2. ENVIRONMENTAL CHEMISTRY & TECHNOLOGY

2.1. Lectures

L01 TOXICITY OF CHLORINATED DISINFECTANTS AND THEIR PRODUCTS IN AQUATIC ENVIRONMENT

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Introduction

In spite of known problems with ecotoxicity of products, the use of chlorine and some chlorinated disinfectants belongs to fundamental method of natural waters treatment at both large-capacity technologies for drinking water supply and individual treatment at exceptional emergency situations including wartime. It is obvious that to the basic requirements on such disinfectants belongs high antimicrobial effectiveness beside lowest possible toxicity of these materials and their products in aquatic environment for humans. The choice of disinfectants to be used for natural water treatment is therefore very limited from wider spectrum of chemicals routinely used for medical disinfection and at emergency situations in foci of extent epidemics in peacetime or after use of biological weapons as well as at decontamination of toxic chemicals in peacetime or after use of chemical weapons. Toxicity and ecotoxicity of disinfectants and of their degradation products is the fundamental property, either determining possibility of their use or disqualifying them for it. It is necessary to take into account that chlorine and its products enter basic environmental compartments due to imissions or as waste in some industries (textile, pulp and paper, wood, chemical industry) routinely using chlorine and its derivatives. Many of these chlorinated products are identical with those occurring at water treatment technologies (e. g. chlorinated phenols).

This work is devoted to the toxicological assessment of selected chlorinated disinfectants and products of their degradation in aquatic environment at water treatment technologies or occurring in waters contaminated by various technological activities or waste waters.

Experimental

Chemicals

Calcium hypochlorite – MTIP Brno
Sodium benzenesulphochloroamide (Chloramin B) – Bochemie Bohumín
Sodium toluenesulphochloroamide (Chloramin T) – Bochemie Bohumín
Trichloroisocyanuric acid – Fluka
Sodium dichloroisocyanurate – Bochemie Bohumín
Chloroform – Merck
2-monochlorophenol (2-MCP)
3-monochlorophenol (3-MCP)
4-monochlorophenol (4-MCP)
2,3-dichlorophenol (2,3-DCP)
2,4-dichlorophenol (2,4-DCP)
2,5-dichlorophenol (2,5-DCP)
2,6-dichlorophenol (2,6-DCP)
2,3,5-trichlorophenol (2,3,5-TCP)
2,4,6-trichlorophenol (2,4,6-TCP) – all these chlorophenols – Riedel de Haen
2,3,4,5,6-pentachlorophenol (PCP) – Sigma Aldrich
Monochloroacetic acid (MCA) – Xenon
Dichloroacetic acid (DCA) – Merck
Trichloroacetic acid (TCA) – Cambrian Chemicals
Luminescent bacteria *Vibrio fischeri NRLLB-11177* lyophilised – Merck
Reconstitution solution NaCl – Merck
Dimethylsulphoxide (DMSO) – Merck

Devices

Luminometer ToxAlert^{R} 10 Merck
Thermostate
Ultrasound bath
Chronometer

Method

For the determination of IC_{50} (i. e. 50 % inhibition of bacterial luminescence): Bacterial suspension is prepared from lyofilised bacteria (stored at –20 to –18 °C in test tubes) after adding 1 ml of reconstitution solution. Revitalisation of bacteria proceeds for 20 min in thermostated bath at 15 °C. For the determination of acute toxicity (expressed as IC_{50}) the mixture of bacterial suspension with revitalization solution is poured into the measuring cuvette from which is taken 0,5 ml into control cuvette. Into the measuring cuvette is given per 0.5 ml of working solution containing ascending concentration of tested chemical. After 15 (or 30) minutes incubation (at 15 °C) the luminescence is measured against control (containing under the same conditions 0.5 ml of distilled water). According to the device programme, per cent of inhibition is directly displayed. On the curve of log C against I %, it is necessary to establish at least 5 points with the inhibition range 5–95 %. Obtained experimental results are treated statistically using probit-logarithmic method in graphic or better numerical programme (MS-Excel) to determine the IC_{50}. In cases of limited solubility it is necessary to use either ultrasound bath or to add (to sample and control) somezsolvent minimally denaturing luminescent bacteria. Optimal results were achieved with DMSO (in the concentrations up to max 2 % v/v). The IC_{50} values are given in Tables I and II.

Results and discussion

At the tested chlorinated compounds following values of IC_{50} (in mg l^{-1}) were determined (Tables I and II).

Table I

Chlorinated disinfectants	IC_{50} [mg l^{-1}]
Trichloroisocyanuric acid	0.39
Sodium dichloroisocanurate	0.91
Calcium hypochlorite	3.10
Chloramin B	18.2
Chloramin T	63.5

Table II

Products of chlorination and analogical chlorinated pollutants	IC_{50} [mg l^{-1}]
MCA	1.38
PCP	1.56
DCA	2.30
2,3,5-TCP	2.36
2,4-DCP	15.30
2,4,6-TCP	30.3
2,5-DCP	33.2
Chloroform	38.0
4-MCP	39.2
2,3-DCP	42.4
2-MCP	45.3
3-MCP	113
2,6-DCP	145
TCA	429

Comparison of determined values shows that chlorinated disinfectants are generally more toxic for luminescent bacteria. This is valid mainly for the chlorinated derivatives of isocyanuric acid that are base for modern disinfectants. This is in agreement with requirements on bactericidal efficiency of disinfectants. Detailed analysis of toxicity in man and higher organisms goes beyond extent of this paper but it is possible to note that we were worldwide the first[1,2] to introduce the mean for individual preparing drinking water under emergency situations for the Czechoslovak armed forces and Civil Protection DIKACID® based on sodium dichloroisocyanurate. This means is substantially better than then worldwide used halazon (contained in Czechoslovakia in tablets depicted as PANTOCID® used till the 1970s) and in the USA used tetraglycine hydroperoxyiodide in all critical parameters (i. e. bactericidal efficiency in *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, acute, subacute and subchronical toxicity of disinfectant and degradation products, solubility, stability in substance and in solution, mechanical properties etc.)[3,4] .

The sequence of inhibition effectiveness generally increases with number of chlorine atoms, so that PCP is the most toxic. Among trichlorophenols, 2,3,5-TCP was found as the most toxic, among dichlorophenols this was 2,4-DCP, among monochlorophenols, 4-MCP was found as the most effective inhibitor. It can be also assumed that substitution in vicinity of hydroxyl leads to lowest toxicities due to shielding hydroxyl group[5]. In the chlorinated acetic acids is the sequence quite reverse. Most toxic is MCA, a little bit toxic is DCA and a dramatic drop of toxicity occurs at trichloroacetic acid.

Confronting the results achieved at chlorophenols with literature data on their ecotoxicity gives a slightly different descendent sequence of toxicity[5,6,7,8,9]:

Bacillus sp.:
2,3,5-TCP > 2,4-DCP > 2,5-DCP > 2,3-DCP > 4-MCP > 3-MCP > 2,6-DCP > 2-MCP
Fungi (16 strains):
2,4-DCP > 2,5-DCP > 2,3-DCP > 2,3,5-TCP > 2,6-DCP > 4-MCP > 3-MCP
Daphnia magna:
2,3,5-TCP > 2,4-DCP > 2,3-DCP > 2,4,6-TCP > 4-MCP > 2,6-DCP > 3-MCP > 2 MCP
Lebistes reticulatus:
2,4,6-TCP > 2,4-DCP > 2,6-DCP > 4-MCP > 2,5-DCP > 2-MCP > 2,3-DCP > 3-MCP

For warm-blooded (rat, man), PCP is generaly the most toxic (p. o. man 29 mg/kg)[10].

Considering toxicity data of other degradation products of chlorination, i. e. chloroform and chloroacetic acids in various aquatic organisms (fish and crustaceans) leads to analogical sequence like in bacterial luminiscence. On the other hand, chloroform is more toxic than DCA while toxicities of DCA and TCA are of the same order for rat and man (p. o.) [11].

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L02 UTILIZATION OF OZONE FOR TREATMENT OF WASTEWATERS CONTAINING BIOLOGICALLY RESISTANT ORGANIC POLUTANTS

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Introduction

Many industrial wastewaters as well as landfill leachate contain high amounts of biologically resistant organics. Ozone oxidation represents one of the alternatives for the removal of these compounds. Combinations of ozonation with other agents, e. g. H_2O_2, the Fenton reagents and UV radiation are also investigated and applied. Combinations of biological and chemical oxidation treatment processes are usually applied in order to fulfil effluent standards[1].

The chemical oxidation processes including ozonation can either lead to the total degradation of chemical compounds to carbon dioxide or to the transformation of organics to more polar molecules. Partially oxidized organics are sometimes more susceptible to subsequent biological oxidation. This is an advantage particularly when biodegradable organics are necessary for biological denitrification and/or enhanced phosphorus removal processes.

The paper is focused on the kinetic studies of removal, transformation and biodegradability enhancement of 1,3-benzothiazole-2-thiol (2-MBT) contained in synthetic wastewater by ozonation.

Experimental

The experimental apparatus is shown in Fig. 1. The ozonation glass columns have a diameter of 0.04 meter and height of 1.7 m. Ozone was produced from oxygen by an ozone generator with maximum production of 625 mg $h^{-1}$of ozone. The mixture of oxygen and ozone was injected at the bottom through porous air diffusers with a constant flow rate of 40 l h^{-1}.

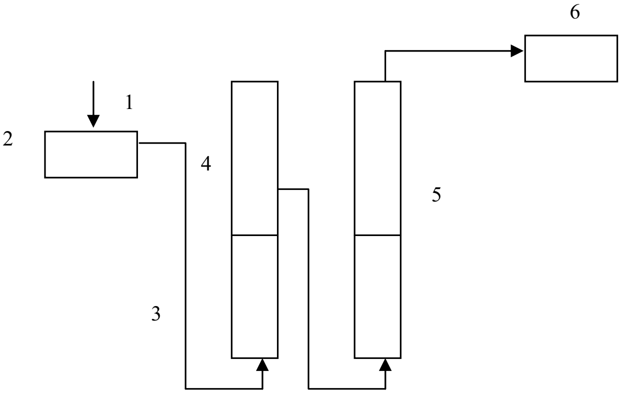

Fig. 1. **Schematic of experimental apparatus: 1 – feed of oxygen, 2 – ozone generator, 3 – feed of ozone, 4 – ozonation column with synthetic wastewater, 5 – ozonation column with solution of KI, 6 – destruction of residual ozone**

Ozonation column was filled by 1 litre of wastewater. The experiments were carried out with the synthetic wastewater contained 1 g l^{-1} of 2-MBT. The system was operated in batch mode.

The efficiency of transferred ozone was determined in liquid phase by an iodometric method[2]. The amount of transferred ozone in clean water corresponded to about 50 % of the generated one. Measurements of COD (chemical oxygen demand) and BOD_5 (biochemical oxygen demand) content were carried out according to standard methods[2]. Respirometric measurements[3] were carried out in order to evaluate an effluence of ozonation products on activated sludge activity.

Selected volumes of hydroperoxide and appropriated amounts of $FeSO_4 . 7H_2O$ were added into ozonation column at the beginning of trials. We applied the value of the H_2O_2/COD ratio[4] 0.45 g g^{-1} and the value of the Fe^{2+}/H_2O_2 ratio[5] 0.047 g g^{-1}.

Results and discussion

We have carried out two sets of measurements. The aim of the measurements performed in the first set was to investigate the kinetics and efficiency of COD removal by ozonation process and ozonation process in combination with H_2O_2 and the Fenton reagents.

COD removal efficiency about 75 % was achieved after 6 hours of ozonation without presence of any other reagent. Hydroperoxide was added to the treated synthetic wastewater sample in the next trial. The aim was to investigate the influence of this compound on COD removal during such combined ozonation process. COD removal efficiency about 77 % was achieved after 6 hours of ozonation in the presence of H_2O_2. The next ozonation trial of the synthetic wastewater was carried out in the presence of the Fenton reagents (Fe^{2+}, H_2O_2). Similarly to the previous experiment COD removal efficiency about 77 % was reached after 6 hours running ozonation in the presence of the Fenton reagents. Time evolutions of COD data obtained during the first set of ozonation trials are summarized in Table I.

Experimental data were fitted by zero (1), the first (2) and the second (3) order reaction kinetic models:

$$\mathrm{COD_t} = \mathrm{COD_0} - k_0 \cdot t \qquad (1)$$

$$\mathrm{COD_t} = \mathrm{COD_0} \cdot \exp(-k_1 \cdot t) \qquad (2)$$

$$\mathrm{COD_t} = \mathrm{COD_0} / (1 + \mathrm{COD_0} \cdot k_2 \cdot t) \qquad (3)$$

COD_t – concentration of COD in wastewater in the time “*t*” [mg l^{-1}]

COD_0 – initial concentration of COD in wastewater [mg l^{-1}]

k_0, k_1, k_2 – the rate constants for kinetics of the zero, 1st and 2nd order [g m^{-3} h^{-1}, h^{-1}, g m^{-3} h^{-1}]

Parameter values of applied kinetic models were calculated by grid search optimization procedure. The residual sum of squares (S_r^2) between observed and predicted values by the model, divided by its number of degrees of freedom ν (the number of observations less the number of parameters estimated) was used as an objective function.

Table I
COD removal during ozonation of synthetic wastewater contained 2-MBT

Time of ozonation [h]	Ozonation COD [mg l^{-1}]	Ozonation with H_2O_2 COD [mg l^{-1}]	Ozonation with the Fenton reagent COD [mg l^{-1}]
0	2391.8	2571.1	2332.0
1	1554.9	1794.0	2033.1
2	1315.8	1674.4	1554.9
3	1196.2	1076.6	1076.6
4	777.7	837.5	837.5
6	598.4	598.4	538.6

According the statistical characteristics values the best descriptions of COD kinetic data measured during ozonation was obtained by utilization of the second order reaction kinetic model. The best fit of COD data measured during ozonation combined with hydrogen peroxide or the Fenton reagents were achieved by the first order reaction kinetic model.

As it has already been mentioned no significant differences of COD removal at different modes of ozonation (without and with presence of H_2O_2 or the Fenton reagents) were observed. Considering additional operational costs related to dosing of hydroperoxide or the Fenton reagents it can be concluded that the catalytic ozonation of 2-MBT is not efficient process.

The rate constants and the values of statistical parameter r_{yx}^2 obtained for ozonation performed in the absence of other any reagents are given in Table II.

Table II
Kinetic parameters and statistical characteristic values

n	k_n	[ ]	r_{yx}^2
0	360.5	g m^{-3} h^{-1}	0.7385
1	0.278	h^{-1}	0.8034
2	1.85	g m^{-3} h^{-1}	0.9745

Based on these preliminary results the measurements with longer ozonation time and ozone supply were performed. The goal of this experimental set was to study the kinetic of COD removal, the influence of partial oxidation on biodegradability of intermediates and their influence on activated sludge respiration activities.

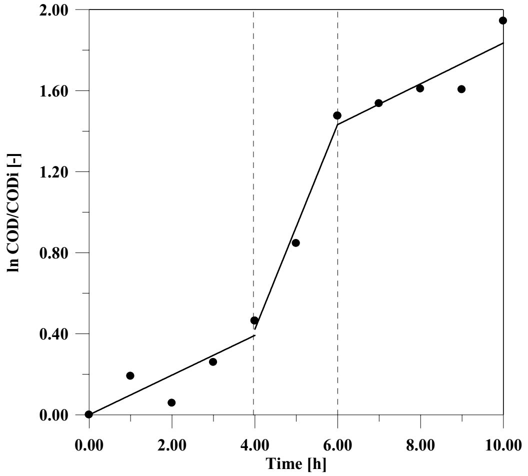

Fig. 2. **Kinetic curve for COD reduction during ozonation**

From the results of this trial it follows that ozonation enables to achieve the reduction in COD about 86 % at ozonation dose of about 5.5 g l^{-1} or 3 g of ozone per gram of removed COD.

The kinetic curve in Fig. 2. shows that the COD removal occurs according to the first-order reaction with respect to the COD with three stages in the oxidation. The first stage takes place within the first more or less two hours of ozonation. The second stage suffers a severe kinetic limitation probably due to lower reactivity of ozone on by-products formed in the first stage.

Table III shows the changes of BOD_5 values during ozonation of 2-MBT. The highest BOD_5 value was observed after 4 hours of controlled ozonation.

Fig. 3. shows the changes in COD and BOD_5 values as a function of the transferred ozone during the ozonation treatment. The COD reduction during this stage causes an increase of the BOD_5 concentration.

It can be seen from Fig. 3. that the maximum value of BOD_5 was achieved more or less at the end of the first stage. The maximum BOD value of about 380 mg l^{-1} was obtained at the ozone dose of 1.8 g l^{-1} and it was approximately constant within this stage.

Table III
Changes in the biodegradability of intermediate products of 2-MBT ozonation

Time of ozonation [h]	COD [mg l^{-1}]	BOD_5 [mg l^{-1}]	$(r_{X,t} - r_{X,e})/r_{X,e}$ [ ]
0	2092.9	0	0
2	1973.3	375.9	0.401
4	1315.8	380.1	0.630
6	478.8	380.1	0.883
8	419.6	251.5	0.103
10	299.5	179.7	0.044

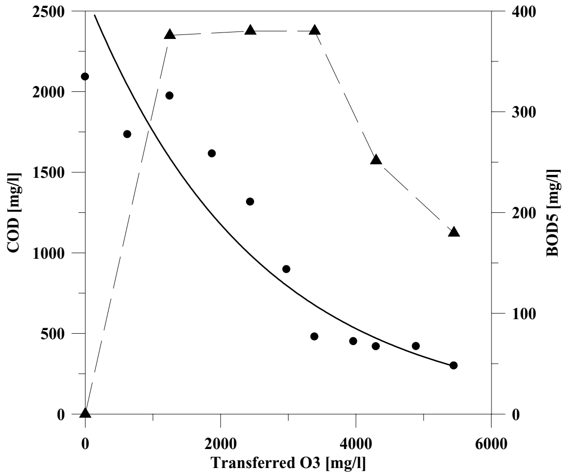

Fig. 3. **Changes od COD and BOD5 values as a function of transferred ozone**

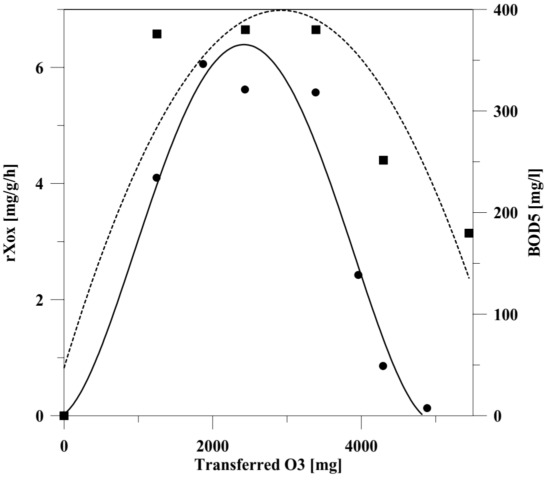

Fig. 4. **Evolution of r_{Xox} and BOD_5 values as a function of transferred ozone**

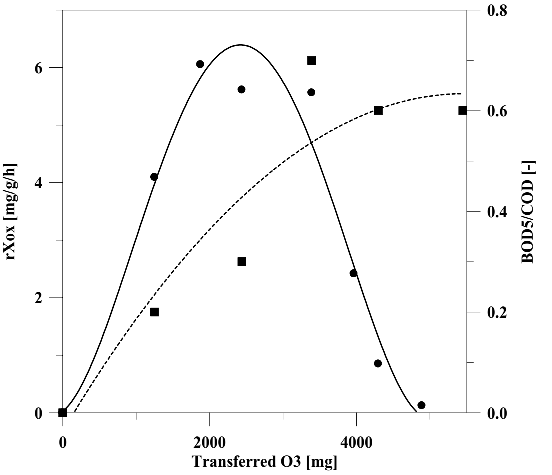

Fig. 5. **Evolution of r_{Xox} and BOD_5/COD values as a function of transferred ozone**

Respirometric measurements with activated sludge cultivated in lab-scale activated sludge model operated at 5 days of solid retention time were performed. The goal was to study the influence of intermediate products resulted from controlled ozonation of 2-MBT on the activated sludge respiration activity.

The values of the relative oxygen uptake rate are given in Table III. These values were calculated by subtracting of the experimental value of specific endogenous oxygen uptake rate $r_{\mathrm{X,e}}$ from the experimental value of total specific oxygen uptake rate $r_{\mathrm{X,t}}$ and dividing the remainder by the experimental value of specific endogenous oxygen uptake rate $\mathrm{r}_{\mathrm{X,e}}$. As it can be seen from Table III, the highest value of the relative oxygen uptake rate corresponds to more or less 6 hours of ozonation.

Fig. 4. displays good correlation also between the values of specific oxygen uptake rate r_{Xox} ($r_{\mathrm{X,t}} - r_{\mathrm{X,e}}$) and BOD_5 values observed during ozonation treatment as a function of the transferred ozone.

As it can be seen from Fig. 4. the maximal value of oxygen uptake rate approximately corresponds to the maximal BOD_5 value.

Fig. 5. shows the evolution of specific oxygen uptake rate (r_{Xox}) values and BOD_5/COD ratio values during ozonation treatment as a function of the transferred ozone. The value of BOD_5/COD ratio is slightly increasing also during the second and the third stage of oxidation (Fig. 5.). This can be explained by gradual decrease of COD value during ozonation.

Conclusions

Ozonation enables to achieve the reduction of 2-MBT in COD terms about 75 % at ozone dose of about 5.5 g l^{-1}. No significant differences of COD removal at different modes of ozonation (without and with presence of H_2O_2 or the Fenton reagents) were observed. Considering additional operational costs related to dosing of hydroperoxide or the Fenton reagents it can be concluded that the catalytic ozonation of 2-MBT is not efficient process.

The COD removal occurs according to the first-order reaction with respect to the COD with three stages in the oxidation. The first ozonation stage with the ozone applied dose of 1.8 g l^{-1} ensures the maximal increase of biodegradability. The maximum value of BOD_5 of about 380 mg l^{-1} was achieved at the end of the first stage. It was observed that the maximal value of OUR correlates with the maximal BOD_5 value.

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L03 APPLICATION OF SEMIPERMEABLE MEMBRANE DEVICES FOR SAMPLING OF POLYAROMATES FROM SOILS

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Introduction

Passive sampling of persistent organic pollutants in the environment becomes more and more popular. No wonder; passive sampling devices are small, simple to operate and inexpensive. No electricity is needed, because the transport of pollutants from the sampled matrix to the collecting medium is driven by the difference in chemical potentials of the compounds between these media. Uptake of pollutants takes place during whole exposition period; therefore there is no risk of missing some episodic emission events. The response of passive sampling devices is mostly time-weighted average. The whole exposition period (usually ranging from several days to several weeks) is covered by one passive sampler, thus one sample has to be analyzed to obtain information about the average levels of pollutants during the whole exposition period. Because these devices combine *in-situ* sampling, extraction and preconcentration, the laboratory treatment of exposed media is usually simple and straightforward and usually only little or even no solvents are required.

Quite large number of various passive devices has been described till now. Among them, SPMDs (Semipermeable Membrane Devices) developed by Huckins et al.[1] are probably most often used. These devices are layflat low-density polyethylene tubes, usually 84 cm of active length and 2.5 cm in width, polyethylene thickness 80 µm, filled with 1 ml (0.915 g) of triolein. They were originally designed as integrative concentrators of non-polar organics in aquatic environments, which mimic the uptake of organic compounds by living organisms; the polyethylene membrane simulates the biomembranes and triolein is the substitute of their lipid fraction[2]. Shortly after the discovery of their ability to effectively uptake organic contaminants from air they were successfully applied for the air sampling[3].

The total amount of pollutants in soil can be divided into two parts: the first is bioavailable part, which can be taken up or transformed by soil biota[4]. The second part is immobilized by bonding to soil organic matter. By standard analytical procedures employing mot often organic solvent extraction the total content of pollutants in soil is determined; this can lead to overestimation of toxicological effects. The bioavailable part can be evaluated either by mild extraction techniques[5] or by the analysis of soil biota[6]. In the second case, the degree of bioaccumulation can be characterized by the biota-to-soil accumulation factor BSAF:

$$\mathrm{BSAF} = C_\mathrm{b}/C_\mathrm{soil} \qquad (1)$$

where C_b is concentration in biota [µg/kg of lipid] and C_soil is concentration in soil corrected for the organic carbon content [µg/kg organic carbon].

In our study we tried to estimate the bioavailable part of polycyclic aromatic hydrocarbons in soils contaminated by polycyclic aromatic hydrocarbons (PAHs) using the passive sampling by SPMDs. The amounts sequestered by SPMDs were compared with the levels found in soil biota (earthworms) and with their total soil concentration evaluated by organic solvent extraction.

Experimental

SPMDs were prepared following the procedure described in literature[7,8]. Briefly, the layflat polyethylene tubing (low-density polyethylene without additives, width 28 mm, thickness cca 75 µm, Cope Plastic, Inc., U.S.A.) was cut into 104 cm pieces, which were heat-sealed on one end. The pieces were purified by dialysis in hexane (48 hours, solvent exchange after 24 hours) to remove small amount of remaining monomer and other impurities. The pre-cleaned tubes were filled with 1 cm^3 of triolein (95 %, Sigma-Aldrich) and after squeezing out of the air and forming of the smooth lipid layer on the whole length of the tubing the other end was heat-sealed. SPMDs were then immediately closed in air-tight containers and stored in deep-freezer at –18 °C.

All sampling experiments were realised at 3 sampling places in the DEZA chemical plant, Valašské Meziříčí, Czech Republic, and in one place its close vicinity. These places differ substantially in the degree of contamination by PAHs.

For exposition in soil, the SPMD arranged in U-shape was placed between two pieces of fine stainless-steel mesh screen (60 × 15 cm, mesh 2 mm). This setup was graved to soil in depth of 20–25 cm. On each sampling place two SPMDs were exposed in parallel. The samples of soil and samples of soil biota (earthworms) were collected simultaneously. After exposition period SPMDs were harvested, closed in air-tight containers and transported to laboratory on ice and stored in deep-freezer until analysed.

Before dialysis the SPMDs were carefully opened on one end and recovery standards were added to the lipid. After re-sealing the sequestered pollutants were released by dialysis in n-hexane for 48 hours with one solvent exchange after 24 hours, using 125 cm^3 of the solvent each time. The dialysate was concentrated on rotary evaporator and nitrogen blown-down. Small amount of residual lipid was removed by GPC on Bio-Beads S-X3 (200–400 mesh, Bio-Rad Laboratories) in a stainless steel column 8 × 500 mm (Tessek Ltd.) using chloroform as a mobile phase at a flow-rate of 0.6 cm^3 min^{-1}. The fraction containing low-molecular lipo-philic organic compounds including PAHs was collected and further cleaned-up by column chromatography on activated silica (Kieselgel 60, Merck) using stepwise elution by n-hexane (non-polar compounds) and n-hexane-dichloromethane 1 : 1 (polyaromates). The second fraction was solvent exchanged to acetonitrile and 16 US EPA Priority Pollutant PAHs were determined by reversed-phase liquid chromatography with water-acetonitrile binary gradient using programmed fluorescence and UV detection.

Earthworms were after collecting cleaned and starved. Before analysis the sample was homogenized with equal amount of Na_2SO_4. Then it was mixed with se Spe-ed^{TM} Matrix-38 and after adddition of intenal standards the mixture was extracted using pressurized solvent extraction on One-PSE extractor (Applied Separations, USA) using mixture acetone-dichlormethane (1 :1, v : v) at 150 °C and 150 bars in two 15 minute cycles. Extracts were nitrogen blown-down and then cleaned-up by GPC and CC and analyzed by HPLC as described above.

The humidity of soil samples was established by drying the sample at 150 °C to constant weight, organic carbon content was determined after heating of the dried soil sample to 400 °C for 3 hours. The extraction of PAHs from soils was realized after drying at laboratory temperature, homogenization and sieving. Pressurised solvent extraction on One-PSE extractor was applied. The extraction conditions and the clean-up procedure and HPLC analysis was the same as for dialysates, as described above.

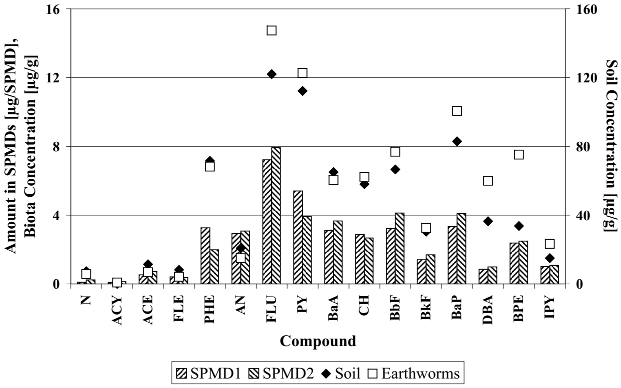

Fig. 1. **Amounts of PAHs sequestered by SPMDs [ng/SPMD] and their concentrations in soil and earthworms [both ng/g] on sampling place 1. Abbreviations:**
N – naphthalene; ACY – acenaphthylene; ACE – acenaphthe-ne; FLE – fluorene; PHE – phenanthrene; AN – anthracene; FLU – fluoranthene; PY – pyrene; BaA – benzo[*a*]anthracene; CH – chrysene; BbF – benzo[*b*]fluoranthene; BkF – benzo[*k*]fluoranthene; BaP – benzo[*a*]pyrene; DBA – dibenzo[*a,h*]anthracene; BPE – benzo[*ghi*]perylene; IPY – Indeno[*1,2,3-cd*]pyrene

Results and discussion

Fig. 1. shows the amounts of PAHs sequestered by 2 SPMDs exposed in parallel in soil at the most contaminated sampling place in the DEZA factory and also the concentrations of these compounds in soil and earthworms from this place.

It is quite surprising that even higher PAHs are effectively transported from soil to SPMDs. The results from other sampling places were quite similar, only the scales on Y-axes were different. The distribution of PAHs in SPMDs, earthworms and soil is quite similar; the only exception is the ratio of phenanthene/anthracene, which is close to unity at SPMDs, but it is around 4 in soil and in earthworms. This can be caused by the difference in uptake by SPMDs due to different shape of molecules.

Similarly to soil biota, SPMDs uptake only the bioavaliable part of soil contamination. To be able to compare the earthworms, which are in equilibrium state described by the *BSAF* value, and SPMDs sequestering compounds under conditions far form equilibrium, we proposed the value *N*:

$$N = UR_{SPMD}/C_{soil} \qquad (2)$$

where UR_{SPMD} is the uptake rate of SPMD [µg/(SPMD × × day)] and C_{soil} is concentration in soil corrected for the organic carbon content [µg/kg organic carbon]. The dependence of *BSAF* on the *N* for phenanthrene on all sampling places is in Fig. 2.

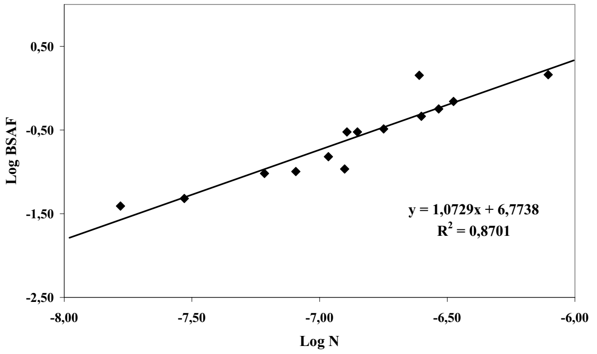

Fig. 2. **Dependence of the biota-soil accumulation factor BSAF on the *N* value for phenanthrene**

The tight correlation of the values *BSAF* for earthworms and *N* for SPMDs is evident. For other PAHs these correlations were quite similar. Therefore, it could be possible to estimate the value of biota-to soil accumulation factor on the base of the amounts of pollutants sequestered by SPMDs from soils and the organic carbon content.

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L04 FISH – USEFULL BIOINDICATORS IN ENVIRONMENTAL MONITORING

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Introduction

Fish is one of the animal species, which is most often used as an environmental bio-indicator to monitor a level of hydrosphere pollution. It is recommended that the monitoring of hydrosphere pollution should be as complex as possible which means that the levels of xenobiotics should be known not only in water but also in sediment and tissues of animals considered to be bio-indicators. Monitoring studies of this kind are sometimes extended to phytoplankton and zooplankton[1]. Fish are often screened for high-risk elements and/or also for various kinds of organic pollutants.

In their thorough monitoring studies aimed at marine fish the levels of high-risk elements in fish living in Finnish coastal waters, in the Baltic Sea and offshore waters of western Estonia were monitored[2,3,4]. According to the available literature fresh-water fish have not been extensively used as a bio-indicator to evaluate a level of contamination in the fresh-water ecosystem.

The Ministry of the Environment and the Ministry of Agriculture of the Czech Republic evaluates contamination of fish annually focussing particularly on determination of high-risk elements, polychlorinated biphenyls and organic chlorinated insecticides. The current levels of particular pollutants are not too high and comply with both national and European limits; an increased level of pollution was found only as a consequence of ecological accidents[5].

There are approximately 100 PCB congeners detected in environmental samples, from which about 50 are toxicologically and/or environmentally important. The maximum residual limit in particular commodities is given as the sum of the seven indicator congeners (PCB 28, 52, 101, 118, 132, 153, 180). In addition the congeners PCB 206 and 209 are also monitored.

The hydrosphere probably contains the substantial part of PCBs present in the environment. Due to their hydrophobic behaviour and the ability to be kept in sediments and/or biota they are detectable in all part of hydrosphere even in areas where the contamination could be kept at the background levels[6].

The load of sediments varies from several tenths of mg/kg up to tens of mg/kg depending upon a distance from contamination sources. Contents in water organisms are increasing with their position in the food chain. Fish are reflecting the state of the pollution very good because its ability to eliminate such contaminants is rather limited. Thanks to the stability of the PCBs and their lipophilicity they are preferably accumulated in the fat tissue. The majority of PCB congeners detected in fish tissues are penta- and/or hexachlorobiphenyls, especially the PCB congeners 118 and 153 (ref.[7]).

There have been two complex studies carried out in the Czech Republic dealing with the systematic monitoring of indicator congeners contents[8,9].

Materials and methods

The fish were taken during the years 2002–2004 from the Smradavka and Koryčany reservoirs in order to find out whether fish can serve as a bio-indicator in the fresh-water ecosystem. The fish were taken by properly licensed workers in compliance with the law relating to the protection of animals against maltreatment. The following species of fish were taken: pike, zander, eel, carp, bream, roach, tench, and silver salmon.

Chemicals

All chemicals used for analytical work were of Analytical Grade (Merck, Germany) except for HNO_3 (65%) which was additionally purified by sub-boiling distillation. H_2SO_4 (96%, Suprapur, Merck Germany) and HCl (37%, Suprapur, Merck Germany) were used. For dissolution and/or dilution of chemicals, fresh deionised water with specific resistance > 0.4 MΩ m was used. Standards of particular elements were prepared from commercial 1 000 mg dm^{-3} stock solutions (Analytika, Czech Republic). Standard reference materials DORM-2 and DOLT-2 (2Theta, CR) have been used in order to check the accuracy.

Anhydrous sodium sulphate, analytical grade, annealed in a muffle furnace at 650 °C for 4 h; Florisil 60/100 mesh, cleaned and annealed at 600 °C; aluminium oxide, chromatography grade, cleaned and annealed at 400 °C. Before use, sorbents were activated in a hot-air drier at 130 °C. Once activated sorbents can be stored in brown wide-neck bottles in a desiccator for 3 days; n-hexane, residue analysis grade; petroleum ether (boiling point 35 to 60 °C), pure, bi-distilled in a ground glass apparatus; diethyl ether, analytical grade, bi-distilled, peroxide-free, and dried over anhydrous sodium sulphate; acetone, residue analysis grade; sulphuric acid 96% (Suprapur, Merck); n-hexane, isooctane (SupraSolv; Merck), elution mixtures n-hexane : diethyl ether (94 : 6), n-hexane : acetone (94 : 6).

Standards and reference materials: PCB congener mixture in isooctane having the concentration of 10 μg of each congener per 1 cm^3 (Promochem, Germany); standard PCB congener mixture in n-hexane having the concentration of 100 μg of each congener per 1 cm^3 (J. T. Baker r); reference material of the Ehrenstorfer company (Germany), Promochem (Germany), or national reference materials of Czech hygienic services.

Pre-analytical and analytical procedure – determination of metals

Samples of fish tissues were wet digested using the mixture of nitric acid and sulphuric acid (2 : 1). The levels of lead, cadmium, copper and nickel were than determined using Graphite Furnace Atomic Absorption Spectrometry (GF-AAS), zinc was determined by Flame Atomic Absorption Spectrometry (FAAS) and arsenic by Hydride Generation Atomic Absorption Spectrometry (HG-AAS). The instrument AAA Solar with appropriate accessories (Thermoelemental, UK) was used within the study. Limits of determination were 2 μg cm^{-3} for each particular element. The accuracy of the analysis was verified using standard reference materials.

Pre-analytical and analytical procedure – determination of PCBs

The whole procedure for the determination of PCBs has been already described in[8].

Results and discussion

The results of metals determinations are summarized in Table I and Table II Average values, standard deviations and ranges are given. Table I provides average level of risk elements in muscles of fish. Seven fish species (pike, zander, eel, carp, bream, roach and tench) were monitored. The number of fish samples in each group was different ranging between 6 (zander, tench) and 28 (eel). It can be seen that the level of contamination in fish taken from reservoir Smraďavka is low

Table I
Content of heavy metals in muscle tissues of fish – reservoir Smraďavka (μg/kg fresh tissue)

Elements		Pike N = 13	Zander N = 6	Eel N = 28	Carp N = 16	Bream N = 12	Roach N = 22	Tench N = 6
As	mean	24.4	32.9	29.8	27.1	23.3	22.5	30.0
	range	19.8–27.6	29.8–37.8	24.5–37.2	23.2–29.8	18.2–29.5	15.3–29.6	27.9–32.3
	SD	2.35	2.94	3.40	1.92	3.94	4.02	1.62
Cd	mean	< 2	< 2	41.2	< 2	< 2	< 2	< 2
	range	n.e.	n.e.	20.0–70.0	n.e.	n.e.	n.e.	n.e.
	SD	n.e.	n.e.	17.1	n.e.	n.e.	n.e.	n.e.
Cu	mean	595	142	583	112	261	249	505
	range	533–658	127–158	463–712	86.0–126	215–302	196–287	482–528
	SD	37.9	12.5	65.2	12.9	26.0	21.6	16.2
Ni	mean	23.3	49.7	125	42.7	113	44.6	127
	range	17.5–34.5	39.0–66.0	89.0–184	25.3–38.3	85.0–158	28.0–70.0	104–142
	SD	3.93	10.1	23.0	14.1	22.7	11.3	14.7
Pb	mean	< 2	< 2	22.2	12.4	< 2	< 2	28.7
	range	n.e.	n.e.	12.0–32.0	10.4–19.6	n.e.	n.e.	24.0–33.0
	SD	n.e.	n.e.	5.59	2.25	n.e.	n.e.	3.50
Zn	mean	3264	5396	6960	7916	7886	2771	7889
	range	2335–4300	3597–6521	4968–12005	5998–10021	5987–10050	1998–4220	4295–12500
	SD	546	1098	1547	1229	1568	506	2780

N – number of analysed samples SD – standard deviation n.e. – not evaluated

Table II
Content of heavy metals in muscle tissues of fish – reservoir Koryčany (µg/kg fresh tissue)

Elements		Pike N = 7	Zander N = 8	Eel N = 46	Carp N = 13	Silver salmon N = 6
As	mean	10.5	18.6	16.5	19.9	9.55
	range	< 2–16.5	10.2–26.9	10.0–22.8	11.6–30.5	< 2–14.65
	SD	1.55	2.12	2.08	3.65	1.16
Cd	mean	< 2	< 2	< 2	< 2	< 2
	range	n.e.	n.e.	n.e.	n.e.	n.e.
	SD	n.e.	n.e.	n.e.	n.e.	n.e.
Cu	mean	142	217	212	245	168
	range	99–187	168–264	145–259	198–3056	104–226
	SD	9.65	14.9	7.53	21.8	19.4
Ni	mean	65.5	75.4	66.9	60.9	38.2
	range	26.5–89.4	41.7–112.3	39.8–99.8	29.5–100.2	20.5–77.6
	SD	11.6	14.8	9.98	12.4	13.2
Pb	mean	< 2	15.7	12.5	18.6	< 2
	range	n.e.	< 2–26.5	< 2–18.6	14.3–26.7	n.e.
	SD	n.e.	9.62	6.55	8.65	n.e.
Zn	mean	1265	3015	2689	2998	987
	range	867–1665	2258–3612	2485–2998	2789–3351	712–1165
	SD	80.7	102	25.9	56.5	412

N – number of analysed samples SD – standard deviation n.e. – not evaluated

and that the current limit for residues which is valid in the Czech Republic was not exceeded in any case. Table II shows the average levels of heavy metals in muscle tissue of fish taken from the Koryčany reservoir. Five fish species (pike, zander, eel, carp and silver salmon) were monitored. The number of fish in each group was different ranging between 6 (silver salmon) and 46 (eel). It follows from the values introduced in this table that the level of contamination in fish taken from reservoir Koryčany is very low and again the current limit for residues which is valid in the Czech Republic was not exceeded in any case.

Levels of the most often monitored risk elements such as cadmium and lead were even below the limit of determination in several cases. The lowest level of all risk elements was found in pike (predatory fish) while the highest levels were detected in tench, which lives predominantly near to the bottom. The measured levels of risk elements were compared with the data published by the State Veterinary Administration of the Czech Republic in 2001 (ref.[10]) and were significantly lower. However, it should be emphasized that the levels found in 2001 all over the Czech Republic were not high and that the hygienic limit was exceeded only once for arsenic (predatory fish – rainbow trout) and once for mercury (barbel).

The results of PCBs determinations are summarized in Table III and Table IV average values and ranges in muscles of fish are given.

It follows from the Table III and the Table IV, that the reservoir Smraďavka is more polluted than the reservoir Koryčany (compare e. g. the sums of congeners). It must be stated, that there is the enterprise Colorlak in this region where, as one of the row materials used in production of paints, Delor 106 was used. Differences resulting from the Table III and Table IV could be ascribed to the fact, that the distance of Koryčany reservoir from the contamination source is larger. In addition there have been not illegal dumps found in the vicinity of Koryčany reservoir which was the case in the vicinity of Smraďavka reservoir. The differences found were not too extensive nevertheless some additional amount samples should be taken and analyzed in order to make reliable statistical evaluation. Anyway the level of PCB congeners are higher than the background in the Czech Republic, where the sum of congeners ranges between 2–5 µg/kg of fresh tissue.

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Table III
Content of PCBs in muscle tissues of fish – reservoir Smraďavka (μg/kg fresh tissue)

PCB congener		Pike N = 5	Eel N = 9	Bream N = 7	Roach N = 5	Carp N = 9
28	mean	1.16	4.85	4.26	0.62	1.53
	range	0.36–1.82	0.88–13.25	0.42–7.35	0.35–1.05	0.78–3.03
52	mean	0.38	1.08	1.30	0.24	0.57
	range	0.23–0.86	0.33–3.25	0.10–3.86	0.08–0.52	0.22–0.78
101	mean	0.62	3.36	4.76	0.48	0.85
	range	0.18–0.98	0.60–9.56	0.21–7.98	0.26–0.82	0.79–0.83
118	mean	0.24	0.63	1.51	0.20	0.39
	range	0.11–0.51	0.37–1.12	0.19–3.68	0.10–0.48	0.33–0.92
138	mean	9.95	11.79	13.63	4.52	6.83
	range	2.48–19.10	5.70–23.14	2.72–24.11	1.04–9.85	4.56–9.88
153	mean	13.45	16.71	22.49	5.71	10.48
	range	3.27–24.66	8.26–31.40	3.96–44.65	1.43–12.52	6.36–14.99
180	mean	6.99	7.51	5.92	0.57	4.38
	range	1.38–13.59	2.93–17.65	1.12–15.72	0.15–2.52	2.43–8.28
∑ of congeners		32.79	45.93	53.87	12.34	25.03

N – number of analyzed samples

Table IV
Content of PCBs in muscle tissues of fish – reservoir Koryčany (μg/kg fresh tissue)

PCB congener		Pike N = 6	Eel N = 8	Bream N = 8	Roach N = 6	Carp N = 8
28	mean	0.71	3.09	3.15	0.36	1.02
	range	0.36–1.64	0.83–14.28	0.32–6.32	0.35–0.98	0.91–1.03
52	mean	0.21	0.83	1.06	0.14	0.37
	range	0.23–0.56	0.40–3.08	0.11–3.00	0.08–0.48	0.37–0.66
101	mean	0.39	2.94	3.85	0.29	0,62
	range	0.18–0.95	0.60–9.36	0.31–7.76	0.26–0.61	0.79–0.83
118	mean	0.10	0.49	0.67	0.10	0.12
	range	0.11–0.36	0.37–0.98	0.19–3.35	0.10–0.32	0.33–0.34
138	mean	9.02	10.95	12.85	3.94	5.04
	range	2.48–19.07	5.70–23.06	2.72–23.97	1.04–9.62	4.56–9.18
153	mean	12.88	15.78	21.33	4.11	9.63
	range	3.17–24.00	8.26–31.40	3.96–44.29	1.44–12.36	6.56–14.28
180	mean	5.98	6.89	4.93	0.16	2.76
	range	1.36–13.19	2.80–17.25	1.01–11.72	0.65–5.52	2.43–6.28
∑ of congeners		29.29	40.97	47.84	9.10	19.56

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L05 COMPARATION OF TOXIC EFFECTS OF RODENTICIDES FOR BIRDS

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Abstract

Pheasants were used to study and compare the toxic effects of rodenticidal substances (zinc phosphide and bromadiolone) for non-target organisms. A total of 20 artificially reared pheasants were used to study each rodenticidal product. The toxic effects were evaluated using pre- and post-exposure biochemical parameters as well as histopathology. Considering zinc phosphide, following changes were noted: lung oedema and hydropericardium due to respiratory failure and dystrophic changes in the liver, kidney and digestive tract. Biochemical parameters reflecting the above signs in surviving birds returned to normal within days. Bromadiolone caused no mortality in experimental birds. There were, however, differences in some biochemical parameters of control and experimental birds.

Introduction

Rodenticides have become important factors with direct and indirect effects on non-target organisms, agro-ecosystems and the whole environment due to their extensive use. Some products may be so toxic as to cause mortality in wild animals. Others may impair their health and enhance the effect of diseases, climatic conditions or predators.

Mammalian species, in particular, may be endangered by anticoagulant rodenticides of the second generation such as bromadiolone and difenacoum[1]. There are increasing numbers of reports of wildlife contamination and toxicosis after the use of second generation anticoagulants[2,3]. Results of the study to evaluate detrimental effects of anticoagulant rodenticides in non-target wild animals were presented. Predators such as foxes and buzzards were found to be exposed via contaminated prey[4].

On a large scale basis, two effective substances, i. e., zinc phosphide and bromadiolone, are used to control overcrowded common vole (Microtus arvalis) populations – the most important pest to agricultural crops in the Czech Republic. Zinc phosphide has also been involved in wildlife toxicity[5,6].

As gamekeepers report cases of intoxication of wild animals, it was our aim to evaluate the toxicity of the two products and compare the toxic effects of rodenticides for birds.

Material and methods

Pheasants (artificially kept *Phasianus colchicus* individuals) were used to study and compare the toxic effects of rodenticidal substances (zinc phosphide and bromadiolone) for non-target organisms. A total of 20 artificially reared pheasants were used to study each rodenticidal product. The toxic effects were evaluated using pre- and post-exposure biochemical parameters as well as histopathology. Avian dietary toxicity tests published in OECD Guidelines for testing of chemicals were employed for the study[7].

Results

Toxicity of the Stutox-I product (zinc phosphide as the effective substance) for the pheasant

The LD_{50} of the Stutox-I product containing 5 % of zinc phosphide was found to be represented by approximately one bait granule in pheasants (average body mass of females and males of 950 g and 1230 g, respectively) . Standard biochemical parameters in blood plasma sampled from individuals after acute intoxication indicated damage to the liver and kidney, cardiovascular system failure and absorption of phosphorus released from the zinc phosphide. Blood collected ante mortem could be characterised by significantly higher levels of ALP and AST as compared to levels prior to the toxin ingestion. The levels were rising from 5.54 to 23.64 and from 6.78 to 28.1 μkat l^{-1} in ALP and AST, respectively. The level of phosphorus in the blood also increased from 1.4 prior to the toxin ingestion to 3.82 mmol l^{-1} in blood collected ante mortem. Values of biochemical parameters in those birds that survived even the thrice of the LD_{50} returned to the normal of pre-exposure levels within a day from the ingestion. There was only one exception to this statement and it was that of impaired renal functions as evidenced by the increasing uric acid levels on day 0, 1, 2, 7, 13, 27 and 34 of intoxication amounting to 136, 147.8, 87, 139.2, 345.9, 374.8 and 644.4 μmol l^{-1}, respectively.

On autopsy of intoxicated individuals there were found green granules or green mash matter of specific odour (garlic or acetylene like) present in the cervical and thoracic esophagus, crop, proventriculus, muscular ventriculus and duodenum. Patho-anatomical findings consisted of hyperaemia of body cavity organs (liver and spleen), blood injected vessels of the gastrointestinal tract (proventriculus, duodenum), congested heart atria – right heart atrium due to circulatory failure, petechiae within the heart muscle, spleen and pancreas, lung hyperaemia and oedema, pronounced hydropericardium, and dystrophic to necrotic changes of the digestive tract mucosa.

Toxicity of the Lanirat Micro product (bromadiolone as the effective substance) for the pheasant

Biochemistry of blood plasma was evaluated in a total of 20 adult males of the pheasant. Mean body mass of pheasants amounted to 1136 g. A group of 10 pheasants served as a control receiving standard feed. Another group of 10 pheasants was for three days fed on the feed ration containing 75 % of the tested product (Lanirat Micro with 0.005 % of bromadiolone) and 25 % of standard feed. Every day each of the experimental pheasants was provided with and ingested a dose of 52.5 g of the Lanirat Micro product. Considering the toxic substance concentration in the product it is clear that during the three days of testing each pheasant ingested a total dose of 7.875 mg of bromadiolone. During the next days the pheasants were fed on the standard feed only. No signs of intoxication or changes in the behaviour of the pheasants were noted during the experiment and the following 14 days. Necropsy performed on pheasants euthanised after that period revealed no gross pathological changes except for some occasional haemorrhages within the breast muscles. Plasma biochemistry was evaluated from blood collected on day 15 after the last day of pheasants having access to the toxic granules. The tested group of pheasants could be characterised by significantly lower ($p < 0.05$) levels of glucose, creatinine and sodium as compared to the control and pre-exposure values. On the other hand, uric acid levels were significantly higher in the experimental group. Levels of total proteins, AST, ALP, ALT, potassium, calcium and phosphorus were not statistically different in the control and experimental group.

Discussion

There are differences in the mechanism of toxic effects of zinc phosphide and bromadiolone. The anticoagulant bromadiolone contained in the Lanirat Micro product acts through a competitive inhibition of vitamin K so as to prevent the production of blood clotting factors in the liver. Intoxicated animals then suffer from blood coagulation deficiency and death due to uncontrollable bleeding. Anticoagulation rodenticides such as bromadiolone are highly toxic for mammalian species. Mortality may be due to the dose of several milligrammes per one kilogramme of body mass. Birds, having somewhat different cascade of blood coagulation compared to mammals, are not so susceptible to intoxication by this group of poisons. It means that cases of bird intoxications by bromadiolone are not so often encountered and that use of these substances to control overcrowded rodent pests poses less risk for birds than mammals.

Following ingestion the effective substance of Stutox-I (i. e., Zn_3P_2) reacts with water and acids in the digestive tract to liberate phosphine gas which enters the vascular bed and blood. It is a cellular and tissue toxin causing direct irritation of the digestive tract, dystrophic changes in parenchymatous organs (liver, kidney) and circulatory and respiratory collapse. Death results from respiratory failure and lung oedema.

Zinc phosphide is a rodenticide of acute toxicity and one Stutox-I granule (containing 8.5 mg of zinc phosphide) represents approximately the LD_{50} for pheasants. It was found, however, that ingestion of one, two or three Stutox-I granules (i. e., up to the thrice the LD_{50}) did not necessarily result in mortality in pheasants. The intake of five or more Stutox-I granules resulted invariably in acute intoxication and death of these pheasants. There are known no data on long-term health problems caused by sub-lethal doses of zinc phosphide intake because this substance is considered only to be a rodenticide of acute toxicity. Because of large-scale use of this preparation for the control of the field vole population in the Czech Republic, possible long-term health effects in free-living bird populations have yet to be evaluated. It was found that the biochemical parameters found in the plasma of individuals obtaining up to the thrice the amount equal to the LD_{50} quickly returned (within a day) to normal values.

The presence of green matter of digested granules as far as the duodenum is contradicting the statement of the product producer saying that consumption of only one granule discourages the pheasant from eating any other ones because of their repellent effect thus limiting the possibility of intoxication.

Evaluating the mortality of test animals during toxicity tests by counting the number of the dying and surviving is only a simple way of studying the effects of a chemical substance. It is always necessary to perform autopsy and evaluate gross and microscopic pathological changes. Clinical biochemistry may be used to evaluate impaired functions of internal organs in living individuals. Toxic effects of zinc phosphide in pheasants are similar to those in other animals and include inflammatory to necrotic changes of gastrointestinal mucosae, hyperaemia and dystrophic changes of parenchymatous organs and death due to circulatory failure. Sub-lethaly intoxicated individuals have only to a lesser degree evident changes, which can also be manifested by disruption of clinical biochemistry values. The health status of such individuals, however, is compromised and their long-term survival or even reproductive success is yet to be evaluated. It is therefore necessary to take all the steps to prevent even sub-lethal intoxications in free living animals.

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L06 EVALUATION OF ENVIRONMENTAL HAZARDS OF RODENTICIDES

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Abstract

Rodenticides are an important group of xenobiotics intended to control rodent pests. On the other hand, they can pose a threat for non-target organisms thus deteriorating the environment. On a large scale basis, two effective substances, i. e., zinc phosphide and bromadiolone, are used to control overcrowded common vole (*Microtus arvalis*) populations – the most important pest to agricultural crops in the Czech Republic. It was the aim of the study to evaluate the hazards of use of rodenticidal baits for non target organisms. As methods we employed tests of the attractiveness of various rodenticidal bait products as well as acute avian toxicity tests. Results of these tests and environmental implications are presented.

Introduction

Rodenticides are an important group of xenobiotics intended to control rodent pests. On the other hand, they can pose a threat for non-target organisms such as birds. Various rodenticides may cause both primary and secondary intoxications in birds. Data on the toxicity of such preparations for birds are irreplaceable and cannot be substituted by tests performed using laboratory rodents because some substances are more toxic for birds than mammals and vice versa[1].

The common vole (*Microtus arvalis*) is naturally occurring in the agricultural landscape of the Czech Republic and its population overcrowding can result in great damage to agricultural plant growths[2]. Control of the common vole population is not possible without the use of effective rodenticidal preparations. These rodenticides are used as granular baits made of alfalfa, sugar, grouts, paraffin wax, specific effective substances and dyes. Though the producers declare their products to be relatively safe for non-target organisms, the granules may attract by their colour and shape some non--target organisms, especially birds, and thus endanger their populations.

It was therefore the aim of the study to evaluate hazards of use of rodenticidal baits for birds as non-target organisms. Results of these tests and environmental implications are presented.

Material and methods

The methods were based on guidelines for testing plant protection products in registration (i. e., BBA methods) published by the Federal Biological Agricultural and Forestry Institute Berlin and Braunschweig[3,4]. Tests on the attractiveness of bait granules and toxicity of rodenticides were performed using the pheasant (*Phasianus colchicus*) in the Game Bird Farm Jinačovice belonging to the Teaching Agricultural Plant of the Veterinary and Pharmaceutical University Brno.

Attractiveness of variously dyed granular baits used as carriers for the rodenticidal substances

Granules not treated by the effective substances in colours of red, green, blue and black but otherwise identical to those used as rodenticidal baits were tested. It was tested whether pheasants consume the dyed granules in equal quantities or prefer some coloration. Testing the attractiveness, variously dyed granular baits and normal feed in measured quantities were presented to individually kept pheasants. In all, 10 adult individuals (i. e., 5 males and 5 females) were used. The ratio of normal feed (i. e., the feeding mixture for pheasants) and tested granules of four colours was equal to 25 % : 75 %. Drinking water was supplied ad libitum.

Avian toxicity tests

A total number of 8 control (4 males and 4 females) and 16 test pheasants (8 males and 8 females) were used. The pheasants were kept individually within outside aviaries of 5 × 2.5 × 2.1 metres. The Stutox-I (zinc phosphide) and Lanirat Micro (bromadiolone) products were tested. The proper testing period was preceded by a week of acclimation of test animals to conditions of testing. During the exposure period we observed whether pheasants feed on the tested granules (of green and red colours, respectively). During this test and the following 14-day period we measured the quantity of feed taken, signs of intoxication and mortality. Autopsy examinations of dead animals that were intoxicated and animals after euthanasia following the 14-day post-exposure period were regularly performed.

Results

Tests on the attractiveness of variously dyed granules used as carriers for the rodenticidal substances

Following the seven-day exposure of pheasants to the tested granules of four colours it was proved that pheasants feed on such granules. In all, 28 % of granules presented were consumed after 2 hours and pheasants mostly fed on blue (41.2 %) and red (34.9 %) granules. Green granules were consumed in the amount of 13.8 % and the black ones were least fed on (13.2 %). After 24 hours, 67 % of tested dyed granules and 37 % of normal feed for pheasants were consumed. Following the seven testing days it may be concluded that the pheasants consumed the dyed granular baits with decreasing frequency from red (38.2 %), black (25.1 %), blue (18.5 %) to green (18.2 %) ones.

Avian toxicity tests with granular baits

The intoxication occurred in 10 (7 males and 3 females) out of 16 *Phasianus colchicus* individuals which had had for three days free access to effective Stutox-I granules (containing zinc phosphide). *Phasianus colchicus* individuals were willing to consume such quantities of rodenticidal baits which greatly exceeded the LD_{50} (full crop of granules). There were no cases of intoxication during the first day of application of Stutox-I granules because fresh paraffin wax coated granules were repellent for the birds until some degree of their surface disintegration and loss of repellent qualities took place.

No mortality was observed in pheasants allowed free access and feeding on red granular baits containing bromadiolone (the Lanirat Micro product).

Discussion

The colour of rodenticidal granules may influence the attractiveness of the product and increase or decrease the risk of primary intoxications of non-target organisms including bird species and game animals. It was found that the red and green granules were the most and least attractive ones, respectively. It may thus be concluded that the use of the red dye to distinguish the rodenticidal product increases the risk that such granules be ingested by non-target birds such as the pheasant. The red granules are nearly twice as much attractive for the pheasant as the green ones. Granules of the Stutox-I product containing zinc phosphide are of green colour. The effective substance of Lanirat Micro is bromadiolone and its granules are of red colour.

Negative aspects of large-scale use of anticoagulants in the agriculture can be summarised as persistence in the environment, accumulation in the organism and high toxicity for mammalian species. Pheasant individuals feeding on red granular baits with anticoagulation rodenticidal substances may thus contain within their bodies a dose sufficient to cause intoxication of a predator mammalian species even when the birds themselves are not yet influenced by the toxin. Contrary to this, the Stutox-I product containing Zn_3P_2 is highly toxic both for mammalian and bird species. Regarding environmental consequences of this product use, it is positive that the zinc phosphide quickly disintegrates and forms non-toxic components which may even be used by plants as nutrients. The relatively higher susceptibility of birds to the zinc phosphide may, nevertheless, result in primary intoxications of pheasants. Secondary intoxications are then less probable due to the mechanism of action requiring the whole rodent or avian cadaver including the entrails containing zinc phosphide to be consumed by the predator.

There remains a question concerning the concentration of the effective substance (5 %) in rodenticidal baits of Stutox-I to be discussed. The fact that one Stutox-I granule represents the LD_{50} for the pheasant is negative from the point of evaluating the hazards posed by this rodenticidal bait to free-living birds. According to the paper by Tkadlec[5] the zinc phosphide in rodenticidal baits is effective when used in the concentration from 2 to 5 %. It is therefore our duty to propose to decrease the rodenticidal bait concentration of zinc phosphide to 2 % in order to decrease the hazards posed by this product to non-target organisms and still preserve its field efficacy. As far as paraffin wax coated granules and the hazards of such products are concerned, we can conclude that the repellent effect was evident only for some time after the application of fresh granules and with the disintegration of the surface of granules the repellent qualities decreased and the pheasants were then willing to consume the granules in great quantities causing acute toxicity. It is, therefore, necessary to test for how long the paraffin wax coated granules remain repellent, when they loose the repellent effect but are still present in the environment and when they completely disintegrate. Another aspect, that has to be mentioned, is the probability that artificially reared pheasants used to feed on granules are at a greater risk than free-living wild birds.

Both products in the application form of granular baits present risk of game animal intoxication when not observing the proper conditions of use, in particular. It is under no circumstances acceptable that piles of granules be left in the fields and present attractive feed sources for game animals such as the pheasant and hare. It should be our endeavour to prevent intoxications of game animals by means of flushing or luring them away from treated agricultural fields.

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L07 EVALUATION OF PCB CONTAMINATION IN FRESH-WATER ECOSYSTEM IN THE PERIOD BETWEEN 1997 AND 2004

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Introduction

Some types of organic pollutants persist in the ecosystem for many years. The disastrous floods that distressed a large part of Moravia in 1997, a considerable part of contaminants may have returned from dumps into circulation and thus cause secondary contamination of commodities of the food chain. No information concerning similar problems was found in the available literature. Regarding the persistence of such compounds in the environment, it is possible to compare our data with findings of the same analytes in the water environment and other compartments of the ecosystem.

The assessment of the contamination of the aqueous environment is often based on findings in tissues of animals typical of hydrosphere. Thus mussels were used as bioindicators of contamination in the basin of the St. Lawrence river. The identified analytes included 7 OCPs, 11 chlorobenzenes, 2 chlorostyrenes, and 63 PCB congeners. An interesting pattern of distribution of PCB congeners was observed in tissues of mussels collected at various sites. While 21 to 27 congeners were identified in the upper segment of the river, this number increased up to 69 (mostly di-, tri-, and tetrachlorobiphenyls) in the industrial area of Cornwall to decrease again to 43 at the inflow into Lac Saint Pierre[1]. A survey carried out in Russia and Uzbekistan in 1989–1990 was based on the examination of sediment, soil and water samples collected in the Moscow region, the Kuban lowlands of the Krasnodar region and in the Samarkand oasis. Analysis of ratios among PCB homologues revealed tetrachlorobiphenyls as the dominant component in all the specified matrices[2]. Further studies were focused on environmental impacts of several pollutants. Thus it was demonstrated that concentrations of PCB, expressed in terms of Aroclor 1254, did not always correlate with values found in fish[3]. Transition of PCB, DDT, and chlordane from water into tissues of molluscs were investigated in California in 1977–1992. During this period, the decrease of DDT and chlordane was more marked than that of PCB. Another finding was that the decrease of PCB concentration in tissues correlated well with the dynamics of concentrations in water[4]. Several authors used contamination of fish as an indicator of pollution of ecosystems. Investigations of the distribution of xenobiotics in tissues of two fish species and mussels living in retention reservoirs of the Seine river demonstrated the highest concentrations in perch followed by roach. The mean concentration in mussels was 26 times lower than that in roach. The pattern of congeners in tissues of the above animals was identical with that found in lower links of the food chain (water, sediments, water plants)[5]. Similarly, the penetration of PCB congeners (147/123, 153, I38/163/164, 180, 182/187) into tissues of marine mammals was demonstrated within a monitoring programme covering the coastal area of Denmark and Norway[6].

The analysis of the above pollutants was based on separation techniques, in particular HRGC/ECD and HRGC/MS, serving for both identification and quantitation. SPE, GPC and column chromatography in Florisil or aluminium oxide was used as pre-analytical clean-up steps.

Experimental

Samples

For sampling of water and sediments three sites were selected namely Napajedla, Spytihněv and Salaška, Czech Republic The samples were collected in an area with intensive agricultural production in the basin of the Morava river during the period 1997–2004. Sampling was carried out six times per each year. The samples of water, sediments were collected simultaneously. The first samples were collected after the drop of water level as soon as it was possible to proceed in accordance with the appropriate standard operation procedure. The whole set consisted of 48 samples of water, 48 samples of sediments.

Chemicals and other materials

Anhydrous sodium sulphate, analytical grade, annealed in a muffle furnace at 650 °C for 4 h; Florisil 60/100 mesh, cleaned and annealed at 600 °C; aluminium oxide, chromatography grade, cleaned and annealed at 400 °C. Before use, the sorbents were activated in a hot-air drier at 130 °C. The activated sorbents can be stored in brown wide-neck bottles in a desiccator for 3 days; n-hexane, residue analysis grade; petroleum ether (boiling point 35 to 60 °C), pure, bidistilled in a ground glass apparatus; diethyl ether, analytical grade, bidistilled, peroxide-free, and dried over anhydrous sodium sulphate; acetone, residue analysis grade; sulphuric acid 96% (Suprapur, Merck); n-hexane, isooctane (SupraSolv; Merck), elution mixtures n-hexane – diethyl ether (94 : 6), n-hexane – acetone (94:6). Glass wool; chromatographic columns 10 × 300 mm with Teflon valves; SPE columns with Florisil and aluminium oxide fillings; yellow-tape paper filters (Filtrak). Standard PCB congener mixture in isooctane with a concentration of 10 μg of each congener per 1 cm^3 (Promochem, Germany); standard PCB congener mixture in n-hexane with a concentration of 100 μg of each congener per 1 cm^3 (J. T. Baker) The accuracy was check by means of Standard Reference Materials BCR-536 Fresh Water Harbour Sediment (Belgium) and NRC HS-1 Marine Sediment (Canada).

Instruments

Ultrasound water bath Bandelin Sonorex Super; water bath with temperature control Biichi B-480; vacuum pump Laboport; vacuum pump Büchi, Vacuum System B-179; hot-air drier Binder; rotary vacuum evaporator Büchi Rotavapor Rl 14; SPE Baker.

Gas chromatographs: Hewlett-Packard (USA) 5890 Series II equipped with ECD ^{63}Ni, split/splitless injector; quartz capillary column HP 5 (5% phenylmethylsilicone), 60 m × 0.25 mm Hewlett-Packard (USA). During the year 2004 analysis were done using the Agilent (USA) 6890 N instrument equipped with dual detector μ-ECD ^{63}Ni and HT 8, DB-17MS (5% phenylmethylsilicone) parallel columns (60 m × 0.25 mm).

Analytical procedure

The whole analytical procedure has been described in[7,8].

Under the conditions of analyses, the following metrological characteristics of the detection limits (means of 10 analyses expressed in μg per ml) for ECD were obtained: PCB 28 – 0.01; PCB 52 – 0.01; PCB 101 – 0.02; PCB 138 – 0.02; PCB 153 – 0.02; PCB 180 – 0.02. RSD ranged from 15 to 22 %

Results and discussion

Concentrations of PCB indicator congeners found both in water and sediments at the sampling localities are presented in the Table I. for locality Napajedla, in the Table II for locality Spytihněv and in the Table III for locality Salaška. It follows from the tables that both low- and high-chlorinated PCBs were detected. This is true especially for the sample obtained within the year 1997, when the important part of contamination originated in secondary contamination from the loaded area. It must be stated, that there is the enterprise Colorlak in this region where, as one of the row materials used in production of paints, Delor 106 was used. In the following years the contribution of the PCB 28 congener was not such remarkable.

Very similar trend was demonstrated in sediments. In samples originated from 1997 to 2000. Content of the PCB 28 congener was higher than the PCB 180 one. Nevertheless the differences were not as clear as in water. Both in water and in sediments very similar trends in PCB congeners concentration were found in the other two localities. (see Table II and Table III)

In order to get better overview through the whole time period 1997–2004 the sums of indicator PCB congeners are given in the Fig. 1. (water samples) and in the Fig. 2. (sediments). It follows from both of the figures mentioned above, that the content of PCB is decreasing in both of these abiotic environments. The fastest decrease in the sum of PCB indicator congeners in water can be seen within the period 1997–2000 especially in the locality Salaška.

As far as sediments are concerned, in comparison with the locality Salaška the decrease of concentrations in sediments in the localities Napajedla and Spytihněv was more

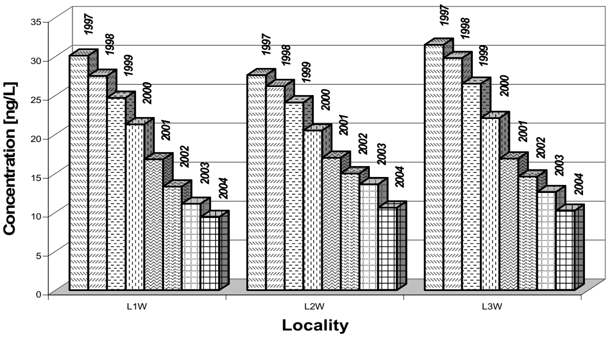

Fig. 1. **Sum of indicator PCB congeners in water (L1W – Napajedla, L2W – Spytihněv, L3SW – Salaška)**

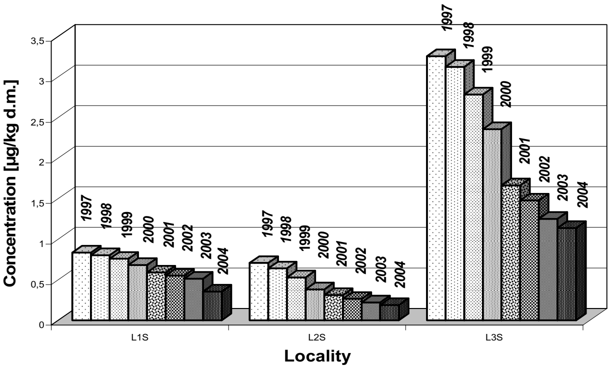

Fig. 2. **Sum of indicator PCB congeners in sediments (L1S – Napajedla, L2S – Spytihněv, L3S – Salaška)**

Table I
Determination of indicator PCB congeners in water and sediments – Napajedla (Sediment μg kg^{-1} dry mater, Water ng dm^{-3})

Year	Congener 28		Congener 52		Congener 101		Congener 118		Congener 138		Congener 153		Congener 180	
	S	W	S	W	S	W	S	W	S	W	S	W	S	W
1997	0.154	3.4	0.009	3.3	0.053	1.6	0.074	3.3	0.229	8.0	0.163	5.7	0.151	4.8
1998	0.153	2.6	0.006	2.8	0.045	1.4	0.072	3.1	0.221	7.9	0.160	5.4	0.144	4.3
1999	0.149	1.9	0.005	1.6	0.039	1.3	0.066	2.9	0.218	7.6	0.152	5.3	0.128	4.1
2000	0.126	1.9	0.005	1.7	0.035	1.1	0.054	2.4	0.200	6.4	0.139	4.8	0.122	3.0
2001	0.095	1.6	0.005	1.3	0.032	1.0	0.051	2.0	0.186	4.9	0.114	4.3	0.105	1.7
2002	0.088	1.4	0.003	1.2	0.028	0.8	0.046	1.7	0.176	2.9	0.108	3.8	0.101	1.5
2003	0.076	1.2	0.003	1.0	0.022	0.6	0.039	1.3	0.168	2.6	0.105	3.2	0.098	1.2
2004	0.059	1.0	0.002	0.9	0.018	0.4	0.03 0	1.1	0.145	2.1	0.099	3.0	0.085	1.1

Table II
Determination of indicator PCB congeners in water and sediments – Spytihněv (Sediment μg kg^{-1} dry mater, Water ng dm^{-3})

Year	Congener 28		Congener 52		Congener 101		Congener 118		Congener 138		Congener 153		Congener 180	
	S	W	S	W	S	W	S	W	S	W	S	W	S	W
1997	0.067	2.9	0.007	2.7	0.056	2.4	0.009	1.7	0.312	6.9	0.128	5.9	0.127	5.1
1998	0.062	2.6	0.006	2.5	0.042	2.7	0.007	1.3	0.308	6.8	0.116	5.4	0.098	4.9
1999	0.054	2.2	0.005	2.3	0.026	2.8	0.006	1.2	0.256	6.0	0.105	5.3	0.075	4.3
2000	0.044	1.9	0.005	1.7	0.014	1.8	0.005	1.1	0.205	5.7	0.069	4.6	0.038	3.7
2001	0.032	1.5	0.005	1.3	0.007	1.7	0.005	0.9	0.176	5.1	0.058	3.9	0.027	2.6
2002	0.028	1.2	0.003	1.2	0.004	1.6	0.004	0.8	0.155	4.5	0.051	3.6	0.019	2.1
2003	0.021	1.1	0.002	1.1	0.003	1.4	0.003	0.6	0.138	4.3	0.037	3.3	0.015	1.8
2004	0.018	1.0	0.001	1.0	0.002	0.8	0.003	0.5	0.124	3.9	0.029	2.8	0.010	1.6

Table III
Determination of indicator PCB congeners in water and sediments – Salaška (Sediment μg kg^{-1} dry mater, Water ng dm^{-3})

Year	Congener 28		Congener 52		Congener 101		Congener 118		Congener 138		Congener 153		Congener 180	
	S	W	S	W	S	W	S	W	S	W	S	W	S	W
1997	0.246	3.8	0.282	3.2	0.170	2.3	0.213	1.9	0.852	8.2	0.795	6.3	0.695	5.8
1998	0.240	3.5	0.286	2.8	0.163	2.1	0.201	1.6	0.854	8.1	0.781	6.0	0.598	5.7
1999	0.218	2.9	0.258	2.3	0.154	1.8	0.171	1.5	0.791	6.9	0.671	5.7	0.519	5.4
2000	0.202	2.3	0.227	2.0	0.106	1.4	0.145	1.4	0.616	5.6	0.569	4.9	0.490	4.5
2001	0.175	1.7	0.183	1.5	0.058	1.1	0.072	1.2	0.425	4.8	0.422	3.4	0.327	3.2
2002	0.152	1.3	0.169	1.2	0.043	0.8	0.062	1.0	0.388	4.3	0.376	3.1	0.286	2.9
2003	0.128	1.0	0.154	1.1	0.032	0.6	0.053	0.7	0.298	3.8	0.306	2.9	0.279	2.5
2004	0.120	0.9	0.123	0.8	0.024	0.6	0.027	0.5	0.312	3.2	0.295	2.2	0.236	2.0

moderate. The overall levels of the PCBs in the sediments from the locality Salaška are higher. It should be mentioned that this locality is situated downstream from the dump of the Colorlak enterprise where wastes containing Delor 106 were deposited. Again the fastest decrease was observed during the period 1997–2000.

Based upon above mentioned results it can be concluded that there is a serious danger for the water ecosystem pollution by the persistent organic substances, which can be liberated from sediments for several years thus causing a secondary contamination. Therefore a systematic monitoring of the environment is necessary.

Conclusions

The long-term monitoring of indicator PCB congeners was done. High resolution gas capillary chromatography was used to determine concentrations of PCB indicator congeners in samples of water and sediments collected in flooded areas of Central Moravia. The eluted chemicals became sources of secondary contamination of the food chain.

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L08 IRON REMOVAL FROM SECONDARY SERPENTINITE RAW MATERIAL BY HIGH GRADIENT MAGNETIC SEPARATION

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Introduction

Former exploitation and the processing of serpentinite ore nearby Dobšiná (Eastern Slovakia) resulted in an accumulation of mine refuse, so called secondary serpentine raw material with a total volume of 3 million m^3. This cone-shaped heap in the vicinity of Dobšiná town with a height of 60 m is a source of dustiness and in such way aggravates the life quality in the town[1]. Moreover, an undesirable effect of the heap is intensified by immission load coming from the mining and mineral processing plant the Siderite, Ltd. Nižná Slaná, located in the same narrow valley as is referred by Bobro et al[2].

The secondary raw material contains about 44 % of SiO_2, 41.3 % of MgO, 6.2 % of Fe, 3.5 % of CaO and 1.35 % of Al_2O_3. The contents of other chemical components such as MnO, TiO_2, Cr_2O_3 and P_2O_5 are not so significant and usually range under 0.4 %[3]. Owing to chemical composition of this material a production of amorphous SiO_2 and $MgCl_2$ is tested. Iron is considered to be a harmful component, mainly from viewpoint of amorphous SiO_2 purity as a final product.

The results of dry magnetic separation were reported by Leško et al[4]. The content of Fe in non-magnetic product ranged from 2.45 to 3.08 % at a mass yield of 37–70 %. Thus, a total material balance is unfavourable owing to relatively low mass yield into non-magnetic product.

The paper analyses the iron removal by the application of wet high gradient magnetic separation (HGMS) in dependence on the grade of grain-opening, i.e. on the grain size of feed into the magnetic separation.

Feed preparation, HGMS conditions and methods of assessment

The preparation of feed consisted of screening on laboratory sieves and grinding in universal laboratory ceramic ball mill with an inner diameter of 20.5 cm and inner length of 21 cm. The ceramic balls with a diameter of 36–42 mm and a density of 2.60 g cm^{-3} were used as grinding bodies. The loading of grinding mill was one third of total volume, including grinded material and balls. The volume ratio of balls to grinded material was 1:1. The original sample in an amount of 50 kg was divided into 3 charges and grinded at various time to prepare 3 grain size feeds to HGMS, namely 90 % under 200 μm, 90 % under 150 μm and 90 % under 100 μm.

Experiments on wet magnetic separation were performed on magnetic separator JONES. Own separation was carried out in a cassette located between separator poles. The cassette equipped by ribbed plates was used to enhance the magnetic field gradient. The induction of magnetic field "B" during the separation tests was 0.165 T at the solids concentration of 200 g dm^{-3}.

The mass yields of separation products were determined. The products have been also subjected to chemical analyses.

Table I
Mass yields of into separation products and their magnetic properties (M – magnetic product, N – non-magnetic product)

Grain size	Product	Mass yield [%]	Volume magnetic susceptibility K . 10^{-6} SI unit
–200 μm	M	25.35	105,419.28
	N	74.65	2,951.16
	Feed	100.00	28,926.83
–150 μm	M	16.51	153,373.03
	N	83.49	2,695.28
	Feed	100.00	27,572.18
–100 μm	M	11.98	209,334.11
	N	88.02	2,722.82
	Feed	100.00	27,474.85

Table II
Chemical composition of separation products

Content [%]	90 % under 200 µm			90 % under 150 µm			90 % under 100 µm		
	M	N	Feed*	M	N	Feed*	M	N	Feed*
Fe^{2+}	3.460	0.110	0.959	5.250	0.090	0.942	7.140	0.080	0.926
Fe^{3+}	13.940	2.400	5.326	19.720	2.330	5.201	26.610	2.370	5.273
Fe_{total}	17.400	2.510	6.285	24.970	2.420	6.143	33.750	2.450	6.199
SiO_2	36.277	46.340	43.789	30.844	45.910	43.423	24.363	46.600	43.936
MgO	34.509	43.310	41.079	29.219	44.230	41.752	23.050	43.540	41.085
CaO	1.469	4.250	3.545	1.147	3.930	3.471	1.063	3.920	3.578
MnO	0.239	0.106	0.140	0.312	0.118	0.150	0.423	0.117	0.154
Al_2O_3	1.153	1.480	1.397	0.964	1.380	1.311	0.888	1.360	1.303
TiO_2	0.045	0.027	0.032	0.055	0.022	0.027	0.063	0.027	0.031
Cr_2O_3	0.580	0.243	0.328	0.701	0.243	0.319	0.831	0.256	0.325
P_2O_5	0.069	0.020	0.032	0.069	0.020	0.028	0.046	0.010	0.014
Ni	0.2330	n.a.	n.a.	0.2230	n.a.	n.a.	0.2190	n.a.	n.a.
Zn	0.0079	n.a.	n.a.	0.0101	n.a.	n.a.	0.0112	n.a.	n.a.
Cu	0.0018	n.a.	n.a.	0.0024	n.a.	n.a.	0.0021	n.a.	n.a.

* calculated from material balance

Table III
Recoveries of observed components into products [%] M + N = 100

Component	90 % under 200 µm		90 % under 150 µm		90 % under 100 µm	
	M	N	M	N	M	N
Fe^{2+}	91.44	8.56	92.02	7.98	92.39	7.61
Fe^{3+}	66.36	33.64	62.60	37.40	60.45	39.55
Fe_{total}	70.19	29.81	67.11	32.89	65.22	34.78
SiO_2	21.00	79.00	11.73	88.27	6.64	93.36
MgO	21.30	78.70	11.55	88.45	6.72	93.28
CaO	10.50	89.50	5.46	94.54	3.56	96.44
MnO	43.36	56.64	34.33	65.67	32.98	67.02
Al_2O_3	20.92	79.08	12.14	87.86	8.16	91.84
TiO_2	36.13	63.86	33.08	66.92	24.10	75.90
Cr_2O_3	44.77	55.23	36.32	63.68	30.64	69.36
P_2O_5	53.95	46.05	40.56	59.44	38.50	61.50

Table IV
Recalculation of main components content in the products on average feed

Grain size	Magnetic products		Non-magnetic products	
	Fe_{total} [%]	SiO_2 + MgO [%]	Fe_{total} [%]	SiO_2 + MgO [%]
–200 µm	17.19	70.92	2.48	89.81
–150 µm	25.24	59.96	2.45	89.98
–100 µm	33.80	47.40	2.45	90.14
feed	6.21	85.02	6.21	85.02

The contents of iron were determined by titration and in the case of the other components the AAS method (Spectr AA-30 Varian) was applied.

The composition of feed and recoveries of individual components into separation products have been calculated according to classical material balance method.

The measurements of volume magnetic susceptibility "κ" of separation products have been performed by means of the Kappabridge KLY-2 Geofyzika Brno, at the following condition: the magnetic field intensity 300 A m^{-1}, the field homogeneity 0.2 % and the frequency 920 Hz.

The separation products with a grain size of 90 % under 100 µm were subjected to XRD study by means of analyser DRON-UM1 under the following conditions: radiation CuK_{α}, Ni-filter, voltage 30 kV, current 20 mA, the size of the steps of goniometer 2° min^{-1}.

Magnetic separation results

The balance of mass yields into separation products and the magnetic properties of obtained products are introduced in Table I. The decrease of feed grain size resulted in gradually reduction of mass yield into magnetic product. A significant growth of magnetic susceptibility value of magnetic product arises from feed grain size decrease. It shows that finer feed into magnetic separation eventuates a cleaner magnetic product, i. e. the content of weakly magnetic or non-magnetic phases is strongly reduced.

The chemical composition of separation products and the recoveries into the products are performed in Table II and Table III, respectively.

According to values in Table II the influence of grain size is the most obvious in the case of iron migration into magnetic products. The grain opening resulted in gradually enhancement of Fe content and reduction of utility components, namely SiO_2 and MgO.

Moreover, an increasing of MnO, TiO_2, Cr_2O_3 and P_2O_5 content in magnetic product comparing with feed can be observed.

A decreasing of Fe content in non-magnetic product as a function of grain opening did not show. But, it can be stated that Fe content always ranged under 2.6 %. Similarly, a significant trend regarding to SiO_2 and MgO contents was not proven too.

The values, presented in Table III, point to the fact that finer grain size of feed results in the increasing recoveries of observed components excepting bivalent iron into non-magnetic product caused by above all the growth of mass yield. Thus, at the feed with the finest grain size the recovery into non-magnetic product exceeds 90 % in the case of SiO_2, MgO, CaO and Al_2O_3. As to MnO, TiO_2, Cr_2O_3 and P_2O_5 it ranges from 61 % to 76 %. Iron concentrates in magnetic product.

For objective comparison of components distribution in the separation process a recalculation of components contents in the product has been performed. The results are introduced in Table IV. So, significant change in chemical composition in the dependence on feed grain size can be observed in magnetic products, i. e. the enhancement of iron content and the reduction of SiO_2+MgO. The variance in non-magnetic products are not so visible, but with regard to mass yields the increasing of feed grain opening results in the total material balance improvement of utility components.

XRD analysis

The products of magnetic separation being obtained from the feed of –100 μm were subjected to XRD analysis. The XRD patterns of magnetic and non-magnetic product are depicted in Fig. 1. and Fig. 2., respectively.

Magnetite Fe_3O_4 was determined as a dominant mineral in magnetic product (Fig. 1.). This mineral is accompanied by antigorite $Mg_{2.25}Fe^{2+}_{0.75}Si_2O_5(OH)_4$, talc $Mg_3Si_4O_{10}(OH)_2$ and magnesite $MgCO_3$.

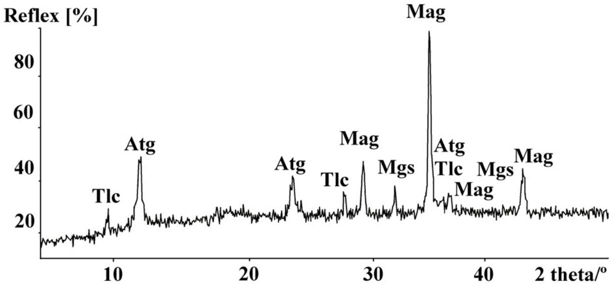

Fig. 1. **XRD pattern of magnetic product (Mag – magnetite, Atg – antigorite, Tlc – talc, Mgs – Magnesite)**

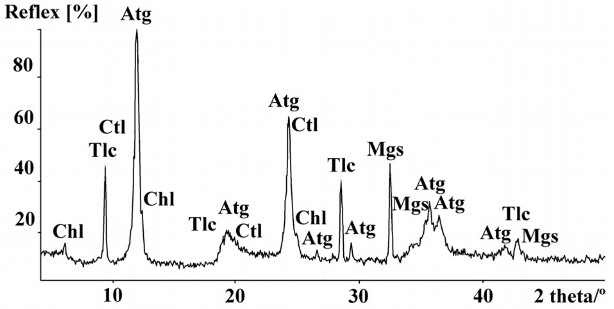

Fig. 2. **XRD pattern of non-magnetic product (Atg – antigorite, Tlc – talc, Chl – chlorite, Mgs – Magnesite, Ctl - chrysotile)**

Antigorite and talc are dominant minerals in non-magnetic product (Fig. 2.). Chlorite $Na_{0.5}Al_4Mg_2Si_7AlO_{18}(OH)_{12}\cdot 5H_2O$ and magnesite $MgCO_3$ represent the accompanying minerals. Chrysotile $Mg_3Si_2O_5(OH)_4$ occurs as an accessory.

Conclusion

The paper analysed the influence of feed grain size on the distribution of chemical components into separation products at constant condition of separation. The obtained results can be summarized as follows:

Iron content was reduced from 6.2 % to 2.5 %.

Decreasing of feed grain size resulted in:

- the growth of mass yield into non-magnetic product,
- the increase of utility components recovery,
- the refining of magnetic product,
- the enhancement of separation process selectivity, i. e. dividing of Fe-bearing minerals from SiO_2-MgO-bearing phases.

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L09 WASTE WATER DECONTAMINATION USING NATURAL ZEOLITE – A MODEL STUDY

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Introduction

The increasing levels of heavy metals in the environment represent a serious threat to human health, living resources and ecological systems. Although there are many sources of heavy metals, some industrial sectors (e. g. mining, metal finishing) are at present those, which contribute the most to environmental pollution with these toxic metals. The main way of contamination of industries is the emission of liquid effluents with relatively low, although harmful metal concentrations (up to some hundreds of mg l^{-1})[1]. Lead, cadmium, chromium, cooper, zinc, mercury and arsenic are considered as “priority pollutants” from the standpoint of potential hazard to the human health. Heavy metals are generally removed from wastewaters by chemical precipitation, ion exchange, coagulation, cementation, electrodeposition, flotation, membrane systems and sorption as well[2–5]. In many cases, these methods are incapable of meeting the more stringent EU legislation for drinking water requirements (i. e. Pb 10 μg l^{-1}, Cd 5 μg l^{-1})[6]. Most of the purification methods listed above are usually effective, but also quite expensive. In the last few decades, adsorption processes have received much concern and became an alternative to conventional precipitation techniques, especially for wastewaters that contain low concentrations of toxic metals and complex forming substaces[7]. The use of low cost sorbent materials could be a good alternative to treat wastewaters contaminated by Cd and Pb. Among the various materials possessing good sorption properties, zeolites are the most promising ones to abstract heavy metal cations from aqueous solutions. Their three-dimensional structure consists of (Si, Al)O_4 tetrahedra which are connected by oxygen vertices forming channels. The negative charge generated from the (Si, Al) isomorphous substitution is counterbalanced by simple cations (i. e. H, Na, K, etc.), which can be easily exchanged by heavy metals, and other toxic cations. Water molecules are also present in their structure. Due to their structure zeolites have high specific surface area[8], good sorption properties and a meso to nano pore size distribution. The main objective of this work was to study the sorption capacity of natural zeolite (clinoptilolite) towards to bivalent Cd^{2+} and Pb^{2+} cations.

Experimental

All chemicals used were of analytical grade. Deionized water was used throughout the experiments. Model contaminant aqueous solutions were prepared by dissolving the nitric salts of the cations ($Cd(NO_3)_2 . 4H_2O$ and $Pb(NO_3)_2 . 3H_2O$) (obtained from Merck, Germany). The solution pH was adjusted with suitable concentration of NaOH and HNO_3. In the frame of this research, the physico-chemical and the sorption properties of zeolite from Nizny Hrabovec (Slovakia) have been investigated. The material was fully characterized by chemical analysis and powder X-ray diffraction (X–ray difractometer DRON–UM 1, CuK_α radiation). The specific surface area of zeolite was determined by low temperature nitrogen adsorption using a Gemini 2360 equipment, whereas a Helos and Rodos apparatus (Sympatec GmbH, Germany) with the wet dispersion base Rodos 11 SROV was utilized for the grain size analysis. The adsorption of Cd^{2+} and Pb^{2+} cations from model aqueous solutions was studied using batch–type equilibrium experiments in a rotary shaker for 24 hours at constant temperature 25.0 ± 0.1 °C. Initial total metal ion concentration range was 20–400 mg l^{-1}. The sorbent concentration was 2 g l^{-1}. The effect of ionic strength has also been investigated using $NaNO_3$ electrolyte concentrations of 0, 0.1 and 0.01 M. The final metals concentration was determined by Atomic Absorption Spectroscopy (AAS using a Varian Spectr AA–30) and the metal uptake was calculated from the difference. Sorption isotherms have been constructed and fitted with Langmuir– and Freundlich– type equations. In order to avoid possible precipitation problems, the thermodynamic equilibria diagrams of the metals have been calculated with the Mineql Plus computer code[9] for a total metal concentration of 100 mg l^{-1}. The precipitation pH was calculated and

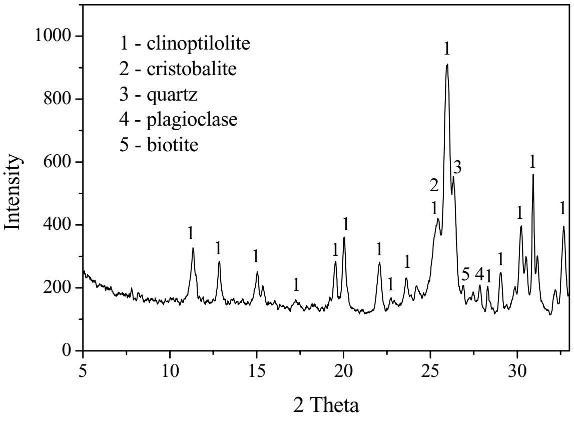

Fig. 1. **Powder XRD pattern of zeolite (Nizny Hrabovec, Slovakia)**

for our experiments a suitable pH, where precipitation was not expected, was selected for further sorption studies. The thermodynamic calculations have shown that Cd remains soluble up to pH value of about 8.4, where it starts to precipitate as insoluble $Cd(OH)_2$ and Pb remains soluble up to pH 5.8, where it starts to precipitate as $Pb(OH)_2$.

Results and discussions

The chemical analysis as well as XRD patterns (Fig. 1.) has shown that the main phase of examined zeolite sample was clinoptilolite (40–60 %). The specific surface area of the sorbent, determined by the BET method, was found to be 22.41 $m^2 g^{-1}$, whereas 100 % of its particles had a size of 0.09 to 0.2 mm.

Zeolites are known as ion exchangers between cations from lattice (Ca^{2+}, K^+, Na^+) and cations (Cd^{2+}, Pb^{2+}) from aqueous solutions. For this reason the general term sorption is used throughout this work, which includes ion exchange and adsorption.

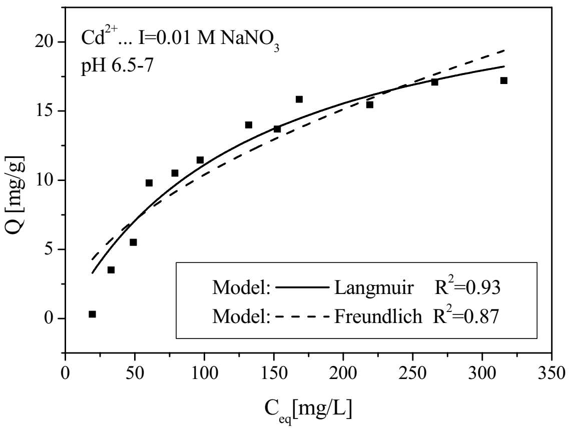

Fig. 2. **Cd uptake by zeolite (sorption isotherm, I = 0.01 M $NaNO_3$)**

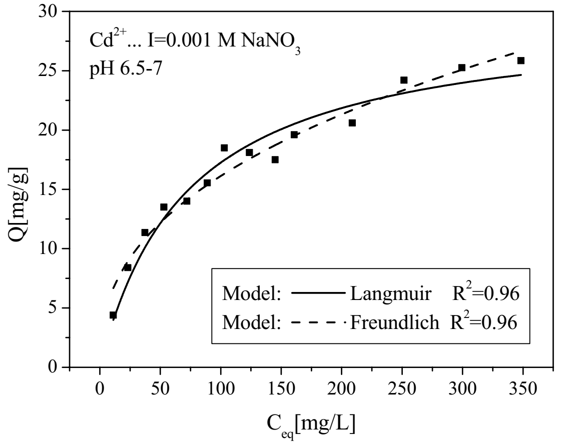

Fig. 3. **Cd uptake by zeolite (sorption isotherm, I = 0.001 M $NaNO_3$)**

The effect of increasing Cd concentration on Cd uptake by zeolite (sorption isotherm) at pH 6.5 to 7.0 and constant ionic strength with 0.01 M $NaNO_3$ is presented in Fig. 2. The experimental data are represented by the solid points. The shape of the curve is between the normal curves for Langmuir and Freundlich type models, so, both models were fitted on the data and are represented by the two lines. Comparison of the coefficients of determination, R^2, shows that the Langmuir model fits better the experimental data. The maximum sorbent capacity was calculated to 25 mg Cd/g of zeolite. This is among the usual values referred in the literature for Cd removal from water solutions.

With a lower ionic strength (0.001 M $NaNO_3$, see Fig. 3.) the results are similar but the maximum capacity is increased to 30 mg Cd/g of zeolite. The sorption isotherm can be explained equally well by Langmuir or Freundlich type models as both have the same R^2 of 0.96.

The corresponding data for Cd removal from aqueous solution by zeolite without addition of electrolyte are shown

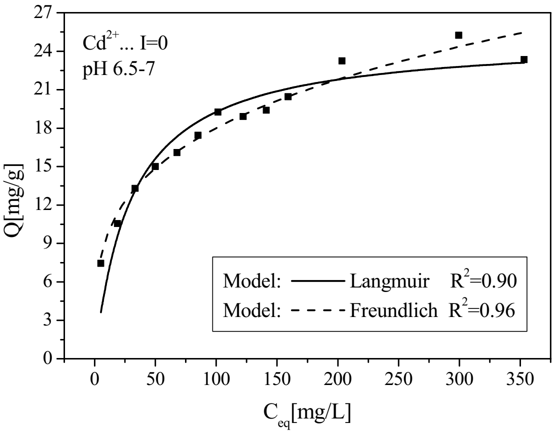

Fig. 4. **Cd uptake by zeolite (sorption isotherm, no electrolyte)**

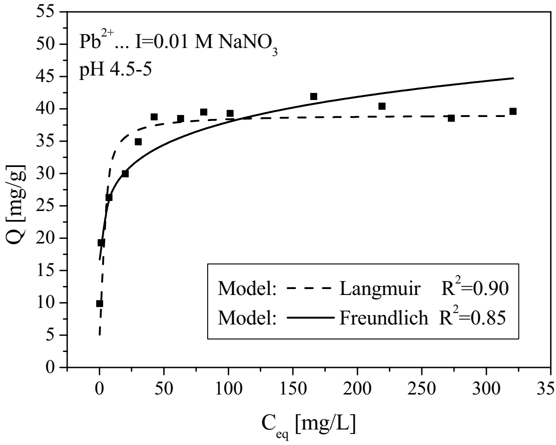

Fig. 5. **Pb uptake by zeolite (sorption isotherm, I = 0.01 M $NaNO_3$)**

in Fig. 4. In this case, the Freundlich model ($R^2 = 0.96$) fits the data better than Langmuir ($R^2 = 0.90$) and the maximum capacity of the material (around 25 mg Cd/g of zeolite) is almost the same with the previous one for 0.001 M $NaNO_3$. Comparing the adsorption curves in Fig. 2.–4., it is noted, that as the ionic strength increases, the removal efficiency of zeolite to cadmium cations decreases. This can be explained quite well if Na cations are exchanged from zeolite surface by Cd. Then with the addition of $NaNO_3$ in the solution the Na concentration increases and inhibits the transfer of Na from zeolite surface to the solution. With 0.001 M electrolyte the increase is not so high to have any measurable effect but with 0.01 M $NaNO_3$ the effect can be seen.

The corresponding results for Pb removal from aqueous solutions at various ionic strength are shown in Fig. 5.–7.

In all cases the Langmuir model fits much better the experimental data. With 0.01 M $NaNO_3$ and pH 4.5 to 5.0 the maximum sorption capacity is calculated to 40 mg Pb/g of zeolite and the data are following well the Langmuir model. It seems that equilibrium has been established too. The coefficient of determination ($R^2 = 0.90$) is not as high as in Cd sorption, however, the shape of the curve confirms well the Langmuir model; the dispersion of experimental data points around the model curve can be explained by the nature of the material which is a natural material containing zeolite as the active sorbent but also other minerals.

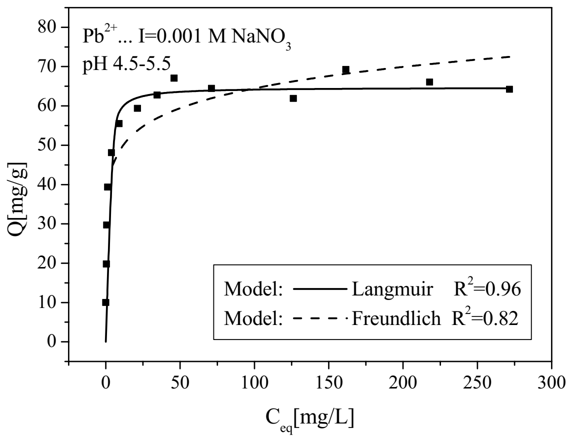

Fig. 6. **Pb uptake by zeolite (sorption isotherm, I = 0.001 M $NaNO_3$)**

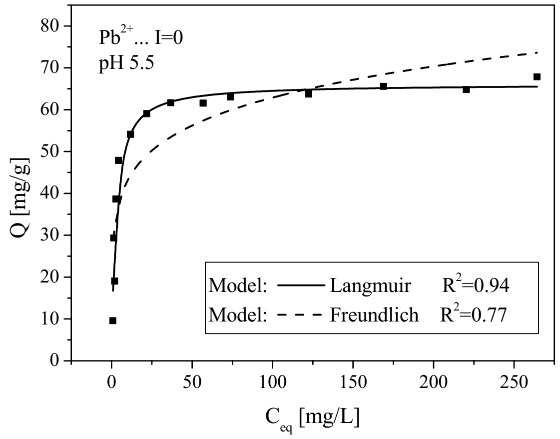

Fig. 7. **Pb uptake by zeolite (sorption isotherm, no electrolyte)**

The experimental data of Pb removal by zeolite for ionic strength 0.001 M $NaNO_3$ and without addition of electrolyte are shown in Fig. 6. and 7. respectively. It is observed that both have a significant increase in the removal efficiency of zeolite and the maximum sorbent capacity is 65 mg Pb/g of zeolite for both experimental conditions. Both curves are well following a Langmuir type model with coefficients of determination (R^2) 0.96 and 0.94 respectively. The shape of the curves shows that equilibrium has been established.

Again a detrimental effect is observed with the increase of the ionic strength by the addition of $NaNO_3$ electrolyte. It is well known that increase of the ionic strength decreases the extent of the effective double layer structure that is created around any particle that comes in contact with water solutions. Also the surface charge of the sorbent is affected. So, it is expected that ionic strength will affect also the ability of the sorbents to remove cations from solutions. Here, the material we have studied contained also Na ions in the structure and it is possible that those Na ions might have been exchanged from Cd and Pb cations from solution.

Comparing the efficiency of the material towards both cations it seems that zeolite is more efficient in removing Pb from solutions than Cd. Given that this is a cheap natural material available in large quantities worldwide and it has been used without any further treatment we can conclude that it is a good canditate for practical use.

Conclusions

The zeolite studied in this work was found to be an effective sorbent for cadmium and lead bivalent cations. Its affinity is higher for lead than cadmium and it is affected by the presence of $NaNO_3$ electrolyte. Increasing quantities of $NaNO_3$ decrease the sorbent efficiency for both Cd and Pb. The best sorbent efficiency was observed with either no electrolyte or a small electrolyte concentration (0.001 M $NaNO_3$) and was found to be around 30 mg Cd/g of sorbent and 65 mg Pb/g sorbent. These capacities and the low price of the material make it a good candidate for practical use.

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L10 THE APPLICATION OF MICROWAVE ENERGY IN WASTE TECHNOLOGY

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Introduction

Microwave energy has found general application in different areas. These include communication, food processing, analytical chemistry, heating and vulcanization of rubber, processing of wide range of materials including ceramics, polymers, composites, powder and minerals.

In the area of solid waste treatment and remediation, microwave power has been explored in vitrification and recycling of electronic circuitry and waste from metallurgic processing and reduction of gaseous pollutants.

Microwave heating is unique and offers a number of advantages over conventional heating such as: non-contact heating, energy transfer (not heat transfer), rapid heating, material selective heating, volumetric heating, quick start-up and stopping, heating which starts from the interior of the material body, higher level of safety and automation[1].

The great attention is devoted to the research of different heavy metals carriers used in wastewater treatment[2]. The Albanian leaching residue (ALR) is waste coming from nickel production in the hydrometallurgical plant in Sereď and was used as a carrier of Cd^{2+} and Co^{2+} ions. This waste has a high content of magnetite (54 %), which is strong absorber of microwaves and this property can be advantageously used for an acceleration of microwave vitrification.

In the legislative of the Slovak Republic, there was accepted the law of the waste treatment No. 223/2001 which establishes analytical methods of checking waste called JMAKO No. 20 (ref.[3]) and 21 (ref.[4]), in compliance with the water leachate of waste for leaching evaluation of solid waste and wastes stabilised by cementation are prepared. These methods use deionized water as leaching medium. There is not the method for chemical durability evaluation of vitrificated waste in the legislative of the Slovak Republic.

Šild[5] has compared a method of leaching in deionized water to TCLP method (Toxicity Characteristic Leaching Method)[6], which applies acid solution for leaching. He has found that pH of leaching medium is important mainly in leaching of heavy metals. The use of deionized water needn't always reveal risk following from long term storage of waste or their usage. From his results it follows that some heavy metals (e. g. Cd, Ni) have leached in a higher amount in an acid solution. Similar results have been reached in our research as well[7]. This is reason why we consider TCLP test as more available for chemical durability evaluating of vitrificated samples.

Experimental part

Materials

The Albanian leaching residue with precipitated heavy metals (Cd, Co), colourless container glass and dolomite were used for the vitrification. Chemical composition of the Albanian leaching residue composition, colourless container glass and dolomite is described in the Table I, II and III.

Table I
Chemical composition of the Albanian leaching residue [wt %]

Fe_{total}	SiO_2	Al_2O_3	CaO	MgO	Cr_2O_3	NiO
45.89	15.03	4.80	3.54	2.21	1.06	0.17

Table II
Chemical composition of the colourless container glass [wt %]

SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	SO_3	BaO
72.4	1.7	0.05	1.7	9.6	13.8	0.6	0.18	0.2

Table III
Chemical composition of the dolomite [wt %]

SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O
0.57	0.19	0.35	18.7	24.6	0.29	0.13

Methods

Material preparation of waste model metals

The solutions of $CdCl_2.2.5H_2O$ a $CoCl_2.6H_2O$ were prepared then the Albanian leaching residue was added. The addition of solution of NaOH was used for the regulation of pH up to precipitation. The precipitate contained 8.7 mg of Cd^{2+} and Co^{2+} ions in 1 g of the Albanian leaching residue. The precipitated samples were dried at 105 °C for 1 hour.

Microwave vitrification

Microwave vitrification was carried out in a microwave oven Panasonic NE 2740 (frequency 2450 MHz, output 1350 W). The vitrification was accomplished in thermal isolated ceramic crucibles described on the Fig. 1. The samples were heated for 40 minutes. When the samples achieved the melting temperature, the sample flowed out of upper crucible A into crucible B. The vitrificated samples cooled in a muffle furnace at 480 °C for 2 hours. The temperature of melt in microwave oven was measured by the infrared thermometer Raytek with temperature range between 400–3000 °C.

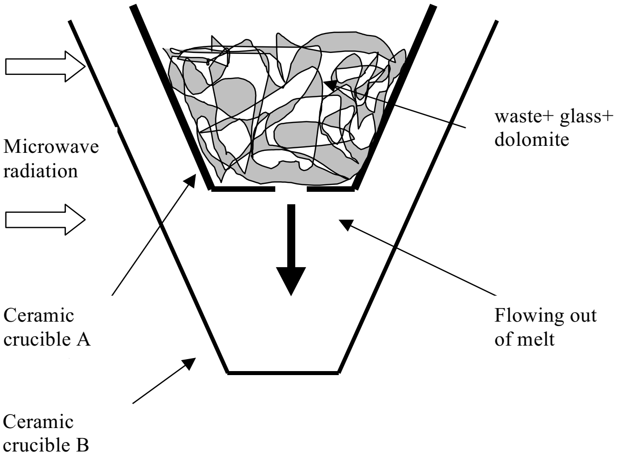

Fig. 1. **Microwave vitrification of waste**

TCLP method

The chemical durability of vitrificated samples was evaluated by TCLP test (Toxicity Characteristic Leaching Procedure), which is based on the use of leaching solutions. The used solutions are a function of the alkalinity (pH) of the solid phase of waste. If the pH is < 5, leaching solution No.1 ($pH = 4.93 \pm 0.05$) is used. Leaching solution No.1 contains of 5.7 ml glacial acetic acid added to 500 ml of the appropriate water and 64.3 ml of 1N NaOH diluted to a volume of 1 liter. If the pH > 5, leaching solution No.2 ($pH = 2.88 \pm 0.05$), that contains 5.7 ml glacial acetic acid diluted to a volume of 1 liter, is used. The leaching solution is added to waste with a grain size less than 1 cm and the ratio: 1 part of a waste (solid phase) to 20 parts of the leaching solution. A sealed flask containing the leaching solution is agitated for 18 hours at 25 °C. The leachate is separated from the solid phase using a 0.6–0.8 μm glass fibre filter. The contaminant concentrations in the TCLP extract were analysed by AAS method.

Results and discussion

The ratios of Albanian leaching residue, glass and dolomite and their chemical composition are shown in Table IV. The glass was added to the mixture for increase of glass-forming additives, the dolomite was added as a modifier. The heating process of sample 1 is depicted on Fig. 2.

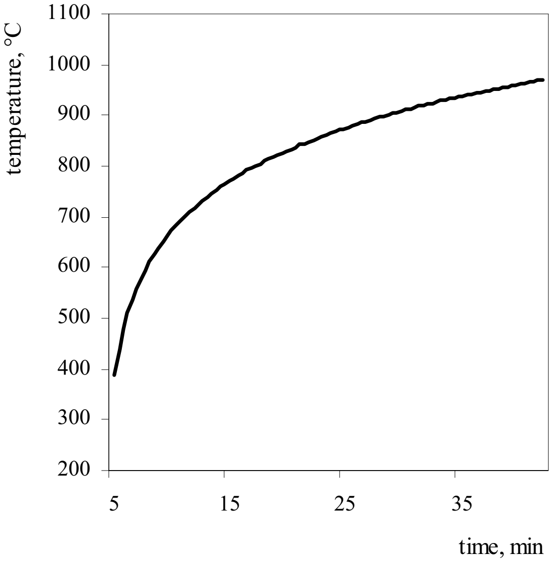

Fig. 2. **The heating process of sample 1 during microwave vitrification**

From the point of view of microwave absorption, glass is transparent material for microwave and it can not be heated at room temperature. There is dependence of absorption rate of microwave radiation of glass on temperature because the absorption of microwave and heating rate rise strongly with increasing temperature. Glass is necessary to activated for microwave absorption by addition susceptor. The Albanian leaching residue is possible to use as a susceptor because of a high content of magnetite which absorbs microwaves at room temperature.

In Table V, a TCLP test results of the Albanian leaching residue with precipitated heavy metals before microwave vitrification are presented. The results reveal that the ions of heavy metals transfer to the TCLP leaching solution.

The TCLP test results of Cd and Co after vitrification are shown in Table VI. From the obtained results follows that chemical durability depends on the SiO_2 content.

Table V
TCLP test of the Albanian leaching residue with precipitated Cd^{2+} and Co^{2+} ions before microwave vitrification [mg l^{-1}]

Cd	Co
540.5	827

Table VI
TCLP test results of vitrificated samples [mg l^{-1}]

Sample number	1	2	3	4	5	6	7
Cd	2.65	1.88	0.73	1.44	1.65	0.57	2.53
Co	1.01	0.52	0.15	0.34	0.36	0.12	0.63

Table IV
Characteristics of samples [wt %]

Sample number	ALR	glass	dolomite	SiO_2	Al_2O_3	MgO	CaO	Na_2O	Fe
1	50	40	10	36.53	3.10	3.66	8.07	5.55	22.98
2	40	40	20	35.09	2.64	5.30	10.18	5.58	18.42
3	35	55	10	45.14	2.63	3.58	8.98	7.62	16.10
4	35	45	20	37.95	2.48	5.28	10.48	6.27	16.12
5	30	50	20	40.82	2.33	5.25	10.78	6.96	13.83
6	30	60	10	48.01	2.48	3.55	9.28	8.31	13.81
7	30	40	30	33.64	2.18	6.95	12.28	5.61	13.85

Conclusion

The obtained results verify the possibility of microwave vitrification of heavy metals carriers that are possible to heat to high temperature by adding the optimal amount of quickly heating material. The vitrificated samples prove good chemical durability, which depends on the content of glass-forming additives.

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L11 ALTERNATIVE CLEAN-UP STRATEGIES IN ANALYSIS OF ORGANOPHOSPHOROUS AND PYRETHROID PESTICIDES IN SPICES

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Introduction

Analysis of pesticides had proven to be the very challenging task for analysts and scientists all over the world. In many cases the complexyty of the matrix is the major limiting factor for trace analysis of these compounds. Typical analyses are based on gas chromathography (GC) with selective detection such as halogen selective detection (XSD), electrolytic conductivity detection (ELCD), flame photometric detection (FPD or pulsed FPD (PFPD), nitrogen-phosphorus detection (NPD) and/or electron-capture detection (ECD)[1,2]. Once a pesticide is detected, confirmation and identification is performed with gas chromatography-mass spectrometry (GC-MS). The use of simultaneous PFPD and MS had been also been reported[3]. Some routine monitoring laboratories use GC-MS in selected ion monitoring (SIM) mode to simultaneously quantify and confirm the identity of a wide variety of GC-amenable pesticides, but multiple injections are still needed. GC-MS analysis in full scan mode can quantify and confirm any number of GC-amenable analytes in a single injection, but matrix interferences continue to act as a bottleneck in this type of pesticide analysis[4]. During the last decade GC-MS/MS methods, mainly daughter ion scan, have also become a routine tool for trace level detection and identification of pesticides. With MS/MS, selectivity is obtained by rejecting matrix interference through the selection of the patern ion, while providing molecular specific information through the daughter ion's spectrum. Combined with the sensitivity of ion-trap MS technology, this method enabled low ppb level informative detection under complex mixture conditions[5]. Several advantages of ion trap MS/MS have been found compared with traditional selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) MS/MS techniques used in sector and quadrupol instruments[6]. Despite this fact we have choosen to test GC-MS/MS quadrupole system under a bidding task i. e. trace analysis of pesticide residues in spices. And so these reprasentatives of very „difficult“ matrix, which content a large amount of voletile and semi-volatile compounds, had been used to further investigate the trace analysis issue of organophosphorus (14 compounds) and pyrethroid (9 compounds) pesticides. As the matrix representatives oregano, cummin and chilly powder had been choosen. The main objectives of this study were (i) to test capacyty of GC-MS/MS analysis and (ii) to develop apropriate method for the clean-up step as the part of whole analytical proces. The extraction in all cases was based on water desorption followed by acetonitrile extraction, plus variety of approaches to SPE using classical sorbents and their combinations were tested. These different techniques wer compared due to recoveries of all analytes, clean-up efficiency and time consumption. For SPE techniques showing to be most promissing the LOQs were determined. The results showed, that there are limitations and gaps on clean-up and chromatografic side of analytical process, which can be partially handled at the detection side i. e. by MS/MS.

Table I
List of transitiens used in GC-MS/MS analysis

Pesticide	Transition/collision energy [mV]	
	I.	II.
methamidophos	141>79(–30)	141>94(–10)
dichlorvos	185>93(–20)	185>184(–10)
acephate	136>94(–20)	183>136(–10)
omethoate	156>95(–30)	156>110(–10)
monocrotophos	127>95(–20)	192>127(–10)
dimethoate	143>111(–10)	229>87(–10)
parathion met.	263>109(–10)	263>136(–10)
malathion	256>93(–10)	256>147(–10)
chlorpyriphos	314>258(–30)	314>286(–10)
methidathion	145>85(–10)	302>145(–10)
ethion	231>203(–10)	384>231(–10)
phosmet	160>105(–20)	160>133(–10)
phosalone	182>102(–20)	182>111(–10)
azinfos met.	160>77(–20)	160>104(–20)
permethrins (I, II)	163>91(–10)	163>127(–10)
cypermethrins (I–IV)	181>127(–40)	181>152(–30)
fenvalerates (I, II)	225>119(–20)	225>147(–10)
deltammetrin	181>152(–30)	253>172(–10)

Experimental

The system used was the Varian 1200 GC-MS/MS (Varian, Walnut Creek, CA, USA). All the experiments were performed using GC capillary column, 30 m × 0.18 mm i. d. 0.18 μm Rtx-5 (Restek, Bellefont, PA, USA). The applied temperature programming was as follows: initial temperature 60 °C, hold for 2 minutes, increased to 280 °C at 20 °C min^{-1} rate, hold 8 minutes. Injector temperature 250 °C and He flow of 1 ml min^{-1}. The detector voltage was held at 1600 volts and the list of MS/MS transitiens, with their collision energies, that were used for analysis are shown in Table I. The sorbents and SPE cartridges (Florisil, ENVICarb and alumina powder) had been provided by Supelco (Sigma-Aldrich, St. Louis, MO, USA) and all 24 pesticides that were used in this project were obtained from Dr.Erenstorfer (Augsburg, Germmany).

Results and discussion

The set of experiments was led under the systhematic aproach which synthetized (i) „core“ scheme (Fig. 1.), that was the same for all different aproaches to combination of extraction solvent-SPE sorbent-elution solvent and (ii) „gradual“ philosophy, that is described as follows:

1. The primer broad set of different aproaches to combination of extraction solvent-SPE sorbent-elution solvent (together 16 variations) were tested and those which showed to be the most promissing under the scope of cleanup efficiency/recoveries (based on comparement of matrix standards with samples prepared using spiked matrix) were choosen to be further investigated.

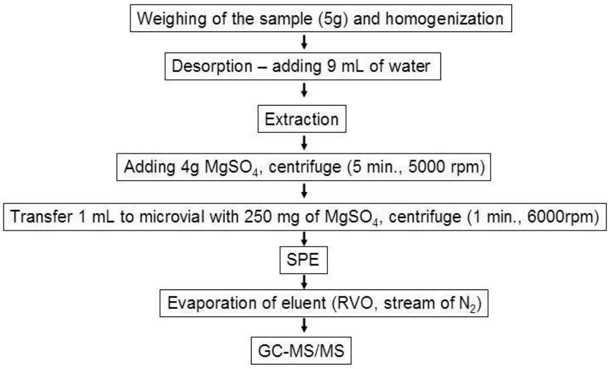

Fig. 1. **The basic core procedures for the methods developed**

2. 4 approaches were choosen and as the fifth, the method using GPC as the cleanup step was used (for resultes see Table II).
3. As the work carried on 2 main aproaches were selected and LOQs for whole analytical method constituing these were measured (see Table III).

Form the variety of different approaches to the extraction and cleanup the appropriate methods for analysis of 24 pesticides (including isomers) were selected. The selection was based mainly on recoveries of the analytes, time consumption and cleanup efficiency. As the best ones these methods were choosen:

(i) From the wiev of cleanup efficiency the method based on hexane extraction step, followed by SPE on Florisil. The drawbacks of this method were poor recoveries of

Table II
Description of selected methods and resultes given

Name and description of the approach	Cleanup efficiency	Time consumption	Recoveries of anlytes	Notes
Metod A1 AcN extraction, SPE (Florisil), elution using hexane	++++++ (best score)	+++	5–90 %	low recoverie for most of the analytes (< 70 %), 6 analytes not detected
Metod A2 AcN extraction, SPE (Florisil), elution using hexane : diethyl ether (9 :1)	++++	+++	5–100 %	3 analytes not detected
Metod B AcN extraction, SPE (Alumina), elution using AcN : toluene (3 :1)	++	+++	56–102 %	–
Metod C AcN extraction, SPE (Alumina+EnviCarb), elution using AcN : toluene (3 :1)	+++	++++++ (best score)	5–111 %	low recoveries for (methamidophos, dichlorvos a acephate)
Metod D EtAc extraction, cleanup using GPC	+ (worste score)	+++++	51–100 %	1 analyte not detected (acephate)

Table III
LOQs for selected methods [ng g^{-1}]

Analyt	Oregano		Chilly powder		Cummin	
	Metod B	Metod C	Metod B	Metod C	Metod B	Metod C
methamidophos	1	1	1	1	1	1
dichlorvos	1	1	1	10	1	5
acephate	1	1	1	1	1	5
omethoate	1	1	1	1	1	1
monocrotophos	1	1	1	1	1	1
dimethoate	10	10	10	10	10	10
parathion met.	5	5	5	5	1	1
malathion	1	5	1	5	5	5
chlorpyriphos	1	1	1	1	1	1
methidathion	1	1	1	5	5	1
ethion	5	5	5	1	1	1
phosmet	1	1	1	1	1	1
phosalone	1	5	5	1	1	25
azinfos met.	1	1	50	1	5	25
permethrins	10	10	25	25	25	25
cypermethrins	25	25	50	25	25	25
fenvalerate	25	25	50	50	10	25
deltammetrin	25	25	50	100	25	25

OP pesticides, which could be partially handled by including diethyl ether into the SPE elution mixture and using acetonitrile extraction step followed by solvent exchange into hexane.

(ii) From the wiev of recoveries the best option was selected to be method based on acetonitrile extraction and SPE on alumina, but this method wasn't providing sufficiently good cleanup so some problems were observed at the GC separation side of the analysis.

(iii) As the best method giving moderate cleanup efficiency combined with recoveries ranging from 85 % to 111 % for most of the analytes, with exception of methamidophos, dichlorvos a acephate, the method using acetonitrile extractin and SPE on combination of ENVI-Carb and alumina was choose.

Conclusions

The limits of quantification that were measured for the two previosly discussed methods were differentiating slightly within different matrixes, but still providing very same resultes. For the early eluting pesticides, even the troublesome ones such as methamidophos, acephate, omethoate, the low LOQs (under 10 ng g^{-1}) were obtained. For the lately eluting ones, pyrethroids, LOQs of higher levels were obtained (25–100 ng g^{-1}). The further experiments will be led to further investigate the potential of SPE on Florisil and to increase number of analytes in established multiresidual methods.

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L12 AGGREGATION OF HUMIC SUBSTANCES IN SYSTEMS WITH DIFFERENT INITIAL PH AND OPTIMUM DOSE OF COAGULANT

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Introduction

Treatment of soft humic waters by coagulation, which have low alkalinity and high NOM content, require pH adjustment to achieve the optimum for coagulation and low residual coagulant concentrations after floc separation. The common design practice is dosing the alkali prior to the coagulant. The question was, how the reverse sequence (i. e. coagulant prior to the alkali) would work and if it might bring some advantages to the whole treatment process? Additional question was, what would be the optimum interval between dosing both chemicals?

Methods

Coagulation test using centrifugation as the separation method[1] was employed. This test did allow us to study formation of NOM particles by Brownian motion (perikinetic coagulation) and has the highest possible degree of reproducibility (in any place in the world) as temperature is the only parameter influencing the kinetics of particles formation. The raw water used for the experiments was taken from the Sous Reservoir in North Bohemia. All experiments were carried out right at the treatment plant Sous. The alkalinity of raw water from the reservoir was 0.1 mmol l^{-1}, colour 51 Pt, mg l^{-1}, absorbance at 387 nm (5 cm) was 0.199, pH was 6.15, natural aluminium concentration was 0.22 mg l^{-1} and COD(permanganate) was 7.2 mg l^{-1}. Residual absorbance at 387 nm is proportional to colour by multiplying by factor 255. The experiments were carried out at five levels of alkaline addition (lime water), giving the range of alkalinities from 0.25 to 0.45 mmol l^{-1} and at six different alum dosages (20–40 mg l^{-1}).

Results

Example of results is given in Tables I and II. The amount of both chemicals added was the same(lime water and alum respectively), only the sequence of dosing was different. We can see that there are remarkable differences between both experiments, especially with respect to the residual absorbance at 387 nm after coagulation. Optimum alum dose is indicated in italics. The "classical" dosing sequence (lime water before alum) gives well known sharp optimum with respect to both, residual absorbance (colour) and aluminium, while the "opposite" sequence performs surprisingly well at both conditions of either slight underdosing or slight

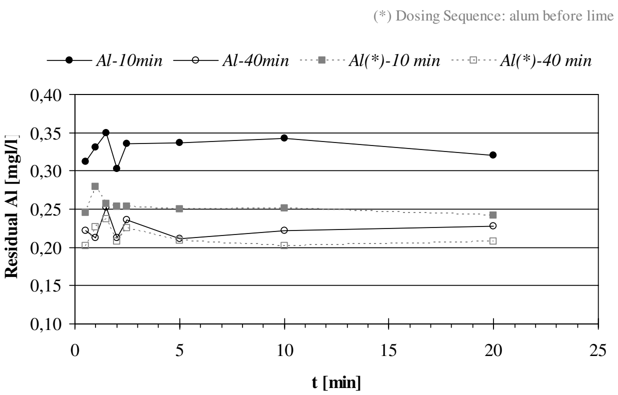

Fig. 1. **Influence of interval between dosing lime water and alum for both dosing sequences tested ("classical" lime *before alum* – black lines, and "opposite" *alum before lime* – dotted lines). Lime addition to alkalinity = 0.25 mmol l^{-1}, alum dose = = 22 mg l^{-1}**

to medium overdosing, especially with respect to the residual absorbance (i. e. water colour). Optimum coagulant dose is practically not influenced by the sequence of dosing.

In the second part of our study, we have studied, what would be the optimum interval between dosing both chemicals. Illustration of results is given in Fig. 1. We can see that at 10 minutes coagulation time, remarkably better results are achieved for the "opposite" dosing sequence (alum before lime). If longer coagulation time would be available, both dosing sequences produce about the same results with respect to the residual aluminium.

Table I
Coagulation test for the "classical" dosing sequence *lime water – coagulant*, 10 minutes total aggregation time (lime addition to total alkalinity = 0.30 mmol l^{-1}, dosing interval between coagulant and lime 1 minute)

Alum dose [mg l^{-1}]	Al dose [µmol l^{-1}l]	pH	Residual Absorbance at 387 nm [5 cm]	Residual Al [mg l^{-1}]
20	60	6.44	0.235	1.803
22	66	6.16	0.140	0.996
25	*75*	*5.88*	*0.049*	*0.288*
30	90	5.55	0.058	0.674
35	105	5.22	0.140	1.017
40	120	5.12	0.182	1.238

Table II
Coagulation test for the "opposite" dosing sequence *coagulant – lime water*, 10 minutes total aggregation time (lime addition to total alkalinity = 0.30 mmol l^{-1}, dosing interval between coagulant and lime 1 minute)

Alum dose [mg l^{-1}]	Al dose [µmol l^{-1}l]	pH	Residual Absorbance at 387 nm [5 cm]	Residual Al [mg l^{-1}]
20	60	6.01	0.218	1.775
22	66	5.85	0.070	0.421
25	*75*	*5.77*	*0.043*	*0.228*
30	90	5.27	0.043	0.620
35	105	5.07	0.041	0.882
40	120	4.98	0.045	1.326

Very interesting is the time interval of about first five minutes between both chemicals addition. In all our experiments, we have observed temporary deterioration of residual alum and colour in periods between 1.5–3 minutes time span of chemicals addition. Until now, we do not have reasonable explanation for this behaviour of the coagulating system.

Generally speaking, the "opposite" sequence of dosing (coagulant before lime) gave better results in residual absorbance (colour) over the whole spectrum of variables studied. This could be important for achieving higher removal rates of NOM in treatment plants. Also the rate of particles formation was faster in case of the "opposite" dosing sequence, especially in the region of lower lime addition (lower raw water alkalinity).

Conclusions

The standard dosing sequence of alkali and coagulant (i. e. lime first, coagulant second) produced poorer results that the "opposite" sequence of dosing (coagulant before lime), which gave better results in residual absorbance (water colour) over the whole spectrum of variables studied. Changing the dosing sequence in treatment plants could be the way of achieving higher removal rates of natural organic matter. Also the rate of particles formation was faster in case of the "opposite" dosing sequence, especially in the region of lower lime addition (lower raw water alkalinity).

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L13 STABILIZATION/SOLIDIFICATION OF HAZARDOUS WASTE BY ASPHALT EMULSION

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Introduction

According to new rules for hazardous waste management in the Czech Republic, no hazardous waste can be deposited to the landfill without previous treatment. There are many techniques of chemical or physical treatment that can be used, such as biodegradation, chemical oxidation, reduction, neutralization, recovery, vitrification and many others[1]. One of the possibilities is also stabilization; Portland cement or lime/pozzolans are mostly used. But also alternative technique may be submitted if it can be demonstrated that the treatment method achieves a measure of performance equivalent to that achieved by classical methods.

Several researchers dealt with stabilization/solidification of waste, which contains heavy metals. Sawada et al.[2] dealt with immobilization of heavy metals contained in fly ash in the mixture of asphalt, sulfur and sodium hydroxide. In this method, the immobilization was mainly achieved by two stabilizations: the solidification by asphalt and the sulfuration of heavy metals. Alba et al.[3] worked on stabilization/solidification of waste incineration residues with Portland cement. Leaching test results showed that heavy metals are immobilized within the binder, whereas chlorides are only partially retained. Fluidized-bed combustion ash was used as a binder for stabilization of metal-hydroxide sludge[4]. The study of Cioffi et al.[5] carried out stabilization of galvanic sludge in a matrix containing calcium silicate, sulphoaluminate and calcium sulfate.

These techniques are mostly very complicated and the results of stabilization do not fulfill the leaching limits in all cases.

Stabilization/solidification of the hazardous waste containing heavy metals by asphalt emulsion (AE) has been tested. The principle of the contaminants immobilization by asphalt emulsion is their encapsulation in asphalt matrix. Waste is mixed with asphalt emulsion; then asphalt emulsion breaks into water and pure asphalt binder. As a result, the waste is coated with the asphalt layer.

Materials and methods

Waste

The hazardous waste comes from destruction of old munitions. The waste sample is dust from the explosion chamber with particle size 0.1–1 cm. The original explosive contained mainly lead azide, lead trinitroresorcinate, lead fulminate, potassium chlorate, barium nitrate, antimony sulfite, trinitrotoluene, pentaerythritetranitrate and trinitrophenylmethylnitramine. The waste sample contains mainly lead, iron, copper, calcium, sulfur, nickel, antimony and traces of titanium. Waste density is 7.1 kg m^{-3}, apparent density 3.1 kg m^{-3}.

Asphalt emulsion

Two different asphalt emulsions were used for stabilization/solidification of waste. The first is anionic asphalt emulsion from the Dutch producer Latexfalt. This emulsion is aqueous anionic slow-setting asphalt emulsion with pH = 11; it contains app. 60 % of asphalt binder. The second is cationic asphalt emulsion from Czech manufacturer Stavby silnic a železnic, a. s., Kolín. This emulsion is aqueous cationic slow-setting asphalt emulsion with neutral pH, it contains also app. 60 % of asphalt binder.

Stabilized waste mixtures preparation

The waste and each portion of asphalt emulsion were weighed to the nearest 0.1 g. For each specimen, 50 g of waste was used.

In case of double addition of AE, the first portion of AE is added to the waste in the first step, and matter is intensively mixed for 5 minutes. Clots resulting from this step are left to dry for 3 days and then second portion of AE is added. The mixture is kneaded for 5 minutes again and then left to dry for another 3 days.

When the technique of the single addition of AE is employed, the waste is simply mixed with asphalt emulsion in a kneader. After that, the resulting sticky matter is left to dry for 5 days.

Leaching procedures

Two leaching tests were used for assessment of S/S efficiency. The acidic leaching test Toxicity characteristic leaching procedure[6] (TCLP) according to U.S. Environmental protection agency and aqueous leaching test according to Czech legislation analogous the German standard[7] were used.

TCLP is a procedure of assessing the potential for hazardous waste to leach in the ground water from the landfill. Extraction fluid # 1 with (buffer with $pH = 4.93 \pm 0.05$) was chosen for leaching of untreated waste and all specimens. The solid phase is extracted in an extractor bottle in the shaking machine; the liquid/solid ratio is 20 : 1. Ambient temperature is maintained at 23 ± 2 °C during the 18 hrs extraction period. After that, the leachate of untreated waste is filtered through a glass fiber filter with an effective pore size of 0.7–1.3 μm. The filtration was not carried out in the case of stabilized waste mixtures leachates, because they were clear.

Aqueous leaching test (AQ) was carried out according to the Czech regulations. Distilled water is used as a leaching medium in this procedure. The liquid/solid ratio is 10 : 1. The specimen is extracted for 24 hours; ambient temperature is maintained at 23 ± 2 °C. After that, the handling with the leachate is the same as in the case of TCLP.

The pH and specific conductivity was determined in all leachates. The concentration of lead was monitored because it exceeds the regulatory levels for untreated waste. Atomic absorption spectrometer was used for metal analyses; advanced mercury analyzer was used for the determination of mercury in the samples of leachate.

Results and discussion

The untreated waste was leached by both TCLP and aqueous extractants. Each procedure was repeated three times. The average results can be seen in Table I.

The highlighted numbers are concentrations of pollutants that exceed regulatory levels. The concentration of lead in the leachate exceeded the limit for both tests, therefore the waste is considered to be hazardous.

Asphalt emulsion was added to the waste in one or two steps; generally, slightly higher amount of AE was used for single addition. Specimens for leaching tests were prepared from these stabilized waste mixtures. Composition of individual specimens and the leaching results can be found in Table II and Table III.

When the leaching results of the aqueous test are compared to the Czech regulatory level, it is apparent, that even single addition of asphalt emulsion to the waste stabilizes the waste successfully.

From the leaching results of the TCLP can be seen, that the waste can be stabilized by double addition of AE to the waste by both anionic and cationic AE. Furthermore, single application of anionic asphalt emulsion Latexfalt stabilizes the waste with success.

Table I
Leaching results of untreated waste*

Leaching procedure		κ [mS m^{-1}]	pH	c_{Hg} [mg l^{-1}]	c_{Ni} [mg l^{-1}]	c_{Cu} [mg l^{-1}]	c_{Pb} [mg l^{-1}]	c_{Sb} [mg l^{-1}]	c_{Ba} [mg l^{-1}]
TCLP	untreated waste	533	5.38	$<4\cdot10^{-5}$	7.90	3.36	*1357.80*	1.43	6.10
TCLP	regulatory level	–	–	0.20	–	–	5.00	–	100.00
AQ	untreated waste	16	11.10	$<7\cdot10^{-5}$	0.03	0.21	*158.53*	<0.5	–
AQ	regulatory level	2000	5.50–13.00	0.05	50.00	–	10.00	–	–

– not determined κ specific conductivity c_{Me} concentration of the metal in the leachate
* presented values are average results from three parallel extraction procedures

Table II
Composition of stabilized waste mixtures and aqueous leaching test results

Technique	AE	m_W [g]	m_{AE1} [g]	m_{AE2} [g]	κ [mS m^{-1}]	pH	c_{Pb} [mg l^{-1}]
single	cationic	50	25	–	2.3	5.70	0.14
	cationic	50	25	–	2.6	6.75	0.16
	cationic	50	25	–	2.3	6.97	0.16
double	anionic	50	7.5	12.5	0.2	5.80	<0.1
	anionic	50	7.5	12.5	0.2	5.10	<0.1
	anionic	50	7.5	12.5	0.2	4.85	<0.1
	cationic	50	10	15	0.7	5.40	<0.1
	cationic	50	10	15	1.2	5.85	<0.1
	cationic	50	10	15	0.5	5.45	<0.1
regulatory level		–	–	–	2000	5.50–13.00	10.00

m_W amount of waste $m_{AE1,2}$ amount of AE in the first, second step added to the waste

Table III
Composition of stabilized waste mixtures and TCLP leaching results

Technique	AE	m_W [g]	m_{AE1} [g]	m_{AE2} [g]	κ [mS m^{-1}]	pH	c_{Pb} [mg l^{-1}]
single	anionic	50	30	–	461	4.93	0.48
	anionic	50	30	–	464	4.94	0.70
	anionic	50	30	–	463	4.94	0.48
	cationic	50	25	–	456	4.87	4.45
	cationic	50	25	–	457	4.87	8.26
double	anionic	50	7.5	12.5	465	4.88	0.24
	anionic	50	7.5	12.5	466	4.89	0.37
	anionic	50	7.5	12.5	465	4.89	0.44
	cationic	50	10	15	461	4.91	0.48
	cationic	50	10	15	465	4.91	0.55
	cationic	50	10	15	466	4.91	0.71
regulatory level		–	–	–	–	–	0.75

Conclusions

A technique of single and double addition of AE was applied for S/S of hazardous waste from the destruction of old munitions in the explosion chamber. Two extraction tests – aqueous leaching test according to Czech regulations and TCLP prescribed by U.S. EPA – were used for assessment of waste hazardousness; concentration of lead in the leachates exceeded the regulatory levels in both cases. Therefore, concentration of lead in the leachates of stabilized waste was considered to be a relevant parameter for assessment of stabilization efficiency.

Concerning Czech regulations, stabilization of the waste was sufficient even by single addition of cationic asphalt emulsion L 03/05 produced in the Czech Republic.

Generally, anionic asphalt emulsions are more suitable for S/S of alkaline waste than cationic AE. From the results of the TCLP extraction test it is evident, that the waste can be stabilized by single addition of AE Latexfalt efficiently. The use of double addition technique decreases the amount of AE, ensures lower concentration of contaminants in the leachates and allows application of either anionic or cationic AE.

The application of the developed techniques is very easy. In practice, the waste is simply mixed with asphalt emulsion and the stabilized waste mixture is then left to dry for several days. After that, the stabilized waste can be landfilled as a non-hazardous waste.

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L14 HOW TO TEACH ANALYTICAL CHEMISTRY AT UNIVERSITIES?

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In consequence of deep changes and rapid development, the modern analytical chemistry has became interdisciplinary science with strong links toward physical chemistry, metrology, chemometrics and information science. Its position among chemical sciences is, however, rather specific. The progress of other experimental chemical sciences is supported by analytical chemistry but vice versa its own progress requires a reliable background of other chemical sciences especially biochemistry, mathematics and physics. Nevertheless, analytical chemistry has also a considerable link to practical problems of the real world, so that it can also be considered a key to progress on national problems and to the safer future of mankind as well. It must be emphasized that analytical chemistry is an independent science and not a kitchen maid who would assist to the chemist in a basic research or solving practical problems of the real world.

Personal views and opinions of various prominent analytical chemists to the contents and tasks of the contemporaneous analytical chemistry were formulated on the basis of a broad competition organized by Fresenius and Malissa Sr[1]. However, I would prefer a quite simple definition, that analytical chemistry develops and applies methods, instruments and strategies to obtain informations on the composition and nature of matter in space and time[2].

Among characteristics of modern analytical chemistry the following items can be outlined:

a) Turbulent development of instrumental and separation sciences and methods.
b) Analytical evaluation of complicated objects of the real world.
c) Search for extremal detection limits, sensitivity and miniaturization, analytical approach to nanotechnologies and ultratrace analysis.
d) Analytical approaches for molecular biology, genomics and proteomics.
e) Analytical chemistry of element speciations and residuals in objects and organisms.

In spite of great efforts, there is no agreement at present in the definition, delimitation or characterization of the detection limit in connection to the analytical method or the analyzed sample which is indispensable for the analytical procedure[3,4]. Similarly no unified approach is available for the analytical selectivity which would enable to characterize exactly interferences[5].

Moreover, analytical chemistry also considerably assists in actual world problems to be solved:

f) Protection of the Environment.
g) Medicine, life sciences, health protection.

h) Development of nanotechnology.
i) Resources, Production of Energy and Food.
j) Material science.
k) Elucidation of criminal acts.
l) Research of remote objects.
m) Monitoring of toxic drugs.

In the frame of structural changes in chemistry education at present (bachelor and master education programs) the teacher of analytical chemistry at universities is faced against a number of difficult problems:

1. The proper position, length and structure of analytical chemistry in the basic and advanced chemistry curriculum.
2. Insufficient content of selected parts of physics, electronics, or instrumental science.
3. To find optimal proportion of method oriented and problem oriented analytical chemistry for solving problems of the real world.
4. To introduce more instrumental analysis and separation sciences.
5. To introduce more chemometrics and computer based analytical chemistry into the program.
6. To find the optimal proportion among methods based on chemical equilibria and instrumental methods.
7. How to manage the increased volume of organic analysis.
8. To introduce new domains of analytical chemistry.
9. To spend sufficient time for methods based on chemical equilibria.
10. Lack of modern but teacher-friendly instrumentation in laboratories for practical courses.

In fact, analytical chemistry should be a weighed mixture of traditional chemical and modern techniques with a solid foundation of scientific knowledge and sufficient flexibility against new techniques. In these connections, the role of experienced teacher and suitable text-books are irreplaceable. The text-book for basic and advanced analytical chemistry represents a complicated task, since it should be a suitable blend of the theoretical background and experimental and instrumental details reflecting the application in the real world[6].

The early Working Party of Analytical Chemistry (WPAC) in the Federation of European Chemical Societies developed considerable activities for the Education of Analytical Chemistry from 1975. This efforts were finalized in a detailed recommendation of a list of compulsory topics for basic curricula in analytical Chemistry accompanied by fixed hours in theoretical and practical courses. Such recommendation was asked to be obligatory for europewide universities especially in the basic curricula in analytical chemistry[7,8]. A comprehensive textbook has been compiled for these purposes[9]. It seems, however, to me this textbook is missing a systematic approach in the treatment of material which is the consequence of an increased number of contributors. In addition, some particular chapters are not balanced in contents and the organisation of the material could be criticised. An opulent attention is paid to COBAC and computer hardware and software. The X-ray diffraction is explained in unnecessary details for analytical classes. An unsufficient attention is given to methods based on chemical equilibria which is felt for titration methods. For biochemical methods and immunoassay more biochemistry would be useful. No gas and organic analysis is given explicitely in the book.

In spite of the WPAC efforts we will give now our list of recommended topics for theoretical analytical classes in the bachelor and master education program which reflects our opinion and experiences from a long-time practice in teaching analytical chemistry. Respecting the 3 years bachelor education program the following theoretical analytical items are considered as most suitable (cf. Table I). Practical applications are always blended with the theory of methods.

Table I
Selected topics valid for teaching modern analytical chemistry

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Theory of analytically significant equilibria and foundation of kinetiks
Gravimetry
Titrimetry
Selected electroanalytical methods (potentiometry, conductometry, electrolysis, polarography, amperometry and biamperometry, coulometry)
Selected optical analytical methods (Atomic and molecular absorption spectrometry (UV/VIS), IR-spectrometry, emission spectrometry, X-ray spectrometry, NMR, fluorimetry, light scattering)
Separation methods (liquid-liquid (solid) extraction, ion exchange, chromatography, electromigration methods)
Principles of chemometry and computer based analytical chemistry (data processing, evaluation of errors and uncertainties of analytical results)
Principles of quantitative chemical organic analysis
Sampling and technical aspects of analysis
Electron spectrometry and microbeam analysis and scanning microscopies
Analytical Aplication of lasers
Mass spectrometry
Principle of activation analysis
Thermal analysis

A particular attention should be paid to the contents of computation courses during the bachelor studies as well as to the modernisation and the contents of practical (experimental) courses. In these connections the extensive training of chemical detection of elements, earlier very popular, recedes into the background. Usually used commercial modern instruments in student laboratories are often rather black boxes joint with a computer. The tendency for shortening the program in practical student courses has a negative influence especially for analytical courses.

Some important problem oriented analytical topics are asked to be included in the advanced master education program which reflect the specialisation and research programs of the Institutes or the contemporaneous trends in analytical chemistry (Table II).

Table II
Some specialised topics for teaching advanced analytical chemistry

Analytical chemistry of the environmental objects
Toxicological and forensic analysis
Analysis of toxic elements and their speciations and residuals
Food analysis
Trace and ultratrace analysis
Material analysis
Quality assurance and quality control for chemical laboratories
Chemical sensors and biosensors
Biochemical analysis
Drug analysis
Immunological analysis (immunoassay)
Analysis of remote objects

An important function play seminars on research problems or actual trends in analytical chemistry with the active part of advanced or postgraduate students or invited researchers.

The analytical laboratory of today in the practice represents optimised and validised analytical procedures comprising research and technological standards with personal skill.

The full presentation of above topics is often limited by the time reserved for analytical classes. In such case the well experienced teacher decides about the approach and extent of particular topics with respect to the profile of the chemistry curriculum of the particular University.

The aim of analytical education at universities is not to produce operators of sophisticated instruments but analysts who know to solve chemical problems of the real world with a critical look into the whole chemistry. The analyst in the practice is often charged by a increased degree of responsibility and is also often asked to propose the solution of a actual real problem involving the results of his analyses.

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L15 PREPARATION OF VERBENONE FROM *TRANS*-VERBENOL BY THE OXIDATION OF MOLECULAR OXYGEN

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Introduction

The oxidation of alcohols to aldehydes and ketones is a fundamental reaction in organic synthesis. For environmental and economical reasons, metal-catalyzed reactions using molecular oxygen as a reoxidant are particularly very attractive. Traditionally, stochiometric and even over-stochiometric amounts of metal oxides or metal salts were used for such oxidation. A number of metal-catalyzed methods have been described as interesting alternatives. Many procedures using metal catalysts such as Co, Cu, Ru, Rh and Pt have been reported[1–5]. Many efforts have been made to find a synthetically useful method for Pd-catalyzed oxidation[6–10]. Many of these studies are quite interesting, but these systems are not applicable to a wide range of alcohols, especially for the effective transformation of primary and secondary aliphatic and aromatic or terpenic alcohols to aldehydes and ketones.

Knowledge

Aliphatic primary alcohols were smoothly oxidized to a corresponding aldehydes selectively in high yields without any formation of carboxylic acids and their esters[2]. A similar type of oxidation of alcohols catalysed by Pd using bromobenzene as a cooxidant was also reported, but the selectivity was low[6,7]. Secondary alcohols were also readily oxidized using the same catalytic system as described above[8,9,10]. Futhermore, even in the reaction of sterically hindered secondary alcohols such as borneol and menthol, the corresponding ketones were obtained in high yields. For the preparation of *cis*-verbenol as 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol (CASN 473-67-8) for pheromones, the basic problem is now the reoxidation of *trans*-verbenol (from the oxidation of alpha-pinene) to verbenone as 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-on (CASN 1830-32-5), because both are very difficult to separate by vacuum distillation. So, we decided to use Pd-catalyzed oxidation for secondary alcohols mainly for terpenic alcohols to obtain corresponding terpenic ketones. The oxidation of these compounds by means of chemical reactants such as chromium oxides, chromates, dichromates, perchromates etc. are very effective (total conversions and often total selectivities), but all reaction residues are very hard reprocessable or disposal wastes[11].

Materials

Palladium dichloride $PdCl_2$ and palladium diacetate $Pd(OAc)_2$ were purchased from SIGMA-ALDRICH Chemical Co. Inc., all others chemicals and SLP for GC were purchased from MERCK s.r.o. Brno, molecular sieves and zeolites were purchased from VÚRUP-SLOVNAFT a.s. Bratislava and were activated by calcination just before use. The concentrated mixture contents trans-verbenol and verbenone (from the oxidation of alpha-pinene) and as a sample for testing the pure cyclohexanol were used.

Apparatus and devices

Oxidation experiments were carried out in a 100 ml stainless steel reactor with electrical heating, metal Pt-thermometer and pressure gauge (manometer). The reactor was filled with reactants, catalyst and oxygen and shaken with a vibrator type *TP-1* (LP Prague, CZ). All analyses were carried out by GC method on the gas chromatograph type Chrom-4 with PC integrator type CI-100 (LP), with dual system and FID detector on the silicone SLP 10 % OV-17/Chromaton NA-W-DMCS with the column 2.000 mm, diameter 3/5 mm, temperature 120–250 °C, gradient 7 °C min^{-1}, injector temperature 250 °C. As internal standards dipropylphtalate and thymol were used[11].

Procedure

The oxidation of trans-verbenol was carried out by means of Pd/C catalyst (prepared from $PdCl_2$ by reduction with formaldehyde), either alone or impregnated with $CoCl_2$, $MnCl_2$ or $CdCl_2$, which were transformed to hydroxides by adding a solution of NaOH, by the known method[12,13]. It is supposed, that the oxidation of verbenol in alkaline media proceeds through an oxidative-dehydrogenation mechanism. Adsorbed hydrogen atoms are oxidized by oxygen atoms sorbed on the palladium surface or dissolved in the palladium lattice. Relatively low conversion in the oxidation of cyclohexanol and verbenol as secondary alcohols may also be result of catalyst deactivation due to blocking of the active sites by side-products formed in the aqueous alkaline solution[13].

The promoting effect of Co, Mn and Cd, used for impregnation of the Pd catalyst is probably connected with their ability to change the stability of the palladium surface toward the oxidation[12,13]. A cyclic allylic alcohols were oxidized by molecular oxygen on the Pd-acetate as catalyst with bromobenzene as cooxidant and with the presence of K_2CO_3 (14) and PPh_3 in DMF or THF as reaction media in molar ratio $Pd(OAc)_2 : PhBr : PPh_3 : K_2CO_3 = 1 : 2 : 3 : 3$ by[14]. In our case the argon was replaced by nitrogen.

A combination of Pd-diacetate with molecular sieve (MS) in pyridine or triethylamine in toluene as a reaction media for the oxidation of secondary alcohols into the corresponding ketones in molar ratio $Pd(OAc)_2$: pyridine = 1 : 5 was used. As it was reported, the palladium-catalyzed aerobic oxidation of these alcohols to ketones using a catalytic amount of Pd-diacetate, pyridine and MS in toluene under molecular oxygen atmosphere represents a conventional method which should have high conversion and selectivity[15,16].

Now the very suitable method for the selective oxidation of alcohols is using palladium(II)-supported hydrotalcite as catalyst. The calalyst was prepared by mixing of Pd-diacetate, pyridine or triethylamine and hydrotalcite in toluene, washing, drying under reduced pressure at the room temperature. The molar ratio $Pd(OAc)_2$: pyridine = 1 : 3 and then ratio Pd(II)-hydrotalcite : pyridine : verbenol = 1 : 10 :10 in toluene was used[17].

The improved method with using the Pd(II)-hydrotalcite as catalyst was used. The oxidation od verbenol with molar ratio of Pd(II)-hydrotalcite : pyridine : verbenol = 1:10 : 20 in toluene under atmospheric pressure of oxygen was performed. Sterically less hindered alcohols were converted to the corresponding ketones much faster than the hindered alcohols. The cyclohexanol was converted into cyclohexanone in about 80 % yield[18,19].

A simple discrimination of the promoter effect in alcohol oxidation and dehydrogenation over platinum and palladium catalysts[20] and common oxidation of alcohols with molecular oxygen on solid catalysts[21] were examined and also discussed.

For the comparison also the zeolite type ZSM-5 instead of hydrotalcite was used, but with lower results. The results of these experiments are summarized in the Table I.

Results and discussion

The oxidation of alcohols in alkaline media proceeds through an oxidative dehydrogenation mechanism. The conversion in the oxidation of secondary (terpenic) alcohols may also form as the results of the catalyst deactivation by means of the active sites by side-products formed in the aqueous alkaline media by dimerization or polymerization of the ketone[12,13].

The oxidation of secondary (terpenic) alcohols by means of Pd(II)-catalyzed oxidation by molecular oxygen give higher conversion and also selectivity. A catalytic cycle including the formation of a Pd(II)-alcoholate followed by the elimination of a Pd(II)-species and a carbonyl compound and then the formation of a palladium hydroperoxide species is also proposed[14–17].

The Pd(II)-supported hydrotalcite acts as a reusable catalyst for the oxidation of these alcohols to ketones in the presence of basic compounds (pyridine, pyrimidine, tert-amines), under atmospheric pressure of oxygen. This method gives high conversion and also selectivity. All these catalysts also have many advantages, such as an ease of

Table I
Oxidation of trans-verbenol into verbenone in various systems

	Catalyst	Medium	Temperature [°C]	Pressure [MPa]	Time [h]	Conversion [%]	Selectivity	Reference
1	5 % Pd/C	1.25 M NaOH	90	0.2	3	44	0.62	12.13
2	5 % Pd/C + 2 % Co – 0,7 % Cd/C	1.25 M NaOH	90	0.2	3	53	0.60	13
3	5 % Pd/C + 2 % Mn – 0,7 % Cd/C	1.25 M NaOH	50	0.2	3	50	0.56	13
4	Pd $(OAc)_2$ + PPh_3 + PhBr K_2CO_3	DMF	120	0.1	10	66	0.85	14
5	Pd $(OAc)_2$ + PPh_3 + PhBr K_2CO_3	DMF	105	0.1	8	80	0.68	14
6	Pd $(OAc)_2$ + pyridine + MS	DMSO	80	0.1	3	68	0.9	15
7	Pd $(OAc)_2$ + E_3N + MS	DMSO	80	0.1	3	65	0.88	16
8	Pd (II) hydrotalcite	toluene	80	0.1	3	75	0.86	17
9	Pd (II) hydrotalcite + pyridine	toluene	80	0.1	3	77	0.85	18.19
10	Pd (II) zeolite + Et_3N	toluene	80	0.1	3	50	0.55	

DMF = dimethylformamide, DMSO = dimethylsulfoxide, MS = molecular sieve

handling and preparation, in addition to good reusability for several times[18,19].

Conclusion

From the presented and checked methods of oxidation of secondary (terpenic) alcohols, the suitable are methods with palladium(II)- catalyzed or palladium(II)-supported hydrotalcite as catalysts and with use of molecular oxygen. *None* of these methods of oxidation *achieved the total conversion and selectivity*. This is possible to obtain in the only step by means of chromium trioxide in acetic acid and hexane, but also with the big amount of the very bad disposal wastes. For this purpose it is necessary to repeat the oxidations by molecular oxygen, because it is very difficult to separate both these compounds owing to their near boiling points[11]. *Trans*-verbenol and verbenone have the boiling points 250 °C and 253 °C (or 115 and 120 °C/1.33 kPa), respectively. For the preparation of *cis*-verbenol for pheromones is necessary to obtain quite pure verbenone. The impurities, such as *trans*-verbenol cause interference of the aggregate effect by the repellent effect in the commercial products of the pheromones against the spruce bark borers, mainly *Ips typographus*.This pheromone is widely used in coniferous forests of the Slovak Republic.

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L16 TRIHALOMETHANE FORMATION POTENTIAL – THE INTERESTING SOURCE OF INFORMATION ON SURFACE WATER QUALITY CHANGES UNDER NATURAL AND TECHNOLOGICAL CONDITIONS

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Introduction

Disinfection of drinking water can be regarded as the single most significant public health measure of the last century. The question of hazardous chemical by-products being formed during disinfection arose in the mid-1970s. Since then it has been the most debated and the most studied subject of the water industry. Leading to the re-assessment of not only drinking water disinfection practice, but also water treatment as a whole. The influence of disinfection by-products (DBPs) on the operation and design of water treatment plants has been increasing since early studies on trihalomethanes (THMs), one family of the disinfection by-products. Methods developed for limiting THMs production within water utilities have concentrated on precursor removal and disinfectant selection. The major technologies for removal DBPs precursors are enhanced coagulation, granular activated carbon adsorption, and membrane filtration.

Predictive models for estimating THM formation exist, but because eventual THMs concentrations cannot be calculated precisely from conventional analyses, methods to determine the potential for forming THMs are useful in evaluating of water treatment processes for efficiency of precursors elimination or water sources for predicting THM concentrations in a distribution system. To obtain reproducible and meaningful results, it is necessary to control such variables as temperature, reaction time, chlorine dose and residual, and pH.

The trihalomethanes formation potential (THM FP) of the waters is characterized as a function of their macromolecular content. Using this method, namely under conditions of standard test, there were found very good relationships between THM FP, the origin, and the macromolecular contents of various waters.

The monitoring of THMs occurrence in treated water from different sources confirmed that only relatively small part of nonspecific organic matter is able to act as precursors of undesirable THMs. In spite of numerous endeavors for prediction of THMs formation from information on water quality, by using mathematical models, it is evident that – for majority of cases – the character of THM FP test will retain empirical.

Several authors in mid-1970s have studied the influence of different technological tools used during drinking water treatment process on THMs level after disinfection by chlorine. Number of publications where the THM FP has been used for characterization of water quality and its changes during different processes increased significantly in the second half of 1990s.

For determination of THMs in water – used for drinking water treatment and treated to potable water supply – in the Czech Republic there was directed congruous standard[1], which is adequately applicable for THMs determination for THM FP test purposes.

Experimental

Instruments and equipments

Gas-liquid chromatograph, Hewlett – Packard 5700A, USA, 1974, with FID for determination of THMs, including of data station for CSW, DataApex, USA, 1997;

Spectrophotometr, UV/VIS Helios Gamma, Spectronics, Great Britain, 2001, in basic version with data station (software: Unicam Vision 32), sets of silica and glass cuvettes of thickness 10 and 50 mm;

Mixing apparatus, MK 6 (Gestra s.r.o., Sedloňov, Czech Republic), 1997 (six-position mixing apparatus with electronic rotations control and automatic operation switching);

Ozone generator, Azcozon VMUS-4, Canada, 1998 (4 g h^{-1} O$_3$ for 5 l min^{-1} dry air flow; 8 g h^{-1} O$_3$ for 5 l min^{-1} oxygen flow).

Technological tests

During laboratory tests of clarifying there was respected proper sectoral technical standard[2] for performing tests and evaluation of obtained experimental data. The original technological installation was assembled for laboratory experiments with ozone, as the agent of surface water treatment, and actual operational parameters of individual tests were specified with regard to technical capabilities of this instalation.

Results and discussion

Utilization of THM FP as a sensitive tool for water quality assessment was the theme of author's dissertational thesis[3]. Some aspects of given problem were published in the paper[4] and preliminary results were presented in a form of lectures[5,6].

Technical equipment of the laboratory enabled quite reliable determination of THMs. There were find satisfactory values of parameters of the method for operational range of concentrations THMs from 50 to 1100 μg l^{-1}. Conditions of THM FP test are adjusted – in the Czech Republic – by relevant technical standard[7].

There were monitored changes of chloroform and free chlorine concentrations only during the study of kinetics of chemical reaction, which are under way of the THM FP test. There was used simplified model of overall reaction kinetics for results processing. From results it follows that THMs formation reflects the content of organic matter of the given type. On the Fig. 1. it is for illustration presented the result of an actual test as a characteristic.

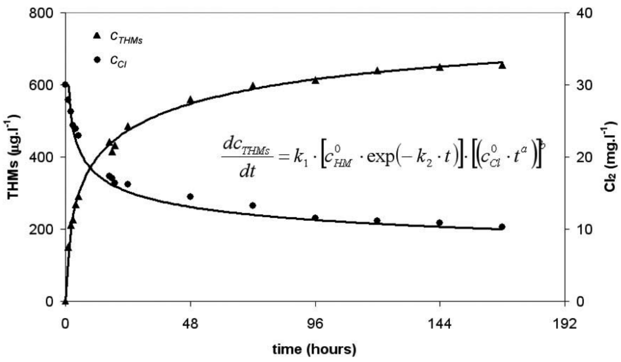

Fig. 1**. Kinetics of THMs formation under conditions of THM FP test**
Sense of model symbols: c_{THMs}, c_{HM} and c_{Cl} are concentrations of THMs, humic matter and free chlorine, in µg l^{-1}, mg l^{-1} and mg l^{-1}, respectively; upper index ^{0} indicate the initial value; t is time in hours; k_1, k_2, a and b are parameters of model in sense of constants

Practical applicability of THM FP test was verified for the assessment of water quality and its changes in laboratory scale and orientationally also for the assessment of water quality from selected water treatment plants. Acquired THM FP values were compared with another indicators of water quality, in order to highlighted the THM FP test capability to reflect changes of chemistry of water with raised content of humic matter.

Results of coagulation tests were evaluated on the basis COD_{Mn}, COD_{Cr}, THM FP, selected spectral parameters and residual concentrations of used coagulant. Assembled data documents testifying capability of the individual used qualitative criteria and possibilities of their comparison.

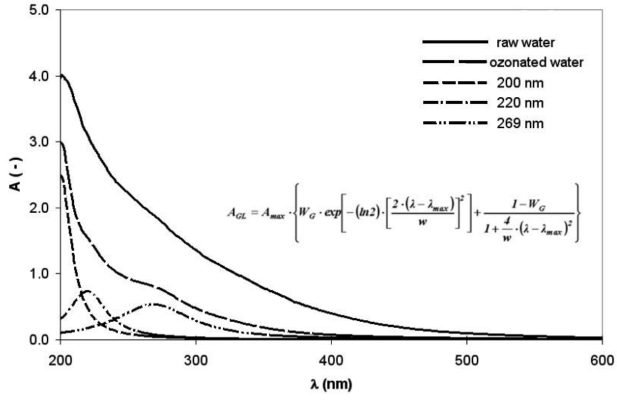

Fig. 2. **Example of deconvolution of UV-VIS spectrum of ozonated water**
In model of individual absorption band is W_G weight of Gauss curve, weight of Lorenz curve is then (1– W_G)), A_{max} is height of band (absorbancy in maximum), w is band half-width (i.e. width of band in one-half of its height), λ is wavelength and λ_{max} then wavelength of band maximum and A_{GL} is absorbancy corresponding to wavelength λ. Curves are responsible for absorption bands with estimated values of model parameters

Attention was focused particularly to search for correlations between ozonization conditions and THM FP values. Deconvolution of UV-VIS spectra was used for acquirement of parameters of presumptive newly formed absorption bands[8]. Example of numeric decomposition of the actual spectrum is presented on the Fig. 2.

For sufficient ozone dose (in conjunction with consecutive well lead coagulation) there were achieved – in compare to simple clarifying – for COD_{Mn} reconcilable, and for THM FP thereafter markedly better, final results.

To sketch in the importance of the THM FP in the assessment of water quality and its changes during natural and technological processes there were compared values of used water quality indicators in testing of real samples of surface water and samples of water from selected water treatment plants. In the overall processing of data acquired in study of above appointed processes, there was observed interesting relation between THM FP and COD_{Mn}. Its sigmoidal trend indicates that THM FP prove distinctly different response to content of organic matter in tested water than COD_{Mn} and it indicates that the content of THMs percursors is apparently slimed more effectively by this process, than the total organic matter content (see: Fig. 3.).

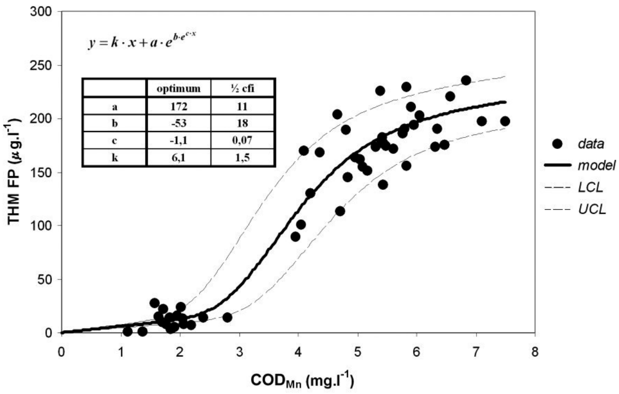

Fig. 3. **The THM FP and the COD_{Mn} correlation**
As a model was used a relation *y* = *k.x* + *a.exp*[*b.exp(c.x)*], where *a*, *b*, *c* a *k* are empirical constants, computed by non-linear regression, *y* is THM FP and *x* then COD_{Mn}, ½ *cfi* is one-half of confidence interval, *LCL* and *UCL* are lower and upper limit of confidence band, respectively, on the level of statistical significance α = 0.05)

Conclusions

The trihalomethane formation potential (THM FP) test presented an empirical method useful for characterization of nonspecific water pollution by organic substances, which are easily liable to chemical and biochemical changes.

Modified method of the THM FP determination in standard test conception was used for water quality monitoring and for effectiveness assessment of drinking water treatment laboratory tests. Clarifying, ozonization and clarifying after preozonation were used for model surface water treating. Obtained values of the THM FP were compared with those of other water quality indicators. The attention was given to the analysis of UV-VIS spectra of tested water samples.

In conjunction with standard and newly developed methods of water analysis, the test of THM FP can serve for intensifying the knowledge of the continuance of natural selfpurification processes in surface waters and for searching of more efficient procedures of surface water treatment.

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L17 DETERMINING BIODEGRADATION OF SYNTHETIC MATERIALS IN BIOTIC ENVIRONMENT

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Introduction

Polyvinyl alcohol is a transparent crystalline polymer exhibiting good mechanical properties. As opposed to other vinyl polymers, it is well soluble in water and at the same time resistant to many organic solvents. Its important feature is biological degradability. It is mostly used as a protective colloid in aqueous dispersion systems, in cosmetics, in medicine in contact lens manufacture, in the pharmaceutical industry. An interesting PVAL application lies in the field of agriculture and agricultural chemistry, where water-soluble films containing disinfection for applying active ingredients on seeds are prepared, or capsules slowly releasing insecticides and fertilisers.

Packing technology utilises PVAL for producing water-soluble films and also multi-wall packing materials. Films of pure PVAL do not require removal from a preparation before use because they both melt together. Accurate dosing of preparation is already carried out during packing, hence repeated weighing during application is unnecessary.

Films of pure PVAL are quite sensitive to atmospheric humidity, these results in greater demands when storing packing films and products packed in this film. There is, therefore, an effort to modify PVAL so that resultant film mainly shows increased resistance to atmospheric humidity on the one hand, and on the other, after application, that it exhibits a shorter biodegradation time chiefly in an aqueous environment.

Modification of PVAL, either chemical or physical, was dealt with in a number of works, for example on blends of PVAL/starch[1], PVAL/lignin cellulose[2,3], PVAL/gelatin[4], PVAL/poly(3-hydroxybutyric) acid[5], PVAL/chitin[5,6] and PVAL/dialdehyde[7], whereas consequences and effect of these processes on biological degradability have not been quite consistently described. Biodegradability of the blends can be affected by physical and chemical processes running during their preparation (heat processing) and need not be an additive quantity, i. e. given by summed up degradability of individual components. They should thus be studied not merely from the viewpoint of required useful properties but also with regard to their further fate in the environment. Biodegradability testing, therefore, has to be an inseparable element when developing new materials.

Experimental

Tested Samples

Tests were run on starting components and on a number of their blends containing 58 – 84 % PVAL (Sloviol P88-08, hydrolysis degree 88 mol %, acetate group content 12 %, produced by Novácké chemické závody, SR), 0–24 % collagen hydrolysate CH (Hykol E, nitrogen content 14 %, molecular weight M_w 1000 g mol^{-1}, produced by Tanex, Jaroměř, ČR), 9–18 % glycerol G (from Lachema, ČR), and 5–22 % starch (natural untreated – Meritena 100, plasticised in laboratory; commercial plasticised starch Mater Bi). Mentioned components were processed in a Brabender mixer for 5 min at 130 °C under atmospheric pressure. Films[8] were prepared from produced blends by blow-moulding.

Determining Biodegradability

Biological degradability of tested samples was studied in an aerobic aqueous environment. Level of pH was held within an interval of 7.0 ± 0.5. Inoculum used was non-adapted and PVAL-adapted activated sludge from the municipal wastewater treatment plant. Actual adaptation of inoculum to PVAL always proceeded for 6 days.

Respirometric test according to ASTM D 5209-92

This determines CO_2 produced by a microbial culture sharing in degradation of the substance under test[9]. The tested sample is the only source of organic carbon and energy in the medium. Produced carbon dioxide is absorbed in a solution of hydroxide and then determined by titration. Biodegradability criterion is the ratio of produced CO_2 to CO_2 calculated from carbon content in initial sample (% – D_{CO2}). Experimental conditions analogous to those of following test.

Modified Zahn-Wellens test (ČSN EN 29888)

This continuously follows decrements of PVAL from cultivation medium (filtrate). Aerobic conditions are ensured through uninterrupted aeration[10]. Biodegradability criterion is the ratio of removed/disposed of PVAL to initial PVAL (% – D_{PVAL}).

Calculation of Substrate Rate Constant "c" According to 1st-Order Kinetics Equation

Measured dependencies $D_{CO2} = f(t)$ and $D_{PVAL} = f(t)$ were described by regression applying an equation for substrate 1st-order kinetics (1):

$$D = D_{\max} \cdot \left(1 - e^{-c\cdot\left(t - t_{lag}\right)}\right) \qquad (1)$$

where is D_{max} regression coefficient, representing limit value D_{CO2}, D_{PVAL} in infinite time (%), c is negative value of rate constant – c_{DPVAL}, c_{DCO2} (hour^{-1}) and t_{lag} shift on time axis to express lag phase (hour).

Calculation of regression coefficients was performed using the least-squares method applying Statistica CZ 6.1 program. In regressive processing of dependencies $D_{CO2} = f(t)$ and $D_{PVAL} = f(t)$, values taken into account were only those corresponding to actual biodegradation (meaning PVAL degradation lag phase – t_{lag} – was subtracted). The regression coefficient achieved in regressive analysis was min. 0.99.

Results and discussion

Modification of PVAL was assessed from two viewpoints: that of processing and ecology. The chief criterion from the processing[8] viewpoint was reducing polymer price, increasing resistance of polymer films to atmospheric/aerial humidity and increasing water solubility; these assessments are not a part of this work. The objective of the environmental viewpoint was to achieve such degradation rate as to bring about in practice, particularly at wastewater treatment plants, potentially fast degradation of PVAL itself.

Utilising waste materials and renewable sources when modifying synthetic polymers is appropriate. For that reason, the substance selected from a number of waste materials to modify PVAL was collagen hydrolysate (CH), which arises following enzymatic hydrolysis of chrome-tanned shavings. Substances selected from a number of renewable sources were lignin 2 (Li), natural untreated starch (S), laboratory-plasticized starch (LPS) and commercial plasticized starch with added caprolactam – Mater BI (PS).

Results are processed from the perspective of a municipal or industrial wastewater treatment plant. Emphasis is placed on lag phase of degradation regarding time during which wastewater is retained in aeration tank, and maximum achieved degradation as seen through residual values on discharge outlet from wastewater treatment plant.

Methodical Possibilities for Observing Biodegradability of Soluble and Insoluble Polymeric Substances in Aqueous Aerobic Environment

An apparatus was designed and constructed enabling to simultaneously execute the ASTM D 5209-92 test and Zahn-Wellens static test. This apparatus enabled to evaluate the biodegradation course by means of two criteria, decrease in substrate and produced CO_2 (D_{TOC}, D_{PVAL}, CO_2), in one reactor while retaining same conditions and, above all, without disturbing the gaseous phase.

As it turned out, combining more methods for determining biodegradability of mixed materials/blends is indispensable for assessing the effect of ingredients on a problem polymer, for example (Fig. 1.)

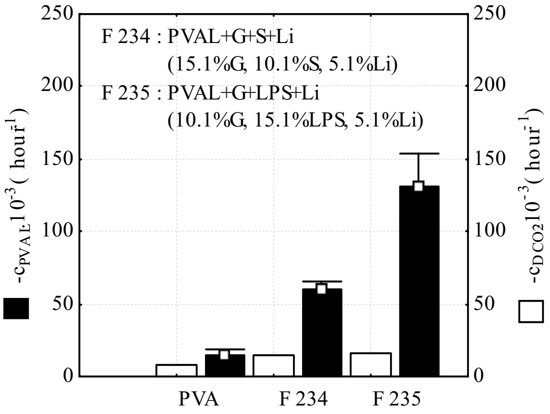


Fig. 1. **Comparing values of rate constants of "c ± 95 % reliability interval" PVAL decrease, and of produced CO_2 of particular films in presence of PVAL-adapted activated sludge**

Observing Biological Degradability of Polymeric Blends based on PVAL and of Their Individual Components

Principal results may be summarised in particular conclusions as follow:

- Physico-chemical interactions between collagen hydrolysate and PVAL do not exert considerable influence on PVAL biodegradation (2nd degradation degree) as evaluated by D_{CO2}.
- During degradation in a PVAL- non-adapted microbial

environment, a lag phase no shorter than at least 10 days always appears. After adaptation, all components undergo degradation without lag. Adaptation is accompanied with an increase in PVAL-metabolising bacteria by about 5 orders (CFU ml^{-1}).

- PVAL-degrading bacteria retain their ability of immediately degrading PVAL (without lag) for a period of at least 14 days of "starving", i. e. since last addition of substrate.
- Biodegradation of PVAL and of mixed films is very sensitive to any changes in environment – temperature and other substrates. At lower temperatures (5 °C), biodegradation of sole PVAL does not take place for as long as 70 days. Presence of a readily degradable substrate leads to prolonging the PVAL-degradation lag phase by as much as 22% when PVAL-non-adapted activated sludge is present.
- It was proved in degradation of mixed films by means of PVAL-adapted activated sludge that the mass ratio of modifying agent (starch, collagen hydrolysate) to plasticising agent significantly affects biodegradability of PVAL itself (Fig. 2.).

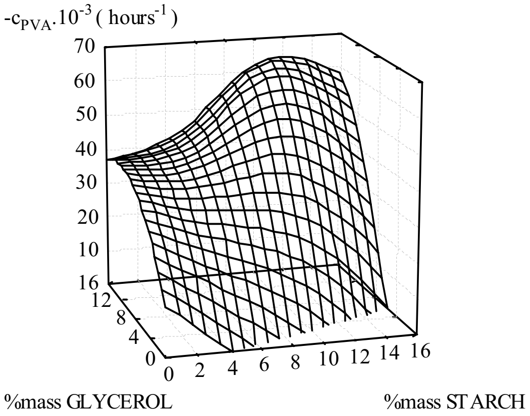
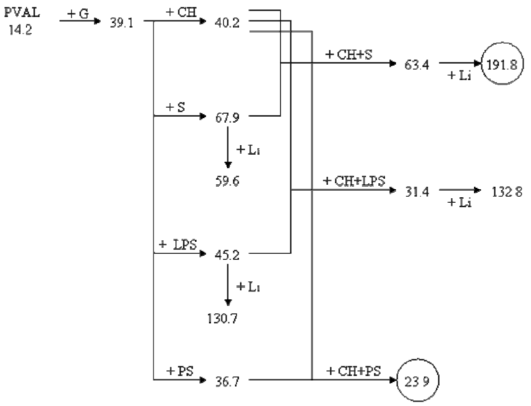


Fig. 2. **Effect of mass ratio of G and S contained in films on PVAL degradation rate – c_{PVAL} (hour^{-1})**



Scheme 1 **Influence of modifying agent on PVAL degradation assessed by c_{PVAL} (hour^{-1})**

For final *assessment of particular modifications* (Scheme 1), combinations with which highest degradation rate according to c_{PVAL} (hour^{-1}) was achieved.

Results presented in Scheme 1 indicate that lignin could be regarded as a potential inductor for PVAL degradation. As is obvious in the scheme, the best combination of modifying agents as regards degradation is G/CH/S/Li at dosages of 2.5 % CH, 15.1 % G, 10.1 % S and 2.5 % Li. In this case, degradation rate was accelerated approximately thirteenfold. Its addition, as far as quantity is concerned, is rather "symbolic" (2.5 %). However, processing and mechanical characteristics of this film were not sufficient for modifying PVAL.

Conclusion

Due to the fact that starch (natural untreated, plasticised) as well as lignin 2 markedly impaired processing and application properties of mixed film, the agent proposed to modify PVAL was collagen hydrolysate (Hykol E). In co-operation with STU, Bratislava, new types of mixed polymers based on PVAL/CH were proposed; these are very well soluble when cold, exhibit higher degradation resistance during processing in an extruder, comparable or similar mechanical properties and at the same time better biodegradability as compared to sole PVAL. They are suitable for use in agriculture, fishing and for some industrial applications.

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L18 THE ROLE OF THE CAROTENOID PIGMENTS IN YEAST CELLS UNDER STRESS CONDITIONS

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Introduction

Carotenoids are familiar to all of us through the orange-red colors of popular foods like oranges, tomatoes and carrots, and the yellow colors of many flowers. They are components of the cells of the red yeasts. Carotenoids especially act in protection against destructive photo oxidation, heavy metals and another stress factors.

The main role of carotenoids is the ability to their antioxidization. This ability is the result of energy transfer reactions in which the energy of triplet state sensitizers by carotenoids or singlet oxygen is transferred to carotenoid molecules in the ground state, forming triplet state carotenoid molecules. For example, presence of the carotene pigments in microorganisms plays a key role for life in the extreme environment. Carotenoid formation by microorganisms is dependent on cultivation conditions[1,2].

Among the all molecules of the carotene pigments, β-carotene is probably the most important as a precursor of vitamin A. Carotenoids may be linked to a sugar by a glycosidic or ester bonds. Such compounds are known for plant, animals, yeasts and bacteria. It is commonly thought that their antioxidant properties functioned as the protection against stress. Metals occur naturally, and several of them are essential components of global ecosystems. They are present in the environment with a wide range of oxidation states and these differences are related to their toxicity. Metals such as copper (Cu) and zinc (Zn) are essential to life, although high concentrations are toxic.

The aim of presented work is to study the role carotenoid pigments under the influence of heavy metals as the exogenous stress factors in some red yeast.

Materials and methods

The yeast strains *Rhodotorula glutinis* CCY 20-2-26, CCY 20-2-33 and *Sporobolomyces roseus* CCY 19-6-4 were obtained from the Czechoslovak Collection of Yeasts (CCY),

Table I
Concentrations of stress elements

Strain	$ZnCl_2$ [mM]	$CuCl_2$ [mM]	H_2O_2 [mM]	$ZnCl_2+H_2O_2$ [mM]	$CuCl_2+H_2O_2$ [mM]
R. glutinis CCY 20-2-26	2	3	6	1.5 + 5	2.5 + 5
R. glutinis CCY 20-2-33	4	3	8	2.5 + 5	2.5 + 5
S. roseus CCY19-6-4	2	3	8	1+5	2.5 + 5

Table II
Percentage content of the carotene pigments in yeast cells without and with stress

Strain CCY 20-2-26	Lutein	Torulen	Lycopene % from total (CP+FPW) carotene	α-karoten	β-karoten	Fytoen
CP	0.85	0.02	1.06	9.62	37.83	46.87
FPW	0.51	0.05	0	0.12	0.04	2.91
CP Cu	0	1.26	0.19	2.13	8.61	86.2
FPW Cu	0.63	0	0	0.11	0	0.86
CP Zn	1.96	1.06	1.08	6.23	46.78	28.93
FPW Zn	0	0.27	0.04	0.51	1.98	11.16
CP H_2O_2	0	2.37	0.29	2.48	6.88	87.97
FPW H_2O_2	0.57	0	0.26	0.23	0.53	3.95
CPZn + H_2O_2	3.21	0.18	0.40	4.01	8.81	83.15
FPW Zn + H_2O_2	0	0	0	0.05	0.07	0.13
CP Cu + H_2O_2	0.42	0.66	0.59	4.57	13.48	52.52
FPW Cu + H_2O_2	0.44	0	0.16	2.31	8.20	16.64

Table III
Percentage content of the carotene pigments in yeast cells without and with stress

Strain	Lutein	Torulen	Lycopene	α-karoten	β-karoten	Fytoen
CCY 20-2-33			% from total(CP+FPW) carotene			
CP	10.22	9.60	0.66	8.15	13.35	24.84
FPW	3.28	1.92	0.72	1.07	3.57	22.61
CP Cu	3.42	7.25	1.55	1.33	4.69	10.47
FPW Cu	32.40	1.58	1.69	3.36	8.04	59.99
CP Zn	1.51	7.35	1.00	6.59	13.47	45.45
FPW Zn	3.70	3.38	1.29	0.84	4.05	11.37
CP H_2O_2	23.52	6.98	0.16	1.29	3.61	18.95
FPW H_2O_2	13.57	13.77	0.33	0.34	2.97	14.07
CP $Zn+H_2O_2$	5.96	5.4	0.48	1.28	5.61	62.86
FPW $Zn+H_2O_2$	5.51	5.60	0.13	0.13	1.26	5.72
CP $Cu+H_2O_2$	4.37	4.32	0.99	6.38	9.13	30.42
FPW $Cu+H_2O_2$	16.72	4.42	1.15	5.34	4.64	15.99

Table IV
Percentage content of the carotene pigments in yeast cells without and with stress

Strain	Lutein	Torulen	Lycopene	α-karoten	β-karoten	Fytoen
CCY 19-6-4			% from total (CP+FPW) carotene			
CP	0	1.52	0.62	4.95	15.19	76.70
FPW	0.78	0.13	0	0	0.05	0.06
CP Cu	1.17	2.74	0.53	2.86	12.52	57.49
FPW Cu	4.09	3.37	0.06	0.21	4.15	10.81
CP Zn	5.52	1.05	1.75	7.03	79.28	18.47
FPW Zn	0.42	0.01	0.13	0.19	1.18	3.41
CP H_2O_2	0	20.97	0.73	4.90	22.86	22.56
FPW H_2O_2	14.26	7.18	0	0	2.94	3.61
CP $Zn+H_2O_2$	1.41	1.02	1.17	7.53	25.32	64.95
FPW $Zn+H_2O_2$	0.40	0	0.05	0.02	0.10	0.27
CP $Cu+H_2O_2$	2.31	1.87	0.88	5.41	20.48	66.34
FPW $Cu+ H_2O_2$	0	0.07	0	0.27	1.64	0.72

Bratislava, Slovakia. The strains were stored at malt-agar at 4 °C.

All strains were growth on synthetic growth medium with 2 % glucose to stationary phase of growth. The stress substances were added on concentration (see Table I) into cultivation medium before cultivation. The biomass was separate by ultrasound on the two part: cells treatment by ultrasound (CP) and isolate fibrillar part wall of the cells (FPW) Total carotenoids were extracted from both part yeast biomass according to Marova[3].

Extracted carotenoids were individually identified and quantified by RP-HPLC using a chromatographic system described previously[4]. Samples (10 microliter valve) were filtered through PTFE filters and injected onto Nucleosil 100 C18 column, 7 mm, 150 × 4.6 mm with guard column 30 × 4.6 mm, 7 mm that had been equilibrated with a mobile phase (methanol/water; 95 : 5). Isocratic elution was carried out at 45 °C by a flow rate of 1.0 ml min^{-1}. Visible detection of lycopene, α-carotene and β-carotene was achieved at 450 nm.

Results and discussions

The yeast strains were stressed at different experimental conditions including the presence of metal ions (Zn^{2+}, Cu^{2+}) and H_2O_2. Tables II–IV show the carotene composition of fibrillar part of wall (FPW) and cells part (CP) of the strains incubated with Zn^{2+} and Cu^{2+} ions and H_2O_2. These values are compared with production of this pigment under optimal conditions. The FPW was 3.8–4.2 % dry weight of CP. Increased levels of carotenoids in FPW were found in the stress presence. It might be explained by the fact that FPW is the site of the primary contact of the cell with its environment. While *R. glutinis* CCY 20-2-26 and *R. glutinis* CCY 20-2-33 can be characterized by high content of β-carotene, *Sp. roseus* CCY 19-6-4 displays increased content of both β-carotene and phytoene. Metals caused substantial change in the carotenoid composition of the treated yeast strains. For instance, we observed increase of luteine and rapid decrease of phytoene in FPW of *R. glutinis* CCY 20-2-33 cultivated under Zn^{2+} ions in comparison to unstressed cultures. On the other hand, level of luteine was very low in *R. glutinis* CCY 20-2-26 and increased amount of α-carotene was found in both CP and FPW of the strains incubated with metal ions. Synergetic action of Zn^{2+} ions and H_2O_2 and also Cu resulted in significant enhancement of fytoene for *R. glutinis* and β-carotene for *Sp. roseus*. Also, improved levels of β-carotene in FPW mainly by Cu^{2+} ions were observed for strains CCY 20-2-33 and CCY 19-6-4. The obtained results can be explained by protection role of the carotene in cells under stress conditions.

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L19 GEL TECHNIQUES IN ENVIRONMENTAL ANALYSES

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Gel techniques, DET, diffusive equilibrium in thin-films technique and DGT, diffusive gradients in thin-films technique, invented by Davison and Zhang in 1991 (ref.[1,2]) are successfully used in environmental analyses for sampling solute species in aquatic systems. Usage of both these techniques is still increasing for the pollution monitoring and environmental studies as trace element cycling, phytoavailability of nutrients and toxic species, for study of processes taking place in sediments and soils. Reliable measurement of trace species in environmental systems is essential for all these studies, but is difficult, partly because the distribution of chemical species often changes during sampling and storage. Both gel techniques overcome these problems providing an in situ means of quantitatively measuring labile species in aqueous systems. DET technique relies a similar equilibration principle as peepers, but rather than confining the solution to compartments, it uses a thin film of hydrogel for equilibration with solutes in waters. Using the DGT technique trace metals are measured easily by accumulation them on a selective binding resin after passage through a well-defined diffusive gel layer. The polyacrylamide diffusive gel layer used in this work has an open structure that allows free diffusion of inorganic labile species and some metal organic complexes. Gel techniques can be used to distinguish between small and large species, which we have ascribed to inorganic and organic species present in aquatic systems. The discrimination of measured species by DGT is also kinetically based according to similar principles to those of anodic stripping voltammetry[3]. When used in solution, it measures those species that can dissociate within the time it takes for ions to be transported through the diffusion layer (typically minutes). When DGT is applied to sediments and soils, metal fluxes are measured and estimation of metal concentration in pore waters is possible under defined conditions. The interpretation of measured results needs more other information about this system[4].

The simple plastic assembly can be deployed in situ in rivers, lakes, and oceans, sediments or soils and effluents where it automatically separates metal ions from solution. Metals are subsequently analyzed conveniently in the laboratory, by direct measurement of the concentration in gel/resin layer with the techniques capable of analyzing solids, such as XRF or PIXE[1]. Alternatively, metal ions in resin layer can be eluted using a known volume of acid solution. ET AAS or emission spectrometry can be used for the determination of the concentration of ions in the acid eluent. The ICP MS is very useful due to its detection power in this instance.

In the lecture our results obtained with both these techniques DET and DGT used for speciation analyses of selected metals in nature waters[5], sediments[6,7] and soils[8] are presented.

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L20 A COMPARISON OF DGT TECHNIQUE WITH BIOMONITORING TECHNIQUE USING AQUATIC MOSS *FONTINALIS ANTIPYRETICA*

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The quantitative determination of metals in the several compartments of aquatic ecosystems constitutes an important task for the identification of pollution sources, evaluation of contamination or decontamination trends and ecological quality control. However, analytical results from river water sampling can give information only on momentary water concentration and represent a partial approach for ecosystem assessment. In addition, collected water samples do not provide accurate information about the bioavailable, i. e. potentially hazardous fraction of trace pollutants.

Living organisms are successfully used to gain time-integrated information about the biological impact of trace metals pollution. Different species of mollusks and bryophytes are described as biological indicators of surface waters contamination by heavy metals[1–8]. From the bryophytes an aquatic moss *Fontinalis antipyretica* (Fig. 1.) is the most used, because it is widespread in most European rivers, it reacts rapidly to changes in water quality and it is able to accumulate pollutants very rapidly and over long periods[9,10].

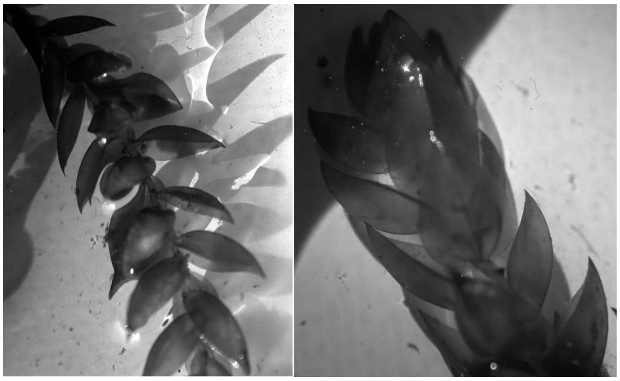

Fig. 1**. The detail of an aquatic moss Fontinalis antipyretica**

In 1994 the diffusive gradients in thin films technique (DGT) was for first time used to measure concentrations of metals in natural waters[11] and it was postulated as possible alternative to living organisms as a means of assessing metal pollution[12].

The DGT technique uses two sheets of polyacrylamide gels closed in a simple plastic piston form unit. First clear (diffusive) polyacrylamide gel is backed by a further thin film of gel, containing cation exchange resin which is selective for trace metals[12]. The resin maintains a zero concentration of trace metals at the side of diffusive gel furthest from the deployment solution. A concentration gradient is established across the diffusion layer between the resin gel and bulk solution contacting the diffusive gel surface (Fig. 2.). Trace metals are collected by resin according to the diffusion of the species through diffusive gel. The mass *M* of metals accumulated in unit area of the resin, which is experimentally determined after DGT deployment, is equal to the integral of the flux over the deployment time *t* (Eq. 1). From Eq. 1 concentration of metals in the bulk solution can be consequently calculated (Eq. 2), if diffusive coefficient in diffusive gel *D* and thickness of diffusive gel Δ*g* are known.

$$M = \int_{t=0}^{t} F(t)\,\mathrm{d}t = \frac{D}{\Delta g}\int c_\mathrm{b}(t)\,\mathrm{d}t \qquad (1)$$

$$c_\mathrm{b} = \frac{M \cdot \Delta g}{D \cdot t} \qquad (2)$$

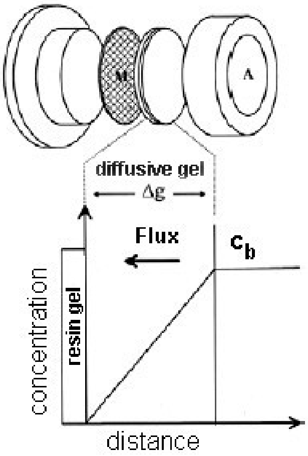

Fig. 2. **The principle of DGT technique. Modified picture of Ernstberger et. al[16]**

Even though more than 60 research articles were published within last 10 years dealing with the use of the DGT technique in environmental monitoring, developing DGT technique and comparing DGT technique with other analytical techniques, the question if DGT technique is able to substitute biomonitoring techniques is still unanswered.

Given lecture will try to clarify this issue, summarizing the results obtained during the one year experiment running at Svitava river within February 2005–2006. This experiment sets on self a task to compare the DGT technique with biomonitoring technique using moss bags. The Fontinalis antipyretica moss species were chosen in this experiment as these are commonly used in strategic environmental monitoring in Czech Republic[13,14] as well in foreign countries[8,9,15] and concentrations of heavy metals, specifically Hg, Zn, Pb, Cr, Ni and Cd were monitored.

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L21 THE IMPLEMENTATION OF THE CONCEPTION OF EDUCATION IN THE AREA OF CRISIS MANAGEMENT

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Abstract

Information about:

- the prepared Bachelor's programme of studies „Protection of population," field of study „Crisis management and protection of population," the starting date, the type and form of studies, the profile of a graduate, programme description and the field of study, courses;
- the accredited educational programme „Planning and management of crisis situations," its form, objectives, target groups, educational courses and the educational plan.

Key words

Crisis management, protection of population, accreditation, programme of studies, educational programme, courses.

Introduction

In accordance with the topic of the conference the paper is focused on a brief presentation of the current involvement of the Chemical Faculty of the Brno University of Technology in teaching disaster and crisis planning and management with a view to the protection of population. In this connection also some partial problems are indicated which occurred during the preparation of university education in the given area.

In January this year the faculty submitted an application for accreditation of the Bachelor's programme of studies „Protection of population," the field of study „Crisis management and protection of population".

Teaching in the accredited educational programme „Planning and management of crisis situations" was begun at the Brno University of Technology in January as well.

The Bachelor's programme of studies „Protection of population" (B9111), field of study „Crisis management and protection of population" (JKOV)

The start of tuition is planned in the winter semester of the academic year 2005–2006 for the full-time form of study.

The profile of a graduate includes:

- an introductory qualification part
- parts expressing acquired knowledge, abilities and habits of graduates.

The qualification part is derived from „The Conception of Education in the Area of Crisis Management," part 3.2. The original proposal of the qualification part also contained an outline of career regulations, depending on gaining practical experience and improving the quality of preparation by subsequent Master's or doctoral study. Because this approach had an exclusively subjective character so far, it was abandoned. Nevertheless, it is becoming evident that preparation of professional growth, especially of crisis top managers, if it is to reach the needed level, cannot be stigmatized by accidentality and pragmatism, but it should be supported by structured career regulations.

Methodology access to working *of other parts of profile* collided on still lurking absence jointly defined and applied theory of the crisis management.

Like basis at their elaboration complex audit of knowledge was hence used, skills and habits crisis managers, implicit in legislative norms (laws, execute public notices of ministries and of others central offices, government decrees and acts) expressing problems crisis management. It showed, that range these knowledge, skills and habits is extremely extensive.

Characteristic of the Bachalour's educational programme and field of study

Programme: Protection population (B9111)

Study in terms of the Bachalour's educational programme is focused on obtaining of graduate universal special worker for region of crisis management and protection population. It is focused on acquirement of needed theoretic principles, generally educational, humanitarian and naturalist discipline, as well as necessary special articles technical and prophylactic alignment, field of knowledge of crisis planning and management, protection population, mastered technical tools and acquired of practical skills of crisis managers.

Natural part of studies is language preparation graduates, acquirement practical habits at short term attachment near appropriate public administration funds repair, components of the integrated rescue system, as well as study and understanding economies accident and crisis situation, their logistical and financial security and driving activity crisis management. Characteristic mark of all period studies is maximum usage of the computer technology.

Characterization of field of study: Crisis management and protection population

Bachalour's educational programme „Crisis management and protection population" on chemical faculty Brno university of technology presents enlargement contemporary spectra bachalour's educational programmes and field of study about unprecedented conceived and oriented educational programme. The focus on enclosure nesting reflected social need expressed government decree No. 417/2002 Coll., them was authorized conception of protection population by the year 2006 with outlook to the year 2015. Basic disposals for its realization includes effort by the year 2006, raise the standard training of public administration, juridical and physical persons, inclusive youth. Further degree of National security council No. 211/2001 Coll., and its amend No. 14/2004 Coll., whereby this conception of the education was authorized in the area of the crisis management. The study offers theoretic pieces of knowledge and practical acquirements in the field of crisis planning and management, protection population. The field of study presents independent, multidisciplinary field of science, whose elaboration, qualified practical application and next development demand graduate specialists. The study is focused on obtaining and acquirement necessary base generally- educational, humanitarian, naturalist and technical articles, as well as understanding and mastered theory management and planning, protection population, especially in extreme and crisis situations, mastered contemporary technical tools needed for organization protectionin population against effects calamities, industrial accidents, weapons of mass destruction, against consequences of terroristic attacks or next undesirable consequencies endangering health and lives of population and property. The conception designed of the Bachalour's educational programme is conformable with actual requirements posed claim on specialized and highly qualified, graduate workers in the area of protection population, crisis planning and management and is conformable with requierements of central organs and needs social practice.

The aim of the programme is to give the graduates a comprehensive and practical education in the field of crisis planning and management in compliance to the resolution of the National security council No. 211/2001 Coll., amended by the resolution No. 14/2004 Coll., „The conception of education in the field of crisis management" and the government decree No. 417/ 2002 Coll., „The conception of protection population by the year 2006 with the outlook to the year 2015" and by doing to participace in improving the quality of theoretical and practical training of public administration and crisis management in companies.

Conclusion

Education of crisis managers at a university level and providing qualification to other people in the given area (see the Conception of education in the area of crisis management, section 3.2.) remains an open issue. The continous improvement of the quality is largely dependent on cooperation and sharing the experience of participating universities. This paper represents another initiative contributing to the topic.

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L22 LET'S GATHER FRUITS IN ELECTRONIC GARDENS OR HOW TO IMPROVE TECHNICAL ENGLISH

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Introduction

It is apparent that many researchers are not aware of some easily available software or internet-based free devices that can dramatically improve their English performance.

There are some handy language supporting aids specialists can use to develop their language skills. Some of the above devices are available at internet free of charge; some other must be bought and installed from CDs.

On-line and off-line dictionaries, glossaries

This article lists devices and tools available on-line starting with general English dictionaries. Two types of them can be distinguished: English-English monolingual dictionaries and Czech-English English-Czech bilingual ones.

The free of charge Merriam-Webster on-line dictionary http://www.m-w.com/netdict.htm provides its users with both graphical and audio pronunciation, enormously useful information that is difficult to find. The dictionary vocabulary of the abridged internet Merriam-Webster form provides also lexical and morphological data, including explication of meaning and references to other hyperlinked on-line devices like Encyclopaedia Britannica etc.

A thorough list of on-line dictionaries containing basic information on items of vocabulary often including pronunciation is available at http://angli02.kgw.tu-berlin.de/call/webofdic/diction1.html. After entering the word you are looking for you are provided with comments on the meaning and hyperlinks to many well-tried reliable on-line dictionaries. A search result for inorganic can be found at the following webpage: http://www.onelook.com/cgi-bin/cgiwrap/bware/dofind.cgi?word=inorganic&type=type_a

Glossaries offer explanation of expressions or phrases from individual branches of chemistry[1].

A detailed list of glossaries aimed at various vocabularies according to branch can be found at http://www.chemistrycoach.com/high.htm#definitions%20of%20chemical. Compared to http://slovnik.seznam.cz/, a limited vocabulary for instant search is purveyed by both www.slovnik.cz and www.slovnik.cz dictionaries.

Several general/chemical dictionaries for off-line use are available at the market. Lingea Lexicon is a widely used general English dictionary with both audio and graphical pronunciation equipped with collocations and user s dictionary. Lingea lexicon 2002 is a technically focused dictionary comprising many chemical and technological terms, unfortunately pronunciation of which was not found important enough to be included. A quite comprehensive set of branch oriented dictionaries can be purchased from the Millenium Company. Pronunciation is incorporated in many of them and they comprise relatively extensive branch-oriented vocabulary, e. g. from the fields of law, chemistry, medicine, biology, military science etc.

Technical texts writing

PhraseBook represents another amazing tool of huge importance. It helps researchers to write journal articles and also any scientific texts they design. PhraseBook, an aid for writing scientific texts, written by PhD authors contains over 4500 words and phrases to help construct a text, from Introducing your work, Arguing for and against, Analysis and explaining, Reviewing other work, Presenting results to Summarizing and Conclusions. In addition, Writing Help sections give advice on style, grammar and punctuation in university and research writing. The PhraseBook also includes a thesaurus, a glossary of university and research terminology, abbreviations and Greek and Latin terms, and a reference section with British and American differences. With the handy PhraseBook every researcher can find writing scientific texts amazing and funny[2].

The www.ruthvilmi.net/hut/LangHelp/Writing/ webpage is hyperlinked to many sites that furnish readers with all the necessary information on writing business letters, technical and academic texts. The webpage also connects to sites with acronym/abbreviation database, grammar exercises and individual technical genres exercises (lecture, presentation, journal article, CV, resume, abstract etc).

Grammar help

Key and exercises equipped grammar help is available at many websites out of which the following one can be recommended: http://web2.uvcs.uvic.ca/elc/studyzone/ . The website offers both explanation of basic grammar items at intermediate level and practical exercises focused on them.

A comprehensive list of grammar problems is provided at http://www.ruthvilmi.net/hut/LangHelp/Grammar/ and http://www.ruthvilmi.net/hut/LangHelp/Grammar/interactive.html websites. The latter contains interactive exercises with keys. Academic text authors can consult all the above sites to check grammar[3].

On-line motivation for language skills improvement

The last points to be addressed are easily available sites of high motivation value that can help their visitors to improve their English free of charge. The Windows Media and Real players hyperlink visitors to music, movie, radio providing servers so that they can watch vidoeclips from movies and listen to music and/or news. Visitors of the http://www.nationalgeographic.com/channel/ will be excited by watching adventures and travel clips videoed by experienced scientists and documentarists. New Zealand kiwi habitat preservation story and humpback life documents belong to the most exciting shows on nature ever presented at the above website.

Conclusions

To conclude, there are many electronic handy tools and devices that can help shape scientists English skill profile either free of charge or at low costs. They comprise on-line and off-line dictionaries, phrasebooks, grammar help, technical writing help and some motivating music and video devices to improve listening skills. The core idea of the article is to make the reader be aware of them and about the necessity to master development of his/her language skills by him/herself because of lack of language teachers able to stay with learners 24 hours a day.

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L23 HYDROGEN SENSORS IN SYSTEMS FOR ALTERNATIVE FUELS

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Introduction

Over the last decade, the expected shortage of fossil fuels, the threat of global warming caused by the CO_2 greenhouse effect, and the problems of worldwide pollution, all have led to an increasing interest in hydrogen based energy systems. Most these are intended for the transport sector, so hydrogen-fuelled vehicles based both on modified combustion engines and fuel cells have become a target for intensive research and development[1]. According to the European Commission White Paper on Transport COM(2001)370, hydrogen, as an alternative fuel, is envisaged to substitute for a significant part of fuel consumption in Europe by the year 2020, and it may also partially substitute for natural gas, using, at least some of the same infrastructure[2]. The technical issues and future directions for hydrogen storage solutions are not yet satisfactorily solved. However, safety considerations imply that adequate monitoring, of the surroundings of any hydrogen facility, will be necessary. In the effective design, operation and maintenance of such facilities, safety will come from the deployment of adequate systems for the detection of unwanted releases of hydrogen, using sensors of appropriate type. Furthermore for fuel cell systems, the use of sensors to control of the purity of hydrogen supplied to the fuel anodes will also be a necessity.

At present, a variety of hydrogen sensors is available which could be used both for safe monitoring and control of fuel delivery to fuel cells, to internal combustion engines, and to other energy conversion systems that are being developed.

This study is related to the testing of hydrogen safety sensors intended to monitor the interior of vehicles, places where vehicles are being refuelled, and where fuel and hydrogen power vehicles are being stored or garaged. The main techniques, which are used in hydrogen sensors, are catalytic pellistor systems, electrochemical (i. e. fuel cell based) and thermal conductivity based approaches[3]. Some modern methods of hydrogen detection are based on new principles such as micro machined semiconductor sensors[4], sensors based on optical properties[5] and methods exploiting the electrical conductivity changes of some metal oxides[6]. For these new techniques gas selectivity, sensitivity, stability against temperature, pressure changes and other environmental influence are critical demands. For some applications, the response time is also a fundamental requirement.

Sensor applications for safety monitoring will need to meet agreed standards and should be tested according to standard procedures, which currently need development. In this respect, standards are available that are based on techniques commonly used for the detection of combustible gases[7], and some papers have proposed various sensor measuring methods and experimental facilities[8,9]. However, the standardised independent testing of sensors for hydrogen energy applications is still yet to be done. A possible way forward is to set tentative or preliminary quantitative targets for the detectors. These should form the basis of specific tests which take into account the properties of the sensors. The requirements include, amongst other things, the measuring range of H_2 in air (the Lower Explosion Limit, or LEL corresponds to 4 mol % H_2), sensitivity to H_2, negligible cross sensitivity to typical pollutants such as CO, NO_x, SO_x, C_xH_y etc, short response time t_{90} (time for achieving 90 % of the steady value), short recovery time t_{10} (time for returning to 10% of the steady value after exposure has ceased) as well as good accuracy.

Facility Layout and Characteristics

Description

The facility, which is schematically depicted in Fig. 1., is designed to give a realistic simulation of a variety of ambient conditions that sensors may encounter during service, and to carry out both dynamic response and long-term stability measurements. It is constructed from Swagelock® tubing and fittings, and manual and pneumatic valves. The gas mass flow

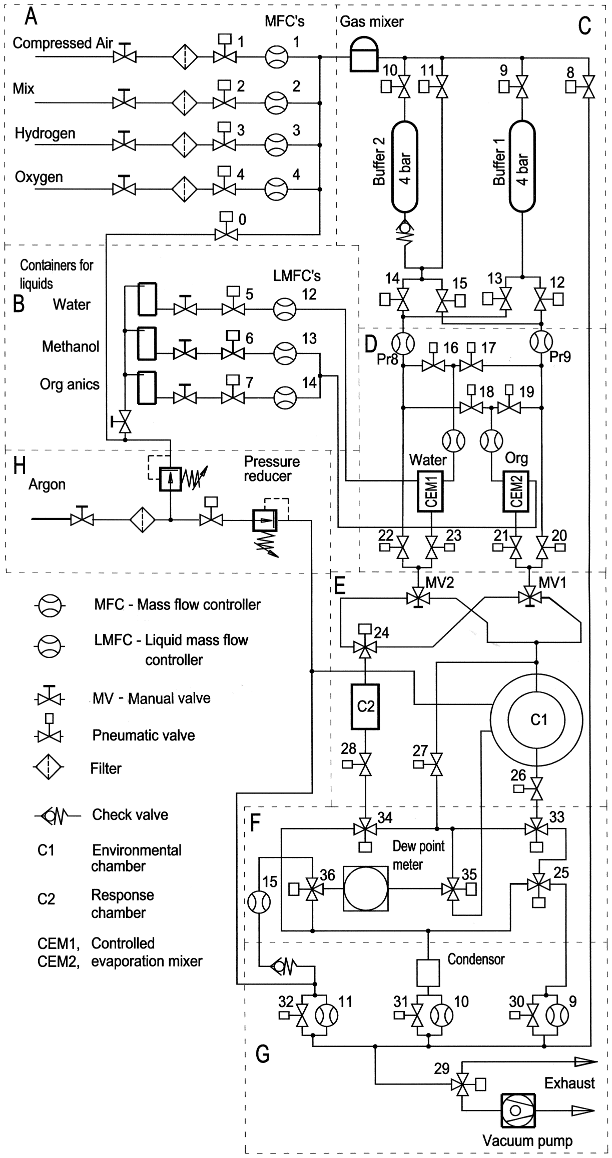

Fig. 1. **Schematic drawing of the sensor testing facility: A – gas inlet and mixing, B – liquid inlet and mixing, C – gas buffering system, D – vapour control system, E – test chambers, F – gas analysis, G – exhaust, H – argon purging**

controllers are Brooks$^{®}$ type, and the liquid mass flow controllers for gas-liquid mixers are of Bronkhorst$^{®}$ type. Humidity is measured independently by means of a dew point hygrometer, model 2002 Dew Prime^{TM} made by EdgeTech. The gas chromatograph is a ct GC of Interscience B. V. with software by EZ Chrom$^{®}$ of Scientific Soft. Inc. connected for gas analysis. The facility can be composed of several sections which are classified according to their functions.

Part A (gas inlet and mixing) is a group of mass flow controllers (MFC), to control flow rates of gases and mix them together. It is connected to gas cylinders or to a central gas distribution system.

Part B (liquids management system) is designed to humidify the gas as well as to prepare mixtures which simulate the environmental pollution of the atmosphere from substance such as alcohols.

Part C (buffers) contains two buffers of volume 3.8 litre, which can be used as reservoirs and to stabilize gas composition. This offers a rapidly available supply of gas for high-speed dynamic measurements.

Part D (pressure/evaporation control system) further prepares the atmosphere for the measurements by setting the pressure at the buffer outlets and regulating the liquid vapour content by means of two installed controlled evaporation mixers (CEM). For test atmospheres, with liquid vapour content corresponding to the dew point of the vapour, above room temperature, the tubing after the mixer outlets is equipped with a heating system, to avoid condensation.

Part E (test points) contains both an environmental test chamber (C1) and a dynamic test chamber (C2). C1enables long-term measurements to be made in highly controlled conditions, where temperature, humidity and gas composition can be gradually varied to simulate natural drifts. This chamber is double-walled, with the temperature being controlled through circulation of a thermostatic fluid between the two walls. For safety, when toxic gases are used, C1 is further contained into another vessel, which is purged with argon. The response chamber C2, enables fast dynamic changes of the gas composition, pressure, and temperature and flow rate, and is intended to be used for sensor response testing.

Part F consists of the analytical equipment, for measurement of the vapour content and gas composition. The level of humidity can be measured with a chill mirror hygrometer, over a temperature range of –50 to 100 °C, step 0.2 °C). The gas chromatograph is connected by means of multiport valve to 11 checkpoints in the facility (not depicted here). The gas analyser multi-column unit, with TCD (Thermal Conductivity Detector) and FID (Flame Ionisation Detector), offers sensitivities of the order of 1 ppm for organic compounds (FID) and approx 10 ppm for H_2, SO_2, and H_2S (TCD).

Finally, part G permits the setting of the proper flow rates for the experiments by regulation of the overall flow, and part H with a vacuum pump serves for argon purging of the tubing in the entire facility.

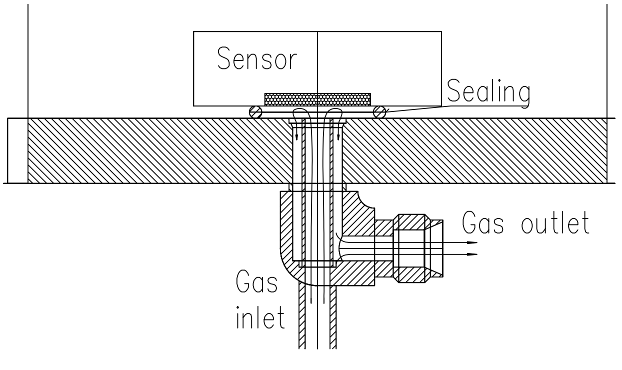

Fig. 2. **Sensor mounting for the response test**

The facility is controlled by National Instruments^{TM} hardware and is programmed in LabVIEW^{TM}. A combination of mass flows, pressures and data acquisition, can be set up at the front panel of the virtual instrument on the screen of controlling computer.

Functionalities and test possibilities

The facility allows handling of 0–2 % vol. H_2 in synthetic air (0 to 50 % LEL), or any concentration of H_2 in Argon, or pure H_2. Hydrogen concentrations down to 100 ppm (0.01 %) can be easily obtained at a flow rate of 1000 sccm by diluting pre-mixed 2 % vol. H_2 in air. Lower concentrations can be obtained by starting with lower H_2 concentrations or by using the buffers as pre-mixers. Concerning humidity, the maximum capacity of the two evaporators is 10 and 100 g H_2O/h, at flow rates of 1 and 10 l min^{-1}, respectively. This allows, in principle, a test range between 10 % relative humidity (RH) at –10 °C (0.025 % vol. H_2O) and 100 % RH at 60 °C (20 % vol. H_2O). At lower or higher temperatures, the accessible test RH increases and decreases respectively: 50 % RH and 10 % RH are the minimum and maximum achievable at –30 °C and at +130 °C, respectively, at a total pressure of 100 kPa.

The test possibilities of this facility can be summarised as follow: It can operate with H_2/air mixtures (dry/moist) in variable concentrations, plus of one gaseous pollutant and one vapour (e. g. CO or SO_x or $H_2S + CH_3OH$ or C_2H_5OH). It enables measurements at temperatures ranging from –40 to +130 °C to be made and elevated ambient humidities. A pressure change between 80 and 150 kPa can simulate variations in altitude, which is particularly useful in view of transport applications such as hydrogen-propelled cars.

Example of test methodology

The experiment presented here was carried out in the response chamber C2, using a sensor of the electrochemical type, with the main scope of verifying the good functioning of the facility. The sensor mounting was arranged according to Fig. 2., corresponding to the so-called "test mask" method. First, the premixed 1 % H_2 in Ar was prepared in the first buffer, to avoid possible hazards in mixing hydrogen directly with air. This allowed further dilution with synthetic air to be carried out, without safety risks, down to any concentration needed. In this manner, a mixture containing 500 ppm H_2 in air was prepared in the second buffer. The calibrated flow meters of 3000 sccm, full range for argon, and 60 sccm for hydrogen respectively, allowed the preparation of a molar fraction 0.0100 ± 0.0002 of hydrogen in argon, and subsequently to obtain a mixture with (500 ± 25) ppm of hydrogen plus 5 % Ar in air, in the second buffer.

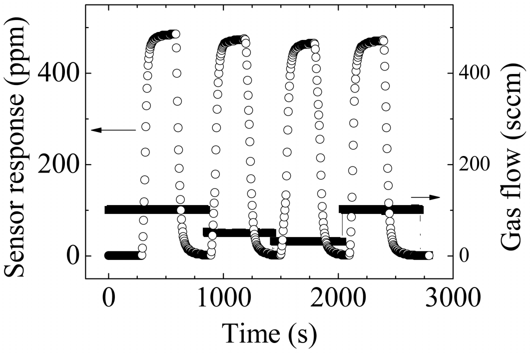

Fig. 3. **Sensor response on rapid change of hydrogen concentration in flowing air at various flow rates**

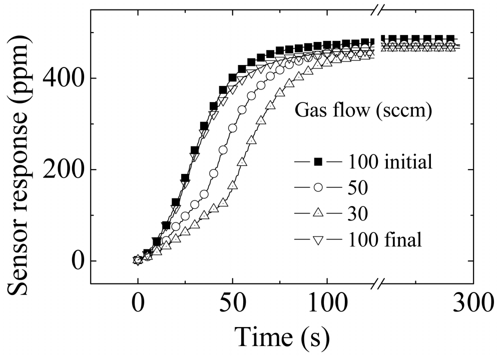

Fig. 4. **Comparison of ascending ramp of sensor response at various flow rates**

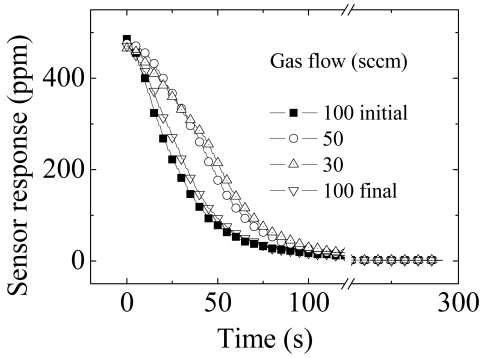

Fig. 5. **Comparison of descending ramp of sensor response at various flow rates**

As shown in Fig. 2., the gas sensor under test was mounted and pressed against the outlet orifice discharging the test gas, so that the dead volume was minimised. Close to it, the three-way valve 24 (in Fig. 1.) was used to switch the flow between gases of different composition, these being clean synthetic air and synthetic air enriched by 500 ppm of hydrogen. The dead volume was estimated to be smaller than 1 ml, which means that the atmosphere to which the sensor was exposed could be completely renewed within one second at every switch of the flow, at a flow rate of 60 sccm at 100 kPa pressure. With reference to Fig. 1., the flow rate was set up by the MFC9 and the test pressure was stabilised by controllers PR8 and PR9 to 100 kPa, so pumping was necessary to maintain the gas flow.

Test results and discussion

The experiment total time was 3000 s. The stable flow of 100 sccm of synthetic air was set up by the MFC9 and, in periodic 300 s intervals, switched with valve 24 from synthetic air containing the hydrogen, and back to hydrogen-free synthetic air. The response of the sensor was recorded and a new cycle was carried out with lower total flow rate.

The results shown in Fig. 3. showed that the test system functioned well being able to define the performance of the sensor under test. Over a total of four cycles at different flow rates, the dynamic response of the electrochemical sensor was in the range between 60 and 90 s, which was in line with the data given by the manufacturer.

Both the response and recovery time showed a significant lengthening at decreasing flow rates, which confirmed the known influence of the gas flow parameters on sensor calibration and testing, especially when this type of set up is used[7]. However with respect to other methods, the procedure used here gives the advantage of allowing rapid mounting of the sample and easy test execution. However, it is important that the mounting of the sensor against the orifice, which can be easily modified on a case-by-case base, needs to be compatible with the mask specified by the manufacturer for the initial calibration of the device.

Further development of the facility and sensor performance characterization is planned, which will cover investigations on long-term drift, hysteresis and dependence on environmental conditions.

Conclusions

The experimental facility for hydrogen safety sensor testing is described. As for environmental tests, this facility can simulate real conditions with high precision. This is obtained by varying the program so as to control the test atmosphere composition, its humidity, the temperature and pressure. As for dynamic response testing, the results of a test example on an electrochemical sensor have been shown. The experimental data show that the dynamic response of the sensor is affected by the gas velocity and pressure. Provided adequate care is used in the mounting of the sensor on the test plate of the test facility. The procedure is suitable for carrying out tests on hydrogen sensors to be used by the automotive industry for the equipment in vehicles, and for the infrastructure used to fill vehicles and store hydrogen.

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