-5 capillar open column was used (30 m × 0.25 mm × 0.25 mm) (Restec, USA). The sprayed area temperature was 250 °C, the ion

Table I

Influence of the fibre type on response of detectors for the monitored analytes. To express it in %, the response within the use of the PDMS fibre = 100 %

	PA	PDMS	PDMS/DVB
Pirimicarb	x	100	x
Vinclozolin	86	100	35
Chlorpyrifos-methyl	93	100	38
Malathion	31	100	22
Tolylfluaniid	86	100	16
Procymidone	56	100	3
Brompropylat	70	100	29
Phosalon	x	100	x

x – analytes were not identified

source temperature was 200 °C and the Transfer Line temperature was 275 °C. The temperature programme was 45 °C for period of 2 minutes, 40 °C min⁻¹ till 220 °C (without retention), 5 °C min⁻¹ till 270 °C (without retention), 270 °C for period of 4 minutes; the total analysis period was 20.5 minutes. The flow-rate of the carrier gas (He) was constant, 1 ml min⁻¹.

Result and discussion

The aim hereof was to optimise the SPME method for determination of pesticides in water. We monitored influence of the stationary phase on the sorption of monitored analytes. We carried out comparison of 3 PDMS fibres (100 µm), PA (85 µm) and PDMS-DVB (65 µm). The results obtained are stated in Table I. From the table it is obvious that the PDMS fibre (100 µm) is the most sensitive for all the monitored pesticides.

Also the influence of temperature on sorption of the analyte was monitored. The following temperatures were tested: 40, 60 and 70 °C in 10 minute sorption. The measured values are stated in Table II.

From the table it is obvious that the highest responses were obtained at temperature of 70 °C, yet this is not true for

Table II

Influence of temperature in sorption on response of the detector for the monitored analytes. Expressed in %, the response in temperature of 70 °C = 100 %

	50 °C	60 °C	70 °C
Pirimicarb	x	x	100
Vinclozolin	40	59	100
Chlorpyrifos-methyl	39	55	100
Malathion	17	33	100
Tolylfluaniid	33	31	100
Procymidone	90	58	100
Brompropylat	20	44	100
Phosalon	x	55	100

x – analytes were not identified.

all the analytes. The temperature of 40 °C seems to be unsuitable for both the sensitivity and a bad repeatability.

Various sorption times were measured (5, 10, 20, 30, 40, 50 and 60 minutes) at temperature of 70 °C with stirring during the sorption. The results are stated in Table III.

From the table it is obvious that in most of the pesticides the response increases with the sorption time. For further experiments, with respect to the attempt to reach the maximum possible sensitivity and not to raise the determination time pointlessly, the 40minute sorption time was chosen. The repeatability of the method was determined based on 5 repetitions and is expressed as a relative standard deviation (Table IV).

Optimisation of the SPME direct method was based on the method for head-space analysis. The optimisation method repeatability was determined based on 4 repetitions and is expressed as a relative RSD standard deviation; the respective values are stated in Table IV.

Based on the above-mentioned RSD values we can say that both the head-space and the SPME methods are suitable for determination of pesticides in water.

Table III

The sorption time influence on response of the detector for monitored analytes. Expressed in %, the response at 40 minutes = 100 %

	5	10	20	30	40	50	60
Pirimicarb	17	32	51	60	100	98	136
Vinclozolin	19	38	61	77	100	116	103
Chlorpyrifos-methyl	37	69	106	108	100	84	115
Malathion	14	61	50	67	100	113	142
Tolylfluaniid	16	34	57	70	100	123	97
Procymidone	16	36	50	72	100	110	152
Brompropylat	10	48	32	58	100	99	112
Phosalon	4	72	15	50	100	49	56

Table IV
RSD in determination of pesticides

	RSD – head-space	RSD – direct
Pirimicarb	15	9
Vinclozolin	7	8
Chlorpyrifos-methyl	5	5
Malathion	9	5
Tolylfluanid	13	9
Procymidone	7	4
Brompropylat	7	7
Phosalon	7	13

Conclusions

The head-space optimised method was described working on the following principle: using the PDMS fibre (100 µm) a 5 ml water sample with 2 µl of methanol with standards were described; the fibre was burrowed in the vial for 22 mm. Similarly, a method for direct SPME was developed, differing from the head-space method by the sample volume – 7.5 ml, sorption temperature – 40 °C and the depth the fibre is burrowed in the vial – 31 mm. The analytical ending in both cases was GC/MS.

The repeatability measurements were carried out for both methods; RSD reached values for head-space 5–15 %, for direct SPME: 4–13 %.

This work was supported by the grants given by Ministry of Education, Youth and Sports of the Czech Republic no. MSM 6046137305 (30 %) and MSM 6215712402 (40 %) and by IGA VFU 8/2004/FVHE (30 %).

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P25 USE OF MICROWAVE DECOMPOSITION IN SPECIFIC ANALYSIS OF WASTE WATERS

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Introduction

Human negative activities have impact on all compounds of the living environment, including hydrosphere. It is known that without water there is no life and any change in water quality can represent a danger for organisms living on our planet; therefore the water pollution issues are of a priority importance. The main way the pollutants get in the water environment is through wastewaters. Among significant producers of polluted wastewater belong the food-processing industry and agricultural primary production^{1,2,6}. Discharge of wastewater is limited by the current legislation and subject to the payment of a fee^{3,4,5}. Therefore, monitoring of selected indices in the wastewater is necessary. As analytes occur in water in very low concentrations, also the analysis issue has to be solved^{7,8}.

Experimental part

The monitoring focused on samples of wastewater coming from the agricultural primary production. The water was sampled in the inlet and outlet of the wastewater treatment plant. Before the testing itself the samples were filtered. They were also decomposed using the MW 520 microwave decomposition unit and TR 420 thermo-reactor (Merck, Germany). The samples were tested in respect to the content of total phosphorus and nitrogen and also the content of nitrates, nitrites, ammonia and COD. The selected parameters were determined by spectrophotometry, using the Spectroquant Nova 60 spectrophotometer (Merck, Germany).

Result and discussion

The wastewater flowing in the WWTP showed changed quality parameters; it had a brown colour and contained a number of solid impurities (straw, hair and bristles), faeces and excrements. The water leaving WWTP was clear, which documented the good functioning of the biological wastewater treatment plant. The following tables contain the values measured in the inlet and outlet. The outlet values show a significant decrease in concentrations of total phosphorus, nitrates and ammonia, COD was reduced up to a half.

Also the influence of the method of decomposition of samples on the phosphorus content in the inlet samples was assessed. In determination of the phosphorus content we tested a number of types of the sample decomposition that

Table I
Inlet values of the individual indices [mg l^{-1}]

	Ptotal MW	Ptotal	Ntotal MW	Ntotal	N-NO ₃ ⁻	N-NO ₂ ⁻	N-NH ₄ ⁺	COD
1	6.0	7.0	6.4	21.2	5.0	0.1	98.0	408.6
2	7.3	9.85	10.2	26.6	2.0	0.05	102.	404.0
3	4.5	5.55	4.1	22.6	5.0	0.019	110.0	551.0
4	10.15	11.5	5.7	30.0	2.5	0.1	98.0	506.0
5	7.9	9.95	3.3	42.8	0.02	0.035	77.0	548.0
6	8.15	9.25	10.7	21.2	2.5	0.17	200.0	542.0
7	9.35	10.0	7.2	42.5	2.5	0.05	110.0	545.0

Ptotal – total phosphorus, **Ptotal MW** – total phosphorus after microwave decomposition, **Ntotal** – total nitrogen, **Ntotal MW** – total nitrogen after microwave decomposition, **N-NO₃⁻** – nitrogen in nitrates, **N-NO₂⁻** – nitrogen in nitrites, **N-NH₄⁺** – ammonia nitrogen, **COD** – chemical oxygen demand

Table II
Outlet values of the individual indices [mg l^{-1}]

	Ptotal	Ptotal MW	Ntotal	Ntotal MW	N-NO ₃ ⁻	N-NO ₂ ⁻	N-NH ₄ ⁺	COD
1	3.0	6.0	22.7	23.4	17.0	0.1	59.0	404.1
2	3.4	4.0	22.2	21.4	16.0	0.05	80.	232.2
3	3.65	3.73	20.4	23.6	23.0	0.028	56.0	248.0
4	3.35	4.45	25.2	25.7	21.0	0.1	10.0	424.0
5	3.3	3.55	24.1	23.4	25.0	0.089	38.0	325.0
6	5.0	5.95	29.3	34.2	15.0	2.71	155.0	432.0
7	5.1	6.1	21.3	36.3	19.0	2.76	53.0	339.0

Ptotal – total phosphorus, **Ptotal MW** – total phosphorus after microwave decomposition, **Ntotal** – total nitrogen, **Ntotal. MW** – total nitrogen after microwave decomposition, **N-NO₃⁻** – nitrogen in nitrates, **N-NO₂⁻** – nitrogen in nitrites, **N-NH₄⁺** – ammonia nitrogen, **COD** – chemical oxygen demand

differed in use of decomposition agents (Oxislov and Crack Set 10) and in the decomposition method. For decomposition we used either the thermo-reactor (60 minutes at 120 °C) or microwave decomposition unit (100 second).

The phosphorus concentrations after decomposition are higher, yet with respect to the fact that the total concentration of phosphorus in the sample was low; it is probable that also content of the undetermined forms of phosphorus is lower; therefore the increase in the phosphorus concentration after the sample decomposition was not significant. Nonetheless, it is obvious that the sample decomposition has a positive impact on determination of the total concentration. The individual decomposition types are comparable. Nearly in all the cases we proved a higher content of the analyte in decomposition in microwave decomposition unit using the Oxisolv decomposition agent.

In addition to that, we assessed influence of the decomposition by microwave decomposition unit and thermo-reactor on the nitrogen content. From the results it is obvious that both types of decomposition have influence on increase in the

total nitrogen content. By comparison of the microwave decomposition and decomposition in thermo-reactor we found that decomposition in thermo-reactor provides better results than the microwave decomposition.

Conclusions

The presented study has proved that it is suitable to include the sample decomposition in the working process particularly in determination of the total phosphorus and total nitrogen content. As during the decomposition process these are released off the fixed bound, we can say that the selected method has really enabled us to determine the total content of analytes in the sample. The obtained values were compared to the respective legislation and it was proved that the limit values had not been exceeded.

This work was supported by the grants given by Ministry of Education, Youth and Sports of the Czech Republic no. MSM 1622700004 (40 %) and MSM 6215712402 (40 %) and by IGA VFU 29/2003/FVHE (20 %).

Table III

Comparison of influences of various types of decomposition on the inlet total phosphorus content [mg l^{-1}]

	Inlet			
	Ptotal	Ptotal MW	Ptotal Crack Set	Ptotal Term
1	6.0	7.5	7.46	7.31
2	7.3	9.85	8.55	9.15
3	4.5	5.55	5.5	5.5
4	10.15	11.5	11.7	11.3
5	7.9	9.95	9.9	10.1
6	8.15	9.25	9.1	9.725
7	9.35	10.0	9.94	9.85

Ptotal – total phosphorus, **Ptotal MW** – total phosphorus after microwave decomposition, **Ptotal Crack Set** – total phosphorus after microwave decomposition with use of the Crack Set 10 decomposition agent, **Ptotal Term** – total phosphorus after decomposition in thermo-reactor

Table IV

Comparison of influences of various types of decomposition on the inlet total nitrogen content [mg l^{-1}]

	Inlet		
	Ntotal	Ntotal MW	Ntotal Term
1	6.4	21.2	30.8
2	10.2	26.6	36.4
3	4.1	22.6	39.9
4	5.7	30.0	40.4
5	3.3	42.8	58.3
6	10.7	21.2	28.6
7	7.2	42.5	60.2

Ntotal – total nitrogen, **Ntotal MW** – total nitrogen after microwave decomposition, **Ntotal Term** – total nitrogen after decomposition in thermo-reactor

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P26 CHROMATOGRAPHIC SEPARATION OF POLYCYCLIC AROMATIC NITROGEN HETEROCYCLES

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Introduction

Contamination of the environment is a serious problem in the recent years thanks to emissions of large scale of inorganic and organic compounds. Significant group of these compounds is group of polycyclic aromatic nitrogen heterocycles (PANHs) related to polycyclic aromatic hydrocarbons (PAHs). These compounds show high toxicity, most of all carcinogenicity and mutagenity. The main sources are combustion of fuels, smoking, and preparing of food (smoking, grilling).

Common chromatographic methods have been used for the determination of the PANHs in different parts of environment. Thanks to the similar sources, PANHs and PAHs are found abreast in the environmental samples. The common analytical methods require some preparative steps before final chromatographic analysis. The preparation steps are often accompanied by contamination of sample or loss of individual sample components.

The aim of this work was to find HPLC separation conditions that allow separation and determination of PAHs along with PANHs in one step without need of prior separation, with low detection limits using fluorescence detection.

Experimental

Materials

PANHs were Aldrich products excluding benzo[*a*]acridine, benzo[*c*]acridine, dibenzo[*a,i*]acridine, dibenzo[*a,j*]acridine, dibenzo[*a,h*]acridine, dibenzo[*c,h*]acridine, 7-H-dibenz[*c,g*]carbazole, that were from Dr. Ehrenstorfer GmbH (Germany). Standard solution of 16 EPA PAHs in hexane was also Dr. Ehrenstorfer GmbH (Germany) product. Acetonitrile used for mobile phase was provided by Sigma-Aldrich (USA). Sodium hydrogen phosphate p.a. was from Lachema (Czech Republic) and potassium dihydrogen phosphate was from Serva (N.Y. USA). Water used in the laboratory was prepared using Simplicity 185 equipment (MILLIPORE, France). Buffers were passed through a 45 μm Nylon 66 filter (SUPELCO, USA) before the use in HPLC system.

Apparatus

The chromatographic apparatus consisted of a HP 1100 series liquid chromatograph (Hewlett/Packard, Palo Alto,

CA, USA) equipped with a models 1100 DAD detector and HP 1046 fluorescence detector. Chromatographic behaviour of individual PANHs was performed by reverse-phase HPLC. Two columns with bonded C18 groups were used for retention behaviour study and separation of mixture of PANHs and PAHs: Ace 5 C18 (250×4.6 mm i.d, 5 μm, Agilent Technologies, USA) and Zorbax Extend C18 (250×3 mm i.d, 5 μm, Agilent Technologies, USA).

Results and discussion

To find optimal separation conditions the investigation of the chromatographic behaviour of the PANHs was necessary. Increasing concentration of acetonitrile as an organic modifier not only decreases retention times of all used analytes by different manner but even retention order changes were observed (Fig. 1.) Acetonitrile content exceeding 40 % v/v causes significant peak's overlap. In the mobile phases with acetonitrile contents lower than 20 % the peak's overlap was observed especially at analytes eluted lately.

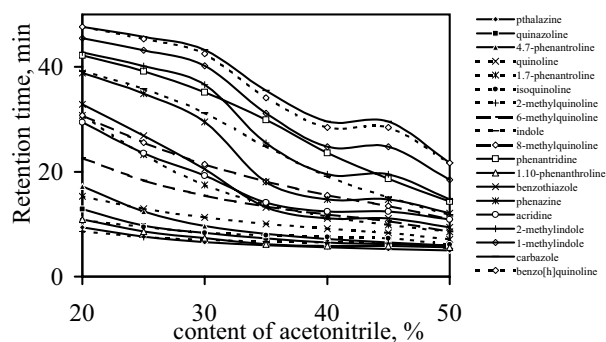


Fig. 1. Effect of content of organic modified on retention of PANHs

Effect of pH of the used phosphate buffer was studied in the range 2.5–6.5. Strong dependency of the retention of some analytes (Fig. 2.) on pH was found.

Ionic strength has not a significant effect on the retention of the PANHs in the measured range of the buffer concentration (Fig. 3.).

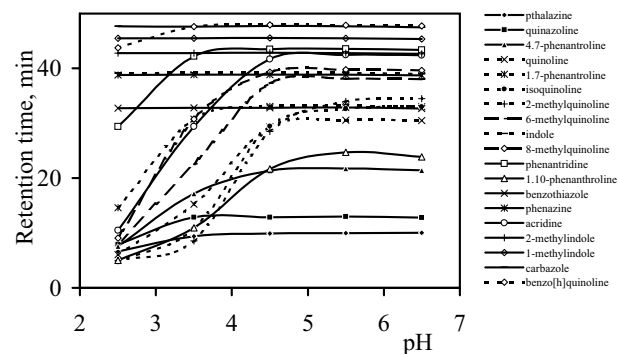


Fig. 2. Effect of pH of mobile phase on retention PANHs

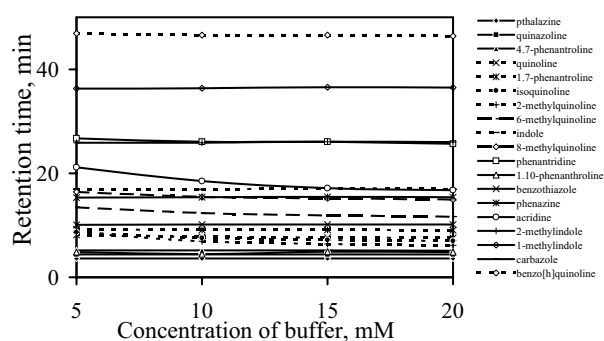


Fig. 3. Effect of ionic strengths on retention of PANHs

A mixture of twenty five PANHs and sixteen PAHs (EPA-PAHs) was prepared for the final separation of the PANHs and PAHs. Gradient of acetonitrile content in mobile phase was used for separation in reverse phase chromatographic systems. Minimum peak's overlap was found in the chromatogram (Fig. 4.). It doesn't matter due to different spectra properties of near eluted compounds.

Low detection limits were achieved especially using fluorescence detector. Detection limits were calculated from calibration curves using Graham method¹. Obtained values are lower in many cases than those published in the literature^{2,3} (Table I).

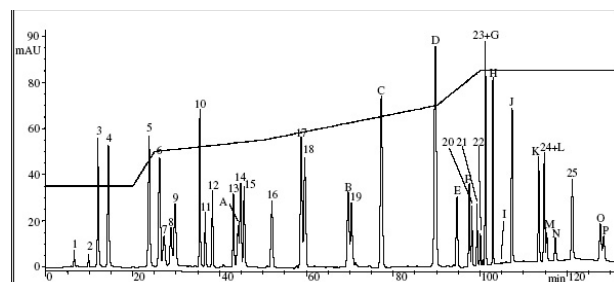


Fig. 4. Separation of mixture of PANHs and PAHs.

Mobile phase: acetonitrile-buffer pH 6.00

Gradient: 0–20 min 35% MeCN, 20–25 min 35–50% MeCN, 25–50 min 50–55% MeCN, 50–90 min 55–70% MeCN, 90–100 min 70–85% MeCN, 100–150min 85% MeCN. Detection UV 231 nm, Flow rate: 0.5 cm³ min⁻¹, column temperature 25 °C, injected amount: 52 ng of each PANH and 40 ng of each PAH. 1 – uracil.

PANHs: 2 – pthalazine, 3 – quinazoline, 4 – 4,7-phenanthroline, 5 – quinoline, 6 – 1,7-phenanthroline, 7 – isoquinoline, 8 – benzothiazole, 9 – 2-methylquinoline, 10 – 6-methylquinoline, 11 – phenazine, 12 – 8-methylquinoline, 13 – acridine, 14 – 2-methylindole, 15 – phenanthridine, 16 – 1-methylindole, 17 – benzo[h]quinoline, 18 – carbazole, 19 – benzo[a]acridine, 20 – 7-H-dibenzo[c,g]carbazole, 21 – dibenzo[a,i]acridine, 22 – dibenzo[a,i]acridine, 23 – benzo[c]acridine, 24 – dibenzo[a,h]acridine, 25 – dibenzo[c,h]acridine

PAHs: A – naphthalene, B – acenaphthylene, C – acenaphthene, D – fluorene, E – phenanthrene, F – anthracene, G – fluoranthene, H – pyrene, I – benzo[a]anthracene, J – chrysene, K – benzo[b]fluoranthene, L – benzo[k]fluoranthene, M – benzo[a]pyrene, N – dibenz[a,h]anthracene, O – benzo[g,h,i]perylene, P – indeno[1,2,3-c,d]pyrene

Table I
Detection limits of PANHs

Compound	LOD [ng/injection]			
	DAD	Literature	FLD	Literature
phtalazine	4.00		No fluorescence	
quinazoline	2.29		3.97	
4,7-phenanthroline	2.26		$2.47 \cdot 10^{-2}$	
quinoline	2.14		12.7	5.00
1,7-phenanthroline	5.00		$6.92 \cdot 10^{-2}$	
isoquinoline	4.49		$3.45 \cdot 10^{-1}$	
benzothiazol	2.82		1.46	
2-methylquinoline	5.08		1.52	
6-methylquinoline	2.08		2.98	
phenazine	2.03		298	
8-methylquinoline	2.10		1.04	
acridine	1.13		$7.35 \cdot 10^{-3}$	$5.50 \cdot 10^{-3}$
2-methylindole	1.78		$2.76 \cdot 10^{-2}$	
phenanthridine	2.09		$8.40 \cdot 10^{-3}$	$7.30 \cdot 10^{-3}$
1-methylindole	1.12		$3.79 \cdot 10^{-3}$	
benzo[<i>h</i>]quinoline	1.11		$3.93 \cdot 10^{-2}$	$7.30 \cdot 10^{-3}$
carbazole	1.11		$8.08 \cdot 10^{-3}$	
Benzo[<i>a</i>]acridine	$9.43 \cdot 10^{-1}$	5.00	$7.60 \cdot 10^{-3}$	$1.10 \cdot 10^{-2}$
Dibenzo[<i>a,j</i>]acridine	$9.73 \cdot 10^{-1}$	$3.00 \cdot 10^{-1}$	$2.09 \cdot 10^{-3}$	$2.30 \cdot 10^{-2}$
Dibenzo[<i>a,i</i>]acridine	2.00	3.20	$3.21 \cdot 10^{-1}$	
Benzo[<i>c</i>]acridine	1.17	$1.10 \cdot 10^{-1}$	$6.00 \cdot 10^{-3}$	$1.10 \cdot 10^{-2}$
7-H-dibenzo[<i>c,g</i>]carbazole	1.22		$5.84 \cdot 10^{-5}$	
Dibenzo[<i>a,h</i>]acridine	$8.75 \cdot 10^{-1}$		$2.93 \cdot 10^{-3}$	$9.50 \cdot 10^{-3}$
Dibenzo[<i>c,h</i>]acridine	$9.50 \cdot 10^{-1}$	$4.20 \cdot 10^{-1}$	$1.11 \cdot 10^{-1}$	$6.20 \cdot 10^{-3}$

Conclusions

The mixture of PANHs and PAHs can be analysed by gradient reverse phase liquid chromatography in one step without prior group separation. Fluorescence detection enables to determine content of individual components with low detection limits. Proposed method is easy-to-use as an alternative method of determination of PANHs in environmental samples.

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P27 INTERLABORATORY STUDIES – EXTERNAL QUALITY CONTROL IN ANALYSIS OF RESIDUES OF MODERN PESTICIDES IN FOOD

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Introduction

Modern pesticides represent an important group of agrochemicals possessing a wide range of physico-chemical properties¹. Unfortunately, under certain circumstances their residues can occur in treated crops. Due to the health concerns, analytical control of pesticide residues is a necessary measure to protect consumers. Because of a large variety of analytes/matrix combinations, application of multiresidue methods (MRM) is the only conceivable solution for flexible identification/quantitation of target analytes. MRMs involve compromise between analytical scope of the method and the quality of the results. Typically, MRM consists of following

steps: (i) extraction – isolation of residues from homogenized sample, (ii) purification – removing of co-extracts from crude extract, (iii) identification/quantitation of target analytes by gas chromatography and/or liquid chromatography, and (iv) positive samples confirmation.

The generation of reliable data is an important issue in this context. In accordance with the provisions of Directive 93/99/EEC, laboratory operations should meet the requirements of a recognized accreditation scheme, complying with ISO 17025 or Good Laboratory Practice (GLP)². One of objective means of assessing and demonstrating the data reliability produced by individual laboratory is participation in proficiency testing schemes³. The ISO definition of laboratory proficiency testing is “determination of laboratory testing performance by means of interlaboratory comparisons”. In these programs assessment of laboratory performance is expressed in the standardized form as a *z*-score, which is defined as , where *x* is result reported by individual

laboratory, $z = \frac{x - \hat{X}}{\sigma_p}$ is a reference value and σ_p is a target

standard deviation, a value representing reproducibility. The value of *z*-score should be $|z| \leq 2$ to be considered as satisfactory⁴. FAPAS[®] (Food Analysis Performance Assessment Scheme) organized by Central Science Laboratory, Department of Environment, Food and Rural Affairs (DEFRA), is one of the most known programs, providing tests in the area of food chemical analysis in a wide range of categories including (i) analytes of “chemical difficulty” – pesticide residues, contaminants, veterinary drug residues and mycotoxins, (ii) nutrient components analyses, (iii) animal feeding stuffs and (iv) special analysis⁵.

Regarding pesticide residues in foods the Council Directives (86/322/EEC and 90/642/EEC) provide for the organization and financial support for regular proficiency testing of the laboratories linked into official control and monitoring programs. The scheme is open also for other EU laboratories working in this field.

The current study presents results of Metrological and Testing laboratory (ICT Prague) in the area of external quality control.

Table I
External quality control in ICT laboratory in the year 2004

examined matrix	Scope (number of pesticides potentially occurring)	identified/quantified analytes	<i>z</i> -score	PT program
wheat flour	16	Chlorpyrifos-methyl	-0.8	FAPAS [®]
		Pirimiphos-methyl	0.1	
		Deltamethrin	0	
		Lambda cyhalotrin	-1.0	
		Permethrin	-0.8	
lemon, puree	51	Diazinon	-0.2	FAPAS [®]
		Fenitrothion	-0.3	
		Metalaxyl	-0.1	
		Methidathion	-0.3	
lettuce, puree	51	Lambda cyhalotrin	-2.1	FAPAS [®]
		Metalaxyl	-1.2	
apple, puree	51	Pirimiphos-methyl	0.4	FAPAS [®]
		Propargite	-0.5	
		Tetradifon	0.6	
tomatoes, puree	57	Azoxystrobin	-1.7	EU-PT
		Bromopropylate	-0.6	
		Chlorothalonil	0.2	
		Diazinon	0	
		Dimethoate	-0.8	
		Endosulphane group	0.7	
		Imazalil	-0.5	
		Procymidone	1.1	
		Thiabendazol	-0.5	

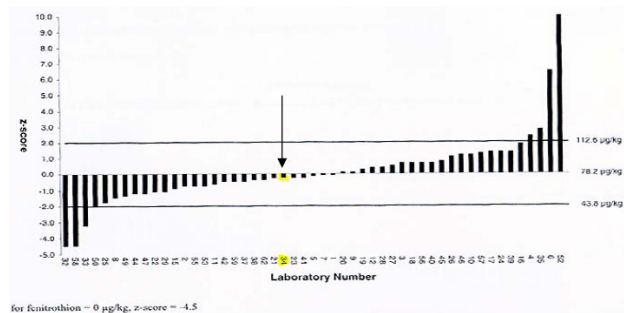


Fig. 1. FAPAS® z-scores (Series 19, Round 37). Laboratory code: 034, z-score for fenitrothion –0.3

Experimental

Multiresidue GC-MS method implemented in accredited Metrological and Testing laboratory (ICT Prague) was validated for 77 pesticides representing different chemical structures (29 organophosphorus, 19 organochlorine, 7 pyrethroids, 5 carbamates, 3 dicarboximides, 4 N-trihalomethylthio- and 10 others). The procedure is applicable for determination of pesticide residues in fruit, vegetable and cereals. It consists of following steps: (i) extraction of sample carried out with ethyl acetate on Ultra Turrax (IKA, Wern, Germany), (ii) clean-up of crude extracts using high performance gel permeation chromatography (ASPEC, Gilson, France); PL-gel column and ethyl acetate:cyclohexane (1 : 1, v/v) mixture as a mobile phase, (iii) identification/quantitation of target analytes carried out by GC coupled to electron capture detector (ECD) and nitrogen-phosphorus detector (NPD), (HP 6890, USA) and (iv) confirmation of positive samples by GC-MS-EI, quadrupole mass analyzer (HP 5973, USA) operated in SIM mode. Both systems were equipped with DB-5MS capillary column (60 m × 0.25 mm × 0.25 µm) and splitless injector. Helium was used as a carrier gas.

Results and discussion

Performance characteristics of the GC-MS method obtained within the validation procedure were as follows: recovery of target analytes in range 79–110 % (with the exception of lambda-cyhalothrin 51 %). Repeatability expressed as relative standard deviation (RSD) ranging from 1 to 20 %. Limits of detection (LOD) were in the range 0.001–0.005 mg kg⁻¹. Calibration was carried out employing matrix-matched standard.

In 2004 Metrological and Testing laboratory (ICT Prague) participated in four rounds of FAPAS® interlaboratory testing and one European Proficiency Testing EU-PT6 organized by University of Almería (Spain). As shown in Table I, satisfactory results (z-scores in the range ± 2) were obtained almost in all tests encompassing a wide range of plant matrices. Of total number 23 z-scores 18 (i. e. 78 %) were lower than 1 what documents low systemic error (good accuracy) of ICT measurements. An example shown in Figure 1 documents proficiency test results obtained within FAPAS® Test (Series 19, Round 37; lemon purée). In particular case (or-

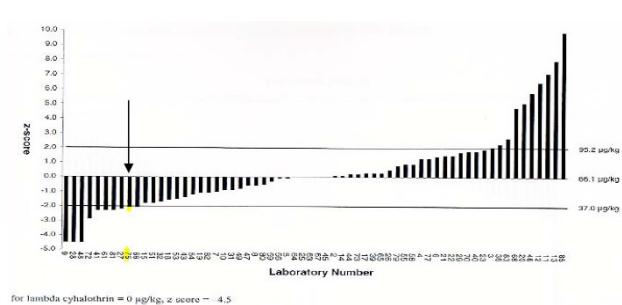


Fig. 2. FAPAS® z-scores (Series 19, Round 35); laboratory code: 075, z-score for lambda-cyhalothrin –2.1

ganophosphate fenitrothion) z-scores are distributed symmetrically.

However, in the FAPAS® Test Series 19, Round 35 (lettuce purée) unsatisfactory value of z-score (–2.1) was obtained for lambda-cyhalothrin. As shown in Figure 2, both overestimated and underestimated results were reported by some laboratories. According to accreditation criteria any identified problem of such kind has to be critically analysed. In this context it is necessary to emphasize that according to FAPAS® requirements results have to be reported uncorrected for recovery. It should be also noted that low (51 %) nevertheless well reproducible (RSD 9 %) recovery of lambda-cyhalothrin is typically obtained by the method described in Experimental. The reason of low recovery value is occurrence of partial overlap of matrix co-extracts and cyhalothrin elution zones during the GPC fractionation. While under these conditions part of this early eluting target analyte is lost (all other pesticides are eluted at higher elution volumes) relatively efficient clean-up is achieved. In practice, being aware this systematic error, correction for recovery is adopted when calculating cyhalothrin concentration. As far as this approach is applied for reporting of cyhalothrin content in FAPAS sample, satisfactory z-score would have been obtained.

In addition to the information shown in Table I, ICT laboratory participated in a special round of FAPAS® test (Series 19, Round 32) focused on trace analysis of pesticide residues in baby food, where LOQs lower than 0.01 mg kg⁻¹ are required (uniform MRL – 0.01 mg kg⁻¹ has been established by EU Directive 1999/50/EC amending Directive 91/321/EEC on infant formulae and follow-on formulae). The results reported as present/absent are evaluated as satisfactory/unsatisfactory respectively. Successful pass for all 36 tested analytes was achieved.

Another interesting information one can recover from FAPAS reports is information on methods used in other participating laboratories. In the year 2004, 84 % of participants were using GC technique for analysis of pesticide residues. Regarding injection techniques, 14 % of participants used split injection, 75 % splitless, 2 % pulsed splitless, 7 % on-column; programmed temperature vaporizer (PTV) was employed by 2 % of participants. MS confirmation of positive results was conducted by 77 % of participating la-

laboratories. For calibration 45 % of laboratories used a single-level calibration while 54 % multi-level calibration. 42 % of participants used matrix-matched standards in comparison to 58 % of participant used standards dissolved in net solvent for calibration.

Conclusions

Regular participation in proficiency testing schemes provides an independent control of quality of data generated in pesticide residue analysis. Because of limited stability of modern pesticides, certified matrix reference materials are not available for control of measurements accuracy, hence in other words proficiency tests are in particular case the only traceability measure.

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P28 SEPARATION PROCEDURES FOR ISOLATION, IDENTIFICATION, AND DETERMINATION OF ESSENTIAL OILS IN DRUGS OF PLANT ORIGIN

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Introduction

Essential oils rank with organic substances with a heterogeneous structure containing volatile and lipophilic components. All are colorless, insoluble in water and readily soluble in polar organic solvents. The typical flavor of essen-

tial oils results mostly from the presence of terpenoid components. In terms of prevailing components, essential oils are classified with groups containing predominantly acyclic and cyclic hydrocarbons, alcohols, aldehydes, ketones, phenols, phenolic ethers, esters, peroxides, or oxides^{1,2,3,4}.

About one third of the 295 plant families contain essential oils in amounts allowing their use in the industry. Most often, they are used in the pharmaceutical and food industries. The effects of herbs, essential oils, and separated and purified components usually differ from each other⁵.

Essential oils are significant components of many pharmaceutical products of plant origin, which are increasingly used as a complement to synthetic drugs known to induce often side effects. From 1987, all drugs of plant origin marketed in the Czech Republic are subject to registration. Essential oils are also used as components of therapeutic cosmetics, which are free of allergic activity⁶.

Essential oils detected in *Melaleuca alternifolia* (tea tree oil) were also tested for inflammatory effects. Analyses by HRGC/MS identified terpinen-4-ol and 1,8-cineol⁷. Antimicrobial activities of selected essential oils were investigated by several authors using *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* as the test microorganism species. Marked antimicrobial activity was demonstrated in thymol and weaker in geraniol and linalool⁸.

Active components are isolated from drugs of plant origin and therapeutic cosmetics usually by water vapor distillation or extraction. The requirements on qualitative analysis are necessarily derived from requirements which the end product must meet⁹. Gas chromatography is preferred for quantitative determination of essential oils. Thermal desorption GC procedure for the determination of menthol in essential oils was tested in two column systems¹⁰. Gas chromatography was also used for the determination of essential oils separated from *Eusteralis deccanensis* Paniobgrahi (*Laminaceae*). This study, focused on investigations of the phytochemical potential, demonstrated that the separated essential oils contained three sesquiterpenes including β -caryophyllene, β -bourbonene, and β -elemene, oxidized monoterpenes (geranyl acetate, neryl acetate), and one sesquiterpene alcohol (δ -cadinol)¹¹. Other authors tested the efficacy of GC/MS for the determination of essential oils isolated from several herbs grown in Greece. Most of the isolated essential oils belonged to the groups of alcohols, esters, and hydrocarbons¹².

Experimental

Materials and chemicals

The following cosmetics, produced by KNEIPP, were analyzed for the contents and composition of essential oils: rosemary and eucalyptus bath salt, rosemary balm and eucalyptus bath, mint oil and herb foot spray. Samples were extracted mostly with chloroform (Merck, Germany) and the extracts were dried over anhydrous sodium sulfate (Merck, Germany). Benzene, ethyl acetate, and toluene were used as the mobile phase. All the solvents were supplied by Merck

and were of residual analysis grade. Anisaldehyde and 4-dimethylaminobenzaldehyde (4-DMABA) were used for the preparation of detection agents. All the chemicals were purchased from Merck and were of chemically pure grade. SPE columns used for clean up, packed with C_{18} , were supplied by Baker (Holland). Certified standards of analytes, tested by mass spectrometry, were purchased from Phoenix (Czech Republic).

Instruments and devices

The SPE apparatus Baker and columns packed with florisol and C_{18} were used for clean-up. Thin layer chromatography was carried out on plates 20×20 cm in size ALUGRAM SIL G; 0.20 mm Silica gel 60 was purchased from MACHEREY-NAGEL, Germany.

HRGC/MS was used for the identification and determination. Mass spectra were recorded under standard EI using the mass detector MSD 5973 with on-line gas chromatograph GP HP 6890. The recordings of mass spectra and TLC chromatograms were evaluated using the standard HP software. Column parameters were as follows: HP 5 MS, 30×0.25 mm; film thickness 0.25 μm ; temperature mode: 1 min at 40 °C followed by increase at 7 °C min^{-1} to 280 °C, hold for 40 min; injector temperature 270 °C, detector temperature 280 °C, ion source temperature 230 °C.

Procedures

The substances under study included mint oil, rosemary oil, eucalyptus oil, chamomile oil, menthol, menthone, cineol, methyl acetate, piperitone, and thymol. The substances were isolated from cosmetics and drugs by water vapor distillation or extraction with chloroform. The extracts were dried over anhydrous sodium sulfate and condensed. The residue was dissolved in a defined volume and cleaned by LC in florisol or aluminum oxide columns (columns and apparatus Baker), or by SPE in columns retaining ballast components.

The presence of the analytes was detected by screening TLC. Five processing systems were tested for their suitability for TLC of essential oils. The best results were obtained with benzene+ethylacetate (19+1) and chloroform+benzene (3+1). Anisaldehyde and 4-DMABA were found to be the best detection agents of the five tested. TLC was chosen because it has been recommended for the demonstration of active components in drugs of plant origin by the Czech Pharmaceutical Code.

HRGC/MS allowed us to identify and determine some components which could not be unambiguously detected by TLC, such as pinene isomers, phelandrene, myrcene, menthone, menthol, piperitone, menthyl acetate, cineol, camphor, isoborneol, borneol, and bisabolol.

Results and discussion

Thin layer chromatography was found to be a suitable method for the identification of components of essential oil-containing drugs. The mixture benzene+ethyl acetate (19+1) was selected as the mobile phase of the five tested.

Solutions of anisaldehyde in acetic and sulfuric acids and of 4-DMABA in acetic and phosphoric acids proved to be the best detection agents of the five variants tested. The patches and zones obtained in our analyses were compared with published data^{2,5}. Both the detection agents were used for the detection of the analytes. R_F values were calculated for each patch and zone and active components were identified in a number of them. Most products of therapeutic cosmetics could not be analysed without preliminary separation by column chromatography or SPE; such procedure can be used only in the case of mint oil. Borneol and cineol were identified in the extract of rosemary bath salt, eucalyptus bath salt, and rosemary bath. The extract of balm bath contained citronelal and citral as the active components. Moreover, patches attributable to terpenic oils (geraniol, linalol, citronelol) were detected. Pale violet patches with $R_F=0.14-0.40$ were detected in the extract of balm bath 4-DMABA as the detection agent. None of the patches were detected as specific components of essential oil-containing drugs. Borneol and cineol were detected with anisaldehyde in the eucalyptus bath. In addition to the two, bornyl acetate was identified with 4-DMABA. No active components were identified in mint oil using the mobile phases and detection agents specified above.

The identity of components in essential-oil containing drugs was verified by HRGC/MS. Specific peaks were identified by comparison with data of the mass spectra library. Our analyses demonstrated that rosemary bath salt contained six components with retention times ranging from 9.48 to 16.95, including cineol, camphor, borneol, linalyl propoate, bornyl acetate and caryophyllene, but only borneol and cineol were identifiable by TLC. The eucalyptus bath salt contained cineol, 3-carene, and linalyl propoate when

analysed by HRGC/MS and only cineol and borneol were identified by TLC. The presence of the latter was not confirmed by the arbitration method, however. Eight components with retention times ranging from 9.39 to 21.40 min, including cineol, isoborneol, borneol, linalyl propanoate, bornyl acetate, caryophyllene, and α -bisabolol were identified in rosemary bath.

Balm bath contained citronelal, citronelol, and geraniol as the major, and citronelol acetate and geraniol acetate as the minor components. Retention times of the identified components ranged from 11.74 to 16.19 min. Rather rich in various components was mint oil in which α -pinene, β -pinene, β -phelandrene, β -myrcene, menthone, menthol, piperitone, menthyl acetate, and caryophyllene were identified by HRGC/MS. The same procedure allowed us to identify β -myrcene, cineol, 3-carene, camphor, isoborneol, borneol, and α -bisabolol with retention times ranging from 8.41 to 21.50 in the eucalyptus bath. The herb foot spray was found to contain cineol, borneol, and isoborneol, which are the major active components of *Rosmarinus officinalis* and thymol, which is the active component of *Thymus vulgaris*. The procedure failed to identify camphor for which another m/e would be necessary. All the identified active components are

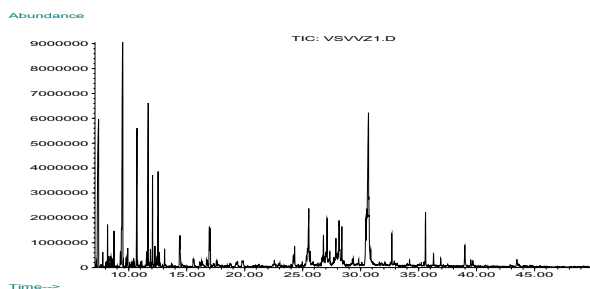


Fig. 1. Recording of chromatographic separation of active components of essential oil-containing drugs contained in rosemary bath salt

Table I
Chromatographic separation of standard Oleum rosmarini

System	Patch (zone) color	R_F	Identification
A	pink	0.23	borneol
	red	0.64	cineol
	pink	0.48	bornylacetate
B	violet	0.24	not identifikated
	greyish violet	0.31	borneol
	pink violet	0.43	not identifikated
	pink	0.58	cineol
	pate violet	0.74	bornylacetate

Legends:

A – detection of anisaldehyde

B – detection of 4-DMABA

Table II
Chromatographic separation of active components of essential oil-containing drugs contained in rosemary bath salt

System	Patch (zone) color	R_F	Identification
A	grey	0.08	borneol
	grey	0.18	cineol
B	violet blue	0.09	borneol
	pink	0.24	cineol

Legends:

A – detection of anisaldehyde

B – detection of 4-DMABA

declared on packings of the products marketed by Kneipp. The following two figures show TIC recordings representing samples of rosemary bath salt (1) and mint oil (6), obtained by HRGC/MS. The recordings show a high number of peaks

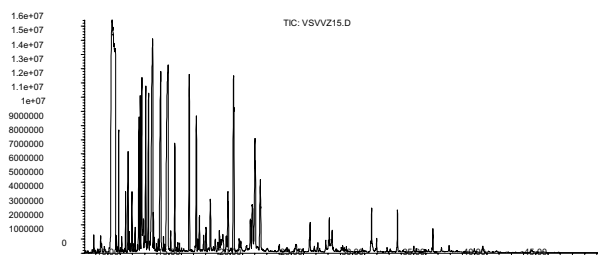


Fig. 2. Recording of chromatographic separation of active components of mint oil

corresponding to six identified active components in Sample 1 and nine peaks of active components in Sample 2. The components were identified using the mass spectra library as a part of the software.

The results demonstrate the suitability of various separation procedures for the isolation, identification and determination of components of essential oils used in the cosmetic and pharmaceutical industries.

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P29 IMPACT OF CONSTRUCTION MATERIALS ON POTABLE WATER QUALITY

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Water as a basic condition of life

Without water there is no life. Average daily consumption is 118,6 litre of water on one inhabitant. We can not introduce modern society without equipment supplying potable and supply water to final user into apartment. Easy availability of water in advanced countries was the cause of expressive growth of consumption, too. There are released chosen data about water requirement in our country and in world on figure 1 and Table I. Hygienic minimum declared by WHO (World Health Organization) is 100 dm³ of water for person and day.

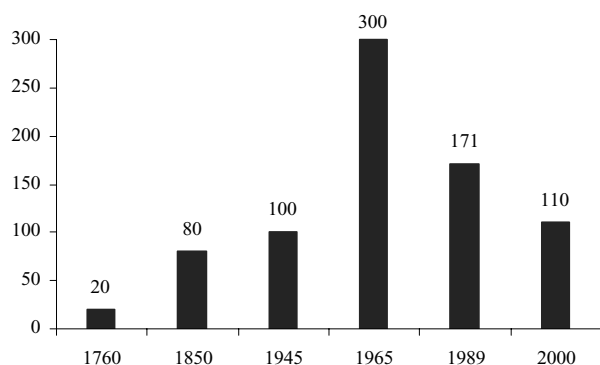


Fig. 1. Progression of specific consumption of water in Czech countries for a person and a day

Table I

Progression of specific consumption of water in the world for a person and a day

Country	Water consumption [dm ³]
USA	300
West Europe	150–200
Czech Republic	120
Developing countries	10

History of water-supply

Question of transport of water from available resources to final user was already resolved in old history. Water pipes has been already known by ancient civilizations. In India 4000 B.C. town houses were equipped with bath, later on Crete or in ancient Greece and Rome some houses were equipped with piping system of distribution cold and sometimes hot water. The first water pipes were built in Czech countries in Vysehrad and Prague castle in 12th century. In

1348 spring water was installed by drilling pine tubes into public fountain on Charles and Wenceslas square in Prague. In 15th century begun water pumping from Vltava, later was filtrated and purified. In Brno the first water pipes associated with pumping device were built in 1415. Water was distributed especially into public fountain on present-day Freedom square and Zelný market. Water was generally supplied by old water pipes into public fountains only, seldom into houses. Inside water pipes sufficient transport of water into the top floors was built in our towns at the end of 19th century and then it was possible to equip flats by bathrooms and squatting closets. In consequence introduction of water into houses water consumption has begun to rise. Present average daily consumption is 90–110 dm³ of water for a person and a day.

Materials used in distribution systems

For distribution of potable water cold and potable water hot different construction materials like steel, cast iron, fiberglass, polyethylene, ethernit, polyvinyl chloride, ferroconcrete are used in the Czech Republic. Implementation of water distribution depends on time of installation and used materials. Materials used in water distribution system in Brno are depicted in figure 2.

Water quality is controled before water is released from waterworks and reservoirs. Potable water is transported to final user by distribution system during several days. Distribution system isn't created only by pipes but by armatures, fixtures and other components. In distribution system different construction materials are used.

In distribution system proceed physical-chemistry process which affects water quality supplied to final user. It starts affecting properties of construction materials of distribution system. Water flows with different speed in distribution system. During stagnation ideal conditions for corrosion process appear. Incrustation or sediments are formed and repeated expansion of microorganism occurs.

Experiment and results

Our work was focused on the research of interactions of water with metal materials. Corrosive stability of six pipe fittings used in water distribution systems and eight construc-

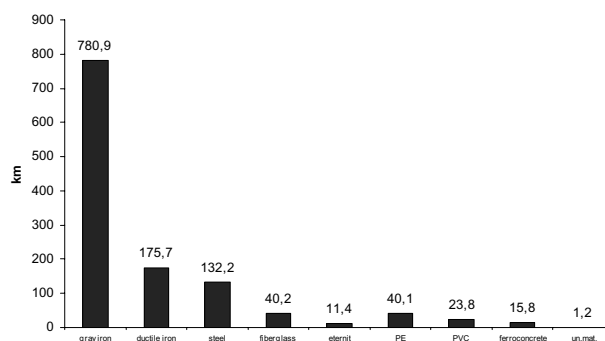


Fig. 2. Constructional materials used in water distribution system in Brno

tion materials (bronze, gray iron and six types of steel classes 11, 12, 15, 17 and 19) was examined. The effect of pH, retention period, structure and quality of surface of construction materials and water composition on content of selected metals – iron, copper and zinc were observed. Water quality was monitored by final users in five parts of Brno. The content of metals in the water was determined by atomic absorption spectrometer SpektrAA 30. It was approved that in dependence on chemical composition of water, constitution of construction materials as well as on temperature of water, pH of water and detention period in distribution system there is a change of water quality. Considerable impact on water quality is possible to adjudicate to unsuitable construction materials.

Conclusion

Average daily consumption of water per person in the Czech Republic is approximately 120 dm³. Potable water is transported to final user by distribution system during several days. In distribution systems different construction materials are used and quality of transported water isn't constant. Moreover, it is necessary to consult the age of current water distribution system and the durability of used materials.

It is possible to conclude that the impact of constructional materials of distribution system on transported water quality is evident and materials used for construction of potable water distribution systems should be chosen carefully. It's necessary to pay attention to these problems.

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P30 SPECTROPHOTOMETRIC MICRODETERMINATION OF PLATINUM

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Introduction

The determination of microconcentrations of Platinum remains actual because of its increasing amount in the environment due of its extended use as automobile catalyst and also the anticancerogenic effect of some Platinum(II) complexes. Recently sensitive spectrophotometric methods on the base of ion associates of Platinum(IV) chloro- and thiocyanatocomplexes with basic dyes, especially Rhodamines called attention^{1,2,3}. Li, Wang, Xi¹ assumed the formation of defined Pt(MoO₃)²⁻ from PtCl₆²⁻ and mentioned high sensitivity when associated with a number of basic dyes, especially Butylrhodamine B and Nile Blue in acid medium and in the presence of polyvinylalcohol (PVA). Unfortunately, questionable results could be used only when their procedure was reproduced.

The less sensitive reaction of PtCl₆²⁻ with stannous chloride in the HCl medium was early recommended for the determination of Platinum^{4,5}.

In this paper the conditions for the formation of the associate of the Platinum(IV)molybdate with Rhodamine B have been studied in detail and its use for the determination of Platinum proved. Furthermore, a sensitive method on the basis of the oligonuclear Tin(II) and Platinum(II)chloride complex in the presence of Rhodamine B and polyvinylalcohol was carried out in aqueous medium. These methods were tested for the determination of Platinum in waters and urine.

Experimental

Absorption double-beam spectrophotometer Unicam UV 500, Thermo-Spectronic, with 1 cm glass and quartz cells. Platinum standard, H₂PtCl₆, containing 1.000±0.002 g l⁻¹ of Platinum in 5% HCl was purchased from Analytica, LTD. Prague.

H₂PtCl₄ was prepared by reduction of 100 µg of H₂PtCl₆ with 1.6 ml of 0.8% Hydrazine hydrate at elevated temperature for 30 min and the solution diluted to 100 ml with 0.1 M HCl. The solution was active for 2 days. Other chemicals used were of analytical grade quality.

Surface water was sampled from Svratka river in a Brno locality, drinking water was taken from Brno Water Supply system. Solutions with the spikes of H₂PtCl₆ were equilibrated for 24 hours. Urine was a day's average sample. No Platinum was available in the original samples. Absorbance

data were averaged from triplicate measurements according to Dean and Dixon¹⁰. Calibration plots were evaluated according to ČSN ISO 8466-1 and 2 norms.

Results

Associate of Molybdato-platinat (IV) with Rhodamine B

The results of previous authors could not be reproduced which was in agreement with previous papers for Nile blue^{15,16}. Precipitates appeared and the published values for molar absorption coefficients were doubtful. In addition, the formation of the simple $\text{Pt}(\text{MoO}_3)_2^{2-}$ from the oligonuclear species⁵ in aqueous medium under given conditions is questionable.

As we could find, satisfactory results were obtained only when Rhodamine B was used as associating agent which gives one defined product with the platinum(IV)molybdate species with λ_{max} at 534 and 576 nm on difference spectrum for strictly maintained conditions in final solutions which are 0.001 M ammonium or sodium molybdate, 0.73 M perchloric acid, in the presence of $7 \cdot 10^{-5}$ M Rhodamine B and 0.06 % PVA. The previous heating of solution containing molybdate and PtCl_6^{2-} is critical and 60 °C for 30 min must be maintained. The absorbance remains constant for at least one hour and has been measured at 577 nm after 15 min. There is no considerable influence between sodium molybdate and ammonium heptamolybdate when used for the reaction. Rhodamine B increases the absorbance till $7 \cdot 10^{-5}$ M concentration only. The influence of perchloric acid is critical and must not exceed 0.73 M concentration. The strict isosbestic points on the absorption curves at 499 nm proves the simple stoichiometry between the Platinum(IV) complex and the Rhodamine in the associate.

The sensitivity of the reaction of Platinum (IV) molybdate with Rhodamine B is however influenced by the age of diluted standard solutions containing hydrochloric acid used. It increases remarkably with the time of aging. Constant absorbance values are observed only after 340 hours. Freshly diluted standards in 5% HCl do not give the reaction with

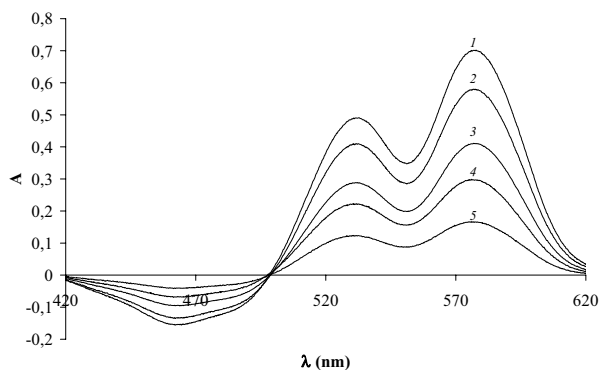


Fig. 1. Difference absorption spectra of Pt(IV) in the presence of $4.23 \cdot 10^{-5}$ mol dm^{-3} Rhodamine B, 0.001 mol dm^{-3} sodium molybdate, 0.73 mol dm^{-3} HClO_4 and 0.06% PVA in dependence on concentration of Platinum(IV). Individual spectra have following concentrations of Pt(IV): 0.2 $\mu\text{g cm}^{-3}$ (1), 0.15 $\mu\text{g cm}^{-3}$ (2), 0.1 $\mu\text{g cm}^{-3}$ (3), 0.05 $\mu\text{g cm}^{-3}$ (4), 0.02 $\mu\text{g cm}^{-3}$ (5)

molybdate and Rhodamine B at low concentration of Platinum even after previous heating. The reason is that more reactive hydroxochloroplatinate and aquochloroplatinate complexes are formed in diluted PtCl_6^{2-} standard solutions even in the presence of hydrochloric acid during growing age¹⁷ which increases the reactivity of such solutions. No influence on the reactivity is, however, observed when simultaneously reduction to Pt(II) takes place. No formation of absorbing ion associate was observed, when Pt(IV) was previously reduced to Pt(II) by hydrazine hydrate.

Associate of Platinum (IV) thiocyanatokomplex with Rhodamine 6G or Rhodamine B

The transformation of PtCl_6^{2-} to $\text{Pt}(\text{SCN})_6^{2-}$ at room temperature is doubtful. According to our experience and the literature¹⁷ the extended heating at 65–75 °C is necessary but the heating at 95° and boiling leads to reduction of Pt(IV) to Pt(II) with the formation of precipitate in the presence of thiocyanate excess. The early described spectrophotometric methods^{2, 3} miss the reproducibility. We have tried to heat the solution of PtCl_6^{2-} for 20–30 min. at 65–75 °C prior to the reaction with Rhodamine 6G and replaced the 0.04% gelatine by 0.18% polyvinylalcohol. The optimised conditions corresponded with 0,1 M NaSCN, 0.04 M citrate (pH 4), $2.3 \cdot 10^{-5}$ M Rhodamine 6G and 0.18% polyvinylalcohol (Mol. W. 15 000–100 000) for the Platinum concentration interval 0.01–8 $\mu\text{g cm}^{-3}$ of Pt(IV). Unfortunately even in such case the absorbance in the difference spectrum decreases rapidly and some fine precipitate was observed in solution, as same as in blank solution.

No interaction with Platinum has been observed if Pt(IV) was previously reduced to Pt(II).

Associate of Platinum (II) oligonuclear complex of Tin(II) with Rhodamine B

An yellow oligonuclear Pt(II) complex of complicated structure is formed from PtCl_6^{2-} with 0.2 M tin dichloride in

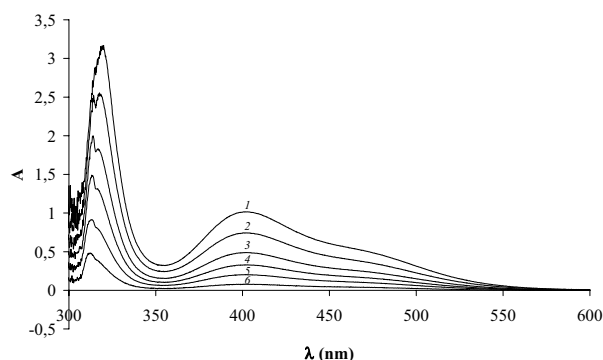


Fig. 2. Difference absorption spectra of the system in the presence of 0.2 mol dm^{-3} SnCl_2 and 1.6 mol dm^{-3} HCl in dependence on concentration of Platinum(IV). Platinum(IV) concentrations: $1 - 25$ $\mu\text{g cm}^{-3}$, $2 - 18$ $\mu\text{g cm}^{-3}$, $3 - 12$ $\mu\text{g cm}^{-3}$, $4 - 8$ $\mu\text{g cm}^{-3}$, $5 - 5$ $\mu\text{g cm}^{-3}$, $6 - 2$ $\mu\text{g cm}^{-3}$

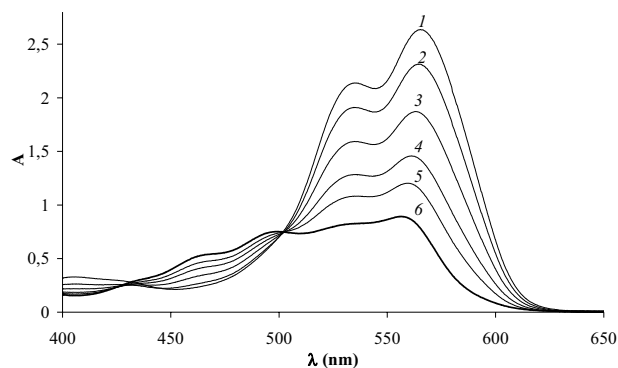


Fig. 3. Absorption spectra in the presence of $5.1 \cdot 10^{-5} \text{ mol dm}^{-3}$ Rhodamine B, $0.16 \text{ mol dm}^{-3} \text{ SnCl}_2$, $1.5 \text{ mol dm}^{-3} \text{ HCl}$ and 0.06% PVA in dependence on concentration of Platinum(IV). Platinum concentrations : 1 – $4 \mu\text{g cm}^{-3}$, 2 – $2.5 \mu\text{g cm}^{-3}$, 3 – $1.5 \mu\text{g cm}^{-3}$, 4 – $0.8 \mu\text{g cm}^{-3}$, 5 – $0.4 \mu\text{g cm}^{-3}$, 6 – determination blank

Table I

Evaluation of various water samples and urine spiked with PtCl_6^{2-} with confidence intervals calculated according to Graham¹¹. All samples were evaluated in triplicate

$c_{\text{spiked}} [\mu\text{g cm}^{-3}]$	Surface water	Drinking water	Urine
	$c_{\text{found}} [\mu\text{g cm}^{-3}]$		
Non-linear plot			
0.4	0.43 ± 0.05	0.43 ± 0.05	0.42 ± 0.05
0.6	0.62 ± 0.05	0.62 ± 0.05	0.62 ± 0.05
0.8	0.83 ± 0.05	0.82 ± 0.05	0.82 ± 0.05
2	2.0 ± 0.1	2.0 ± 0.05	2.0 ± 0.05
Linear plot			
0.4	0.44 ± 0.06	0.44 ± 0.06	0.44 ± 0.06
0.6	0.63 ± 0.05	0.62 ± 0.05	0.62 ± 0.05
0.8	0.82 ± 0.05	0.82 ± 0.05	0.82 ± 0.05

Table II

Detection limits, concentration ranges and molar absorption coefficients of determination methods under various conditions

Method	Concentration interval of Pt [$\mu\text{g cm}^{-3}$]	Detection limit $X_D^{\beta} [\mu\text{g cm}^{-3}]^a$	Detection limit $X_b [\mu\text{g cm}^{-3}]^b$	ϵ [$\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$]
Amonium molybdate	0.02–0.2	0.058	0.021	$8.31 \cdot 10^5$ (577 nm)
Sodium molybdate	0.02–0.2	0.042	0.010	$9.11 \cdot 10^5$ (577 nm)
SnCl_2 (402 nm)	0.5–25	0.51	0.004	$7.96 \cdot 10^3$ (402 nm)
SnCl_2 (320 nm)	0.5–15	0.60	0.013	$2.73 \cdot 10^4$ (320 nm)
SnCl_2 -RhB (non-linear)	0.05–3	0.13	0.010	$1.74 \cdot 10^5$ (571 nm)
SnCl_2 -RhB (linear)	0.05–1	0.19	0.010	$1.57 \cdot 10^5$ (571 nm)

^a Confidence limit for type II errors (according to Graham¹¹)

^b $\bar{x}_{\text{blank}} + 3 \cdot s_{\text{blank}}$

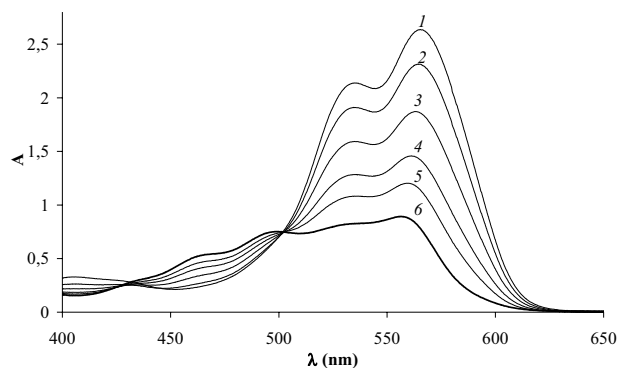


Fig. 4. Absorption spectra of the system in the presence of $5.1 \cdot 10^{-5} \text{ mol dm}^{-3}$ Rhodamine B, $0.16 \text{ mol dm}^{-3} \text{ SnCl}_2$, $1.5 \text{ mol dm}^{-3} \text{ HCl}$ and 0.06% PVA in dependence on concentration of Platinum(IV) : 1 – $4 \mu\text{g cm}^{-3}$, 2 – $2.5 \mu\text{g cm}^{-3}$, 3 – $1.5 \mu\text{g cm}^{-3}$, 4 – $0.8 \mu\text{g cm}^{-3}$, 5 – $0.4 \mu\text{g cm}^{-3}$, 6 – determination blank

1.6 M HCl having $\lambda_{\text{max}} = 402 \text{ nm}$ and 320 nm and several maxima in UV region for $\lambda < 335 \text{ nm}$.

The calibration plots are strictly linear for a broad interval of $0.5\text{--}15 \mu\text{g cm}^{-3}$ but the sensitivity and detection limits are rather low (c. f. Table II).

The determination is not disturbed for spikes of $0.5\text{--}5 \mu\text{g cm}^{-3}$ Pt into 25 ml of surface or waste waters and urine.

Thus, we have studied the ion association of this complex with rhodamine B in acid aqueous solution in the presence of 0.06% polyvinylalcohol to increase the sensitivity of this method. The difference absorption spectra have λ_{max} 535 and 571 nm.

The concentration of Rhodamine B should not exceed $5.1 \cdot 10^{-5} \text{ M}$. The optimised solutions contained 1.5 M HCl , 0.16 M SnCl_2 , $5.1 \cdot 10^{-5} \text{ M Rhodamine B}$ and 0.06% polyvinylalcohol. There is an instant formation of the associate but the absorbance was measured after 15 min. The slightly curved calibration plots obey the polynomial of 2nd degree for the interval of $0.05\text{--}3 \mu\text{g cm}^{-3}$ Pt but becomes almost linear

for the short interval 0.05–1 $\mu\text{g cm}^{-3}$ of Pt. The evaluated parameters are summarised in Table I and II.

In this case, Pt(II) and Pt(IV) give the same response since Pt(IV) is reduced during the complex formation and the aging of diluted standard solutions is without effect.

This procedure is also suitable for the determination of Platinum in drinking or surface waters or urine when spikes of 0.4–2 $\mu\text{g Pt(IV)}$ per ml were applied.

Methods on the basis of $\text{Pt(SnCl}_3\text{)Cl}_2^{2-}$ and its associate with Rhodamine B are suitable for their outstanding reproducibility and sufficient sensitivity and partial selectivity, which can be used for the determination of limited amounts of Platinum in waters and some body liquids.

Noteworthy, the reaction of the presumed $\text{Pt(MoO}_4\text{)}_3^{2-}$ with Rhodamin B is most sensitive under selected conditions but of very limited selectivity and its use for the practical determination of Platinum is questionable.

Some detection limits according to Graham¹¹, IUPAC recommendation¹⁸ etc.¹³ have been compared in this work.

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P31 SPECTROFLUORIMETRIC DETERMINATION OF MICROCONCENTRATIONS OF THALLIUM

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Introduction

Thallium is more toxic than lead and is relatively slowly removed from living organisms after intoxication¹. Recently increased Thallium levels have been observed in environment, especially in soils and waters.

A violet fluorescence was reported for Thallium(I) in aqueous solutions containing considerable amounts of chlorides which is being influenced by a number of components^{2–5}. The formation of Tl(I) chlorocomplexes was assumed to be the reason for this fluorescence. The fluorescence is suppressed when Thallium is oxidized to Thallium(III).

In this paper the conditions for a method were studied in details and optimized and tested for the determination of Thallium in various kinds of water.

Experimental

A single beam spectrofluorimeter Aminco Bowman[®], series 2 with a xenon lamp working in a continuous regime, 4 nm slits and constant photomultiplier voltage was used for the measurements.

Thallium(I) nitrate in 2% HNO_3 (Czech Metrologic Institute-Analytika, spol. s r. o.) and aqueous Thallium(I) sulphate (Merk-Shuchardt Chemicals Co.) standard solutions containing 1 mg of Tl in 1 ml were used for quantification.

Samples of river waters from various depth and of mineral water Mattoni were spiked with Thallium(I) after

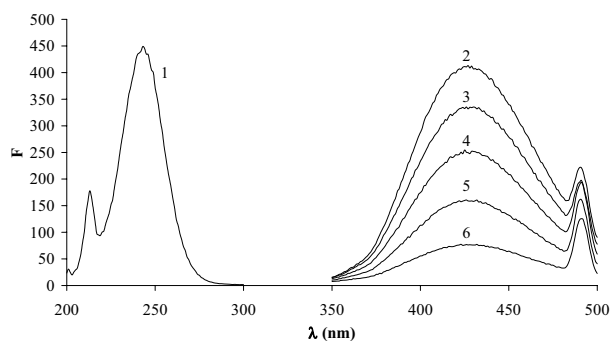


Fig. 1. Excitation and Emission spectra of Thallium(I) in the presence of 1.87 mol dm^{-3} HCl and 3 mol dm^{-3} NaCl in dependence on concentration of Tl(I). 1 – Excitation spectrum of the system with 0.2 $\mu\text{g cm}^{-3}$ of Tl(I), 2–6 – Emission spectra of the system in dependence on concentration of Tl(I) (2 – 0.2 $\mu\text{g cm}^{-3}$, 3 – 0.16 $\mu\text{g cm}^{-3}$, 4 – 0.12 $\mu\text{g cm}^{-3}$, 5 – 0.08 $\mu\text{g cm}^{-3}$, 6 – 0.04 $\mu\text{g cm}^{-3}$)

Table I
Detection limits, concentration ranges and sensitivity equivalents of determination under various conditions

Conditions	4.9 M NaCl, Ti ₂ SO ₄	3 M NaCl, 1.87 M HCl, Ti ₂ SO ₄	3 M NaCl, 1.87 M HCl, TiNO ₃
X_D^α [$\mu\text{g cm}^{-3}$] ⁶	$8.2 \cdot 10^{-6}$	$2.2 \cdot 10^{-5}$	0.0093
X_D^β [$\mu\text{g cm}^{-3}$] ⁶	0.004	0.005	0.023
X_b [$\mu\text{g cm}^{-3}$] ^{7, a}	0.00015	0.00022	0.0042
Concentration range of Tl(I) [$\mu\text{g cm}^{-3}$]	0.004–0.2	0.005–0.2	0.02–0.2
SE [$\text{cm}^3 \mu\text{g}^{-1} \text{V}^{-1}$]	7.86	5.95	2.40

$$^a \bar{x}_{\text{blank}} + 3s_{\text{blank}}$$

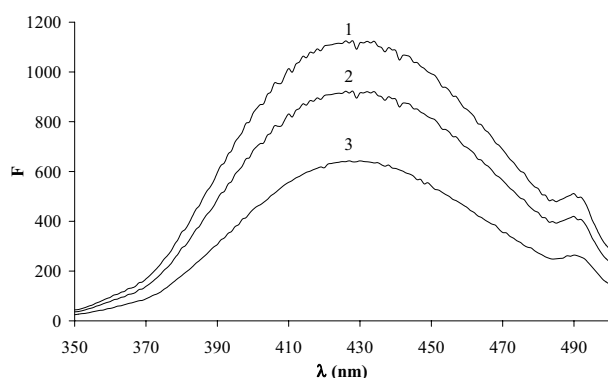


Fig. 2. Comparison of the emission spectra of solutions with 4.9 M NaCl (1), 1.87 mol dm⁻³ HCl and 3 mol dm⁻³ NaCl (2) and 7 M HCl (3) in presence of 0.2 μg cm⁻³ of Tl(I)

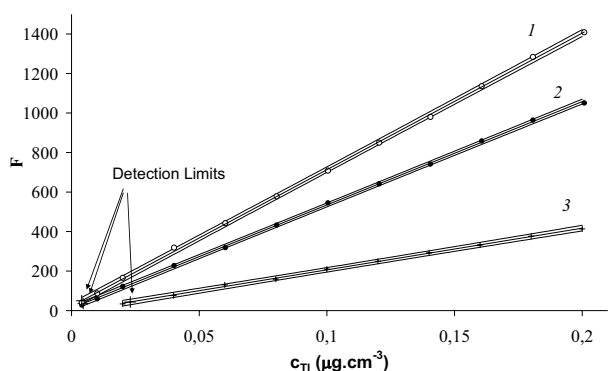


Fig. 3. Comparison of the calibration plots with confidence intervals and highlighted detection limits X_D^β calculated according to Graham⁶

- 1 – Calibration plot with 4.9 M NaCl and Ti₂SO₄
- 2 – Calibration plot with 1.87 mol dm⁻³ HCl, 3 mol dm⁻³ NaCl and Ti₂SO₄
- 3 – Calibration plot with 1.87 mol dm⁻³ HCl, 3 mol dm⁻³ NaCl and TiNO₃

acidification to 0.1 M HCl and equilibrated for 24 hours. No Thallium was present in used waters, prior to spiking.

Results

The intensity of instant fluorescence with $\lambda_{\text{max(em)}}$ 427 nm and $\lambda_{\text{max(exc)}}$ = 245 nm (Fig. 1.) strongly depends on the concentration of sodium chloride or HCl but completely extinguishes after one hour irradiation.

The maximal fluorescence intensity is reached for nearly saturated 4.9 M sodium chloride solution. In mixtures, NaCl and HCl mutually substitute, but for the determination the mixture of 1.87 M HCl and 3 M NaCl was finally elected. 1.2 M NaCl, KCl and NH₄Cl show a similar effect on the fluorescence in the presence of constant 3.6 M HCl, but LiCl is considerably less active. The use of single HCl till 7 M concentration without sodium chloride is unsuitable because only low fluorescence intensity is involved (Fig. 2).

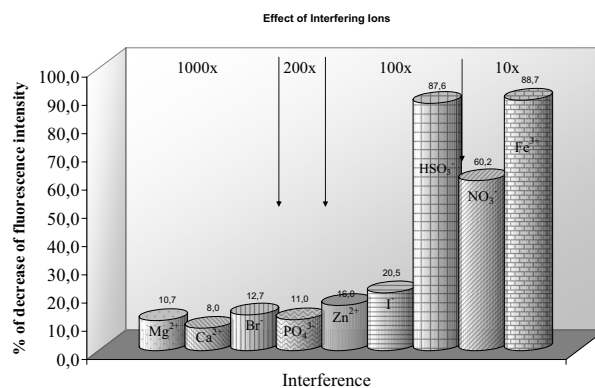


Fig. 4. Extent of interferences for selected ions under optimized conditions 1.87 mol dm⁻³ HCl, 3 mol dm⁻³ NaCl and 1 μg cm⁻³ of Tl

For TiNO₃ in the optimised conditions 1.87 M HCl and 3 M NaCl or in 4.9 M NaCl the suitable interval for the determination reaches 0.02–0.2 μg cm⁻³ of Tl, which is

considerably influenced even in the presence of maximum as little as $6.25 \cdot 10^{-5}$ M NO_3^- . When Tl_2SO_4 has been used the increased sensitivity enables to determine $0.004\text{--}0.2 \mu\text{g cm}^{-3}$ of Tl under the same conditions. The fluorescence was always measured at the $\lambda_{\text{max(exc)}} = 245$ nm and $\lambda_{\text{max(em)}} = 427$ nm after 10 min from the mixing of components. Selected calibration plots are collected in Fig. 3.

The sensitivities expressed as a quotient of linear regression coefficient of calibration plot and photomultiplier voltage are collected in Table I as well as the detection limits calculated according to Graham⁶ and from the blank solutions⁷.

$$SE = \frac{k}{U}$$

where SE is sensitivity equivalent, k is the linear regression coefficient of calibration plot and U is constant photomultiplier voltage.

The interferences of selected ions in solutions with 1.87 M HCl and 3 M NaCl are evaluated in Fig. 4.

For $0.1 \mu\text{g cm}^{-3}$ of Tl, Al^{3+} , SO_4^{2-} , non-ionic surfactants such as Brij 35 and Triton X 100 do not interfere under optimal conditions, considerable interference with the fluorescence was observed for nitrate, sulphite and Fe^{3+} as well as hydroxylamine hydrochloride, ascorbic acid and hydrazine hydrochloride which cancel the fluorescence. Only lower interferences has been found for Mg^{2+} , Ca^{2+} , Br^- , PO_4^{3-} and I^- (Fig. 4.).

Determination of Thallium in natural waters

The calibration plots for the optimal conditions with 1.87 M HCl and 3 M NaCl for the interval of $0.004\text{--}0.2 \mu\text{g cm}^{-3}$ of Tl were considerably influenced by the kind of natural water, especially by the presence of Fe^{3+} . Thus, calibration plots for the particular natural water must be used for the evaluation.

Table II

Values of Tl(I) found in spiked samples of surface waters with confidence intervals calculated according to Graham⁶. Quotient k/k_w represents rate between linear regression coefficients of calibration plots in demineralised and surface water

$c_{\text{spiked}} [\mu\text{g.cm}^{-3}]$	Mattoni	Mostiště	Úhlava
	mineral water	water ^a	river water
$c_{\text{found}} [\mu\text{g.cm}^{-3}]$			
0.02	0.022 ± 0.002	0.025 ± 0.002	0.020 ± 0.002
0.08	0.081 ± 0.002	0.084 ± 0.002	0.083 ± 0.002
0.14	0.146 ± 0.002	0.141 ± 0.002	0.144 ± 0.002
k_D/k_w	0.935	0.915	0.988

^aDepth water from reservoir

Results for three concentration spikes of Thallium(I) in triplicate for three kinds of natural waters with their confidence intervals are collected in Table II. Each value was calculated from the linear regression of corresponding calibration plot measured in each water. Calibration plots were evaluated by the standard addition method and simultaneously different Tl(I) spikes were used as samples.

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P32 THE EVALUATION OF METALLIC CONTAMINATION OF MUSHROOMS FROM SELECTED LOCALITY OF BOHEMIA

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Introduction

Toxicological and environmental studies have prompted interest in the determination of toxic elements in various components of ecosystems. The mushrooms are one parts of forest ecosystem. Wild growing mushrooms have been a popular delicacy in many countries, mainly in central and east Europe. For instance, collecting mushrooms has become a national hobby in the Czech Republic and Poland. But edible mushrooms surely do not constitute a significant portion of the human diet. Recent studies in this field are focused on two ways: screening of mushroom fruiting bodies as bioindicators of environmental pollution and studying of their ability to accumulate high levels of some trace elements, mainly cadmium, mercury, copper, zinc, lead and other risk metals. Occurrence of high metal contents in mushrooms, principally edible, is of considerable importance, since they might constitute a possible toxicological hazard. Trace metals in high concentrations in some edible mushrooms might contribute to chronic poisoning of organisms (mammals and particularly humans) and serious healthy problems.

Experimental part

Mushroom samples were collected in different areas of Bohemia (Pardubice, České Budějovice, Mostecko) and Moravia (Výsočina). Species of *Xerocomus*, *Suillus*, *Lecinium*, *Cantharellus*, *Agaricus*, *Macrolepiota*, *Armillariella*, *Calocybe*, *Russula*, *Lactarius*, *Lycoperdon* were content in the collection of edible mushrooms. Fresh mushrooms were frozen before laboratory analysis. Each sample was dried at 80 °C for 10 h then it was dried at 105 °C to the constant mass. Samples were dissolved in mixture of 2 cm³ concentrated HNO₃ (suprapur, Merck) and 50 cm³ deionized water and heated on 70 °C for 1 h. After cooling, 2 cm³ concentrated HNO₃ (suprapur, Merck) and 1 cm³ H₂O₂ were added again and heated on 60 °C for 30 min. Dissolving samples were filtrated and deionized water added to the volume of 50 cm³. Blank samples were treated in the same way. The ICP spectrometer IRIS AP (Thermo Jarrell Ash, USA) was used for Determination of metals. The concentration of Hg was determined by hydride technique.

Results and discussion

The ability to accumulate metals depends on many factors (especially the distance of the source of environmental pollution, species of mushrooms). The ability of mushroom to accumulate heavy metals was confirmed in relation to indication polluted and unpolluted area. The amounts of heavy metal contents are related to species of mushroom, the collected site of the sample. Species capable accumulate risk metals enourmously were determined. The genera *Agaricus*, *Russula* seem to be highly accumulating of cadmium, mainly species wild growing on generally polluted locality. Heavily accumulating species are *Calocybe gambosa*, *Lepista nuda* and *Agaricus arvensis* for mercury. High levels are characteristic for genera *Agaricus*, *Macrolepiota*, *Boletus*. Extremely high lead levels of mercury were observed in species *Russula* and *Macrolepiota* in the highly polluted locality (Mostecko). The genera *Lycoperdon perlatum*, *Macrolepiota rhacodes* and *Lepista nuda* are highly accumulating lead species. High accumulation of arsenic was designated in genera *Russula* and *Macrolepiota*, and higher amount of arsenic was detected in genus *Russula* from the polluted locality. Metal concentrations of wild-grown edible mushroom species were determined in different morphological parts of fruit-bodies. The highest concentration of Zn, Pb, Cu, Cd were found in caps and metals Cr, Fe, Mn, Ni were accumulated in stipes of genera *Xerocomus subtomentosus* and *Xerocomus chrysenteron*. But higher concentration of Cu, Cd, Mn, Ni were obtained from caps and lower concentration of Cr, Fe, V, Pb, Zn were obtained from stipes of *Russula aeruginosa*. Obtained results were compared with maximal permissible concentration of metals in mushrooms in Czech Republic stated by law. Exceeded limits were found in the case of Cd, Pb, Zn, Ni, Fe, Cu and As, Hg. The concentrations of Cd, Pb and Zn were exceed several times in every localities.

Conclusion

This work confirmed high level of toxic metal in edible mushrooms and identified localities of pollution of selected

areas of the Czech Republic. The fact that toxic metals are present in high concentrations in fruiting bodies of edible mushrooms of Czech Republic and exceeded limits of metals is serious and important in regard with the environmental and diet problems.

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P33 CHARACTERISATION OF HUMIC SUBSTANCES EXTRACTED FROM LIGNITE

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Introduction

Soil organic matter is a critical factor in most soil types and agroecosystems, for sustainable and productive land management. This could be to reflect not only soil carbon content but also soil moisture retention, nutrient availability and resilience to erosion. Generally, it is the substrate for most soil biological activity. Slovak agricultural soils are generally poor on humus content. Lignite is one of the alternative sources of organic carbon and humic substances which can be applied by different ways to soils. The main objective of this work was to determine the content and forms of extractable carbon substances from lignite. Humic substances were extracted from lignite by different extractants from light to strong.

Results and discussion

Twelve samples has been taken from main lignite layer in distance every 50 m. The mine is located at West Slovakia. The lignite samples were analysed for determination of basic parameters characterising forms of organic carbon and sorption properties. Total organic carbon (C_t) was determined by wet combustion method, average value 37.7±3.5 % (with ash) and calculated content C_t (ash free) was 58.6±4.1 %. A major criterion for the quality of organic matter is its C/N

ratio. Content of total nitrogen (N_t) was 0.32 ± 0.03 % and calculated ratio $C_t:N_t = 122.8 \pm 29.9$. Similar results were published by Fagbenro and Agboola¹, but in lignite samples with very acidic in reaction with a pH of 2.0, and over 90 % content of organic matter with more than 78 % extractable humic substances. In our case study, reaction of lignite samples were higher with mean pH values from 5.14 to 5.52 (in H_2O vs. KCl solution). From sorption properties point of view very important informations were obtained about real sorption parameters in lignite samples with specific size distribution of coarser and lighter particles ($MWD = 0.6843$ mm, calculated mean weight diameter).

The values of cation exchange capacity were determined high (622.7 ± 16.4 mmol kg^{-1}) with low values of exchangeable acidity (0.83 ± 0.15 mmol kg^{-1}) and high of hydrolytic acidity (154.8 ± 38.9 mmol kg^{-1}). The sum of basic cations (469.1 ± 16.4 mmol kg^{-1}) was high and base saturation of lignite samples was on medium level (75.40 ± 5.07 %). The significant positive linear correlation was determined between pH values and hydrolytic acidity and base saturation. All parameters excluding high ash content and its high variability we can assess positively and lignite samples like potentially good soil conditioner. Very important information from environmental point of view are water soluble forms of organic carbon in soils. The hot water extractable carbon has proved to be an appropriate criterion for the characterisation of the decomposable carbon.

This is the reason why we determined water dissolved organic carbon content at $20^\circ C$ ($DOC_{20} = 175.0 \pm 22.2$ mg C kg^{-1}) which was lower 2.5 times than hot water soluble carbon ($C_{hws} = 451.0 \pm 74.4$ mg C kg^{-1}), this forms of humic substances, probably fulvic acids are very mobile in soil profile. Klucakova, Pekar² studied solubility and dissociation of lignitic humic acids in water suspension and their results suggest that dissolving of solid humic acids in water environment is more complex than conventional solubility behaviour of sparingly soluble solids. The effects of alkali concentration and extraction time on chemical composition of humic acids extracted from lignite were studied by Platonov et al.³. We decided to compare levels of extraction humic substances in alkali solutions as 0.1 mol dm^{-3} NaOH and 0.1 mol dm^{-3} NaOH + 0.1 mol dm^{-3} $Na_4P_2O_7$ (mixture used in method Kononova-Belchikova for extraction and fractionation of soil humic substances). The positive linear correlation was determined between quantities of extracted organic carbon by used alkali extractants, but only about 31% of carbon from total carbon content (31.03 ± 4.14 % vs. 31.16 ± 4.09 % in NaOH) was extracted as humic substances. Generally, we have experience and same results were published, also in many research works, where the mixture of mixture NaOH and $Na_4P_2O_7$ extracted from soil sample more humic substances than NaOH solution with the same concentration. There was no significant linear correlation between level of extracted humic substances and ash content in lignite samples. Higher content of humic acids (25.7 ± 3.9 %) and lower of fulvic acids (5.5 ± 2.3 % of total organic C) were extracted by NaOH

solution from lignite samples. We expected higher level extraction of humic acids by the mixture of NaOH + $Na_4P_2O_7$, where the complexation influence of $Na_4P_2O_7$ would be increases solubility of humic acids binding strongly with basic cations. The quality of humic substances is often noted as the ratio of humic and fulvic acids carbon. Higher values of this parameter were determined in NaOH extract (5.7 ± 2.9) what is more positive than lower values characterizing composition of humic substances where the mixtures extractant were used (HA:FA = 3.9 ± 2.4). All parameters characterising forms of organic carbon, including fractional composition of organic material, and sorption properties influenced by specific size distribution, excluding the high ash content and its high variability, we can assess positively and to advise this lignite samples like potentially good soil conditioner.

Conclusion

From this short paper, it would be appear that there is now sufficient experimental evidence, about organic carbon forms and basic chemical properties of tested lignite samples, available to predict positive effects of applying lignite to disturbed and derelict land. The high adsorption capacity and favourable content of extracted humic acids give assumes of positive influence in optimal rate of lignite to agricultural soil reclamation and for managing organic carbon level in arable soils.

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P34 CONTENTS OF HEAVY METALS EXTRACTED FROM LIGNITE

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Introduction

The Slovak Republic has minor coal resources of relatively low quality. Recoverable reserves of subbituminous coal and lignite are estimated at 251 million tons, which are high in sulfur and ash. Humates and humic acid derivatives are a diverse family of products, generally obtained from various forms of oxidized coal. Coal-derived humus is essentially the same as humus extracts from soil, but there has been a reluctance in some circles to accept it as a worthwhile soil additive. In part, this stems from a belief

Table I
Statistical values of microelement contents extracted in 2 mol dm⁻³ HNO₃ (A) and HF+HClO₄ (B)

Microelement		Mean [mg kg ⁻¹]	Maximum	Minimum	Median	Sample standard deviation	Population standard deviation
Cu	A	18.2	21.3	12.1	19.1	2.55	2.45
	B	29.5	38.8	20.8	29.9	4.71	4.51
Zn	A	24.0	32.2	14.9	24.6	5.68	5.44
	B	30.9	38.8	20.4	33.9	5.94	5.68
Mn	A	160.3	197.0	139.9	159.6	18.4	17.6
	B	186.6	261.4	152.3	186.6	29.0	27.8
Co	A	8.27	11.0	5.66	8.36	1.77	1.69
	B	14.0	17.1	10.4	14.3	2.19	2.10
Fe	A	4597	6545	3498	4178	1042	998
	B	10307	13106	7916	10226	1418	1358

that only humus derived from recently decayed organic matter is beneficial. It is also true that the production and recycling of organic matter in the soil cannot be replaced by coal-derived humus. Many studies have shown positive effects of humates, while other studies have shown no such effects. Generally, the consensus is that they work well in low organic matter soils. In low amounts they do not produce positive results on soils high in organic matter. At high rates they may tie up soil nutrients. One of problems to keep safe and sustainable agriculture is heavy metal pollution on agricultural land¹. Fundamentally, income of heavy metals to agricultural land should be prevented. Possibilities of removing pollution by using of accumulator plants and developing technologies which preventing translocation of heavy metals to edible parts in plants are intensively studying from a plant nutritional point of view, long time. The large amounts of organic matter that are required to restore soil fertility are more easily applied in plant residues or organic manures, but these sources are limited. Lignite is one from alternative sources of organic carbon which can be applied by different ways to soils, like soil conditioner, but with variable contents of ash and heavy metals. Substances and heavy metals exhibit different binding forms in soil, which depend on how heavily a substance is bound to the soil and how readily can be taken up by the plants, whether it is available for soil organisms or can be relocated with soil leachate. In this context soil-related characteristics and processes (above all pH value, soil texture, humus content, redox conditions) play an essential part in addition to substance-related properties². When we want evaluate the substances in soils it is necessary for heavy metals to choose the extraction method appropriate to the proposed aim. It will be possible by using specific extraction methods for determination of different binding forms in which a metal is present in soil. For soil protection it is appropriate the efforts needed to carry out a sequential extraction. It is rather necessary to determine the total contents or a similar fraction

as parameter of the total presumable hazardous potential. By describing the potentially mobilizable substance contents it indicates the ecological-relevant fractions of metal contents in soils. The mobile fraction of heavy metals includes the proportion soluble in water, the fraction being easily exchangeable due to unspecific adsorption, and the readily soluble metal-organic complexes.

Results and discussion

Twelve lignite samples has been taken from main lignite layer in distance every 50 m, in mine Baňa Záhorie, located at West Slovakia. There were analysed for determination of basic parameters characterising organic carbon and nitrogen, content of ash and selected heavy metals like Cu, Zn, Mn, Co and Fe. The total organic carbon (C_t) was determined by wet combustion method with average value 37.7±3.5 % and content of total nitrogen (N_t) was 0.32±0.03 %. From total contents of organic carbon and nitrogen we calculated ratio C_t:N_t=122.8±29.9. Ash content in samples of lignite were varied about approximately value 34.0±5.4 %. The total content of elements were determined in HF+HClO₄ mixture and available forms in 2 mol dm⁻³ HNO₃. The highest total and available iron content and the smallest cobalt content in both forms were determined in analysed samples of lignite (Table I). Very similar low total content of microelements Cu and Zn were determined, with higher level of available zinc content (77.7 %) than copper (61.7 %) in comparison to total contents. The total manganese content varied from 152.3 to 186.6 mg kg⁻¹ and available forms in range 140–160 mg kg⁻¹. Statistical evaluation obtained results shown little differences between calculated mean and median values characterising available and total contents of elements – Cu, Zn, Mn, Co and Fe. The highest statistically significant value of linear correlation coefficient was calculated between available and total contents for Zn, Cu, Fe and Mn. The relationship between total and available content cobalt had no significant correlation. We know that the main factors which

influence solubility, and therefore environmental mobility are pH, cation exchange capacity, the organic matter and the water and thermal regime of the soil. We obtained complex of interesting informations about organic carbon forms and others above mentioned elements which enable us positively evaluate lignite from this layer as good soil conditioner.

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