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Agnieszka ULANOWSKA[1], Tomasz LIGOR[1], Monika MICHEL[1,2]
and Bogusław BUSZEWSKI[1*]

HYPHENATED AND UNCONVENTIONAL METHODS FOR SEARCHING VOLATILE CANCER BIOMARKERS

ŁĄCZONE I NIEKONWENCJONALNE METODY POSZUKIWANIA LOTNYCH BIOMARKERÓW CHORÓB NOWOTWOROWYCH

Abstract: Volatile organic compounds produced inside the body provide valuable information about human state of health and they are detected in breath, blood and urine samples. Therefore, volatile biomarker analysis seems to become accurate and fast method for tumour detection. So far, there are known several volatile organic compounds (VOCs) recognized as potential cancer biomarkers. For the detection of VOCs different analytical techniques are used. The most popular is gas chromatography coupled with mass spectrometry (GC/MS). More recently, selected ion flow tube mass spectrometry (SIFT-MS), proton transfer reaction mass spectrometry (PTR-MS) and ion mobility spectrometry (IMS) are also applied for biomarker research. Besides typical instrumental methods used for VOCs analysis, unconventional methods such as sensitive canine sense of smell can be used. In recent years, this very sensitive scent is also used for cancer biomarker detection. Dogs are trained to recognize the smell of skin, breath or urine samples from patient with different kind of cancer from the control group. The application of dogs' smell for the preliminary screening of tumour in human body is painless, noninvasive and fast method. Additionally, it does not need the preconcentration of analytes before the analysis.

Keywords: analytical methods, canine scent, biomarkers, volatile organic compounds, cancer

Introduction

Cancer diseases are leading a few million deaths worldwide every year. Early detection of tumour increases the chance to survive. So far, there are known many different methods used for tumour diagnosis. The most popular is computer tomography, nuclear magnetic resonance, positron emission tomography, mammography and single photon emission computed tomography [1]. However, these methods are expensive and could be harmful for patients. Biomarker analysis is complementary method and seems to be screening method for early cancer detection. Molecular biomarker is defined as a molecule which reflects the

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pathological state of the organism and it can be characteristic pharmacologic response to a therapeutic intervention. The expression of estrogen receptors (ER), progesterone receptor (PR), p53, B-cell lymphoma-2 (Bcl-2), cyclin E, cytokeratin 5/6, human epidermal growth factor-2 (HER-2/neu) oncogenes and the Ki-67 index of proliferative activity are protein biomarkers used in medicine [2, 3]. However, huge number of various classes of compounds present in the tissue makes protein biomarkers searching extremely difficult. Therefore, analysis of substances present in exhaled breath seems to be easier to perform, faster and non-invasive method which can be used for cancer detection [4, 5].

In exhaled air more than 2000 different compounds can be detected [6]. These substances are in the most cases volatile organic compounds (VOCs) but nonvolatile organic compounds (proteins, leukotrienes, etc.) and inorganic volatile compounds (carbon oxide, nitrogen oxides, carbonyl sulfide) are also identified. Their exhaled amount may change depends on age, state of health, life style, gender. However, in pathological states of the organism some additional compounds are produced and/or their concentration is changed [7].

Gas chromatography with flame ionization detector (FID) or with mass spectrometry (MS) has been applied for detection and quantitation compounds existing in breath. [8, 9]. Different mass spectrometry techniques such as proton-transfer-reaction mass spectrometry (PTR-MS) [10], selected-ion-flow-tube mass spectrometry (SIFT-MS) [11], ion mobility spectrometry (IMS) [12] allow to on-line breath testing.

Except analytical methods, canine sense of smell can be use for cancer detection [13]. At first, dog’s olfactory system was used in finding bombs, drugs and people [14]. Nowadays, trained dogs smelling different probes (ie skin, urine, breath) are able to recognize samples from people with cancer and healthy volunteers.

The aim of this review is the presentation of instrumental analytical methods and canine scent which can be used for detection of volatile cancer biomarkers. Substances recognized as potential biomarkers have been also present.

Volatile organic compounds proposed as cancer biomarkers

Volatile organic compounds present in exhaled air have both origins - endogenous or exogenous. First are produced during different biochemical processes which occur in living organisms and second can be undertaken with food, inhaled with the air or absorbed by the skin. Only the first group can be considered as a diseases marker. There are known metabolic pathways of formation only limited examples of endogenous substances. Saturated hydrocarbons (ethane, pentane) and aldehydes (acetaldehyde) are generated during lipid peroxidation of fatty acids (Fig. 1) [5]. According to Spanel et al formaldehyde is a potential biomarker of bladder and prostate cancer [15]. Concerning to another aldehydes such as heptanal and hexanal they are supposed to be characteristic for patients with breast or lung cancer [16-21]. Acetone is one of the most abundant compound in breath and an important metabolic marker in breath [22]. It is ultimately formed by the decarboxylation of acetoacetate, which is derived from lipolysis or lipid peroxidation (Fig. 2). Acetone has been recognized by Phillips et al [18] as a potential marker of lung cancer. Isoprene (2-methyl-1,3-butadiene) is a hydrocarbon always present in breath which is a by-product of cholesterol synthesis [23] (Fig. 3). Poli et al found that in breath of patients with lung cancer concentration of isoprene was higher than in control group.

Volatile organic compounds chosen as hypothetical cancer biomarkers are summarized in Table 1.

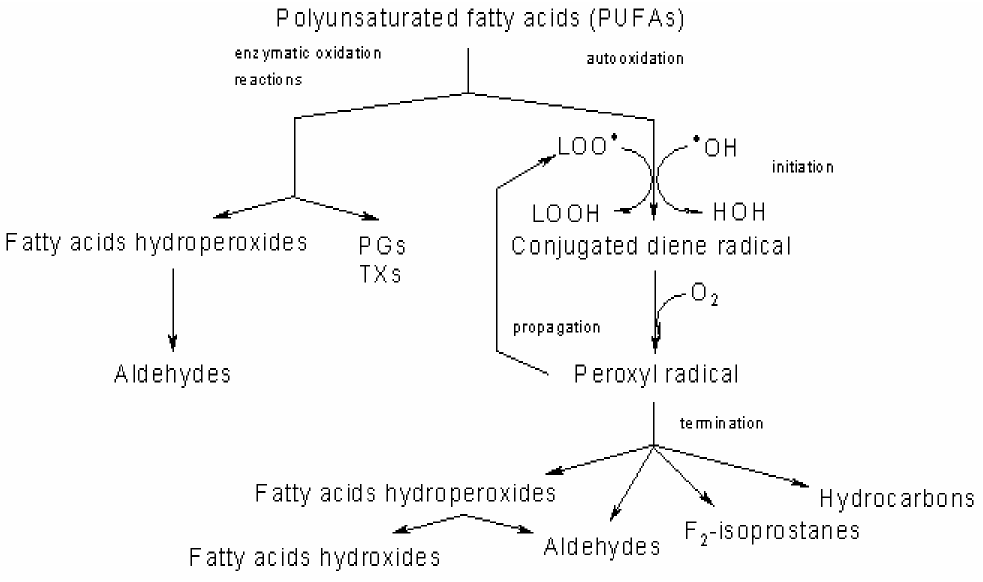

Fig. 1. Products generated in polyunsaturated fatty acids oxidation [4]

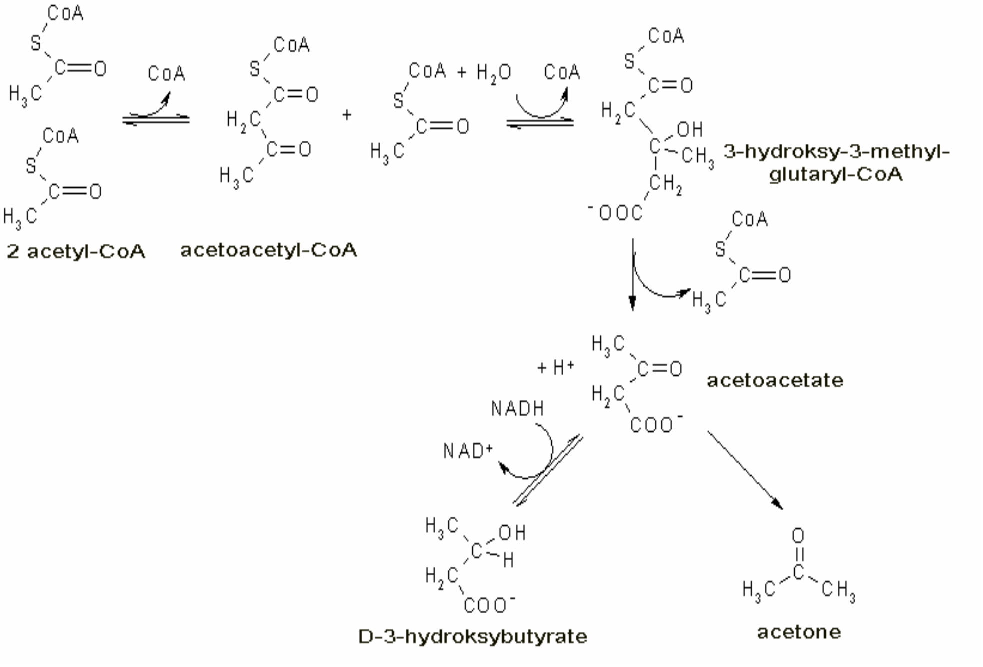

Fig. 2. Biochemical pathway of acetone generation with excess of acetyl-CoA [24]

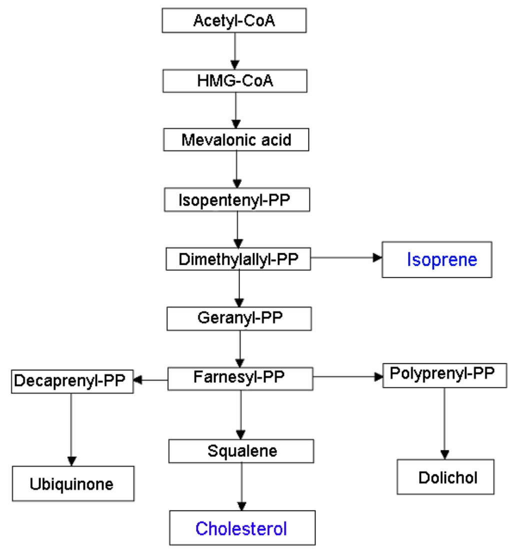

Fig. 3. Biochemical pathway of isoprene generation [5]

Table 1

Volatile organic compounds recognized as cancer biomarkers

Compound	**Type of cancer**	**Applied technique for VOCs analysis**	**Concentration level**	**References**
isoprene	lung	SPME-GC/MS SPME-GC/FID	[ppb/ppt] [ppm]	[14, 15]
pentane	lung	SPME-GC/MS	[ppb/ppt]	[14]
octane	lung	SPME-GC/MS	[ppb/ppt]	[14]
decane	lung	SPME-GC/MS SPME-GC/FID	[ppb/ppt] [ppm]	[14, 15]
undecane	lung	SPME-GC/FID	[ppm]	[15]
methylcyclopentane	lung	SPME-GC/FID	[ppm]	[15]
formaldehyde	bladder, prostate, lung	PTR-MS	[ppb]	[17, 18]
heptanal	lung, breast	SPME-GC/FID SPME-GC/MS TD-GC/MS	[ppm] [ppb/ppt] [ppt]	[15, 19-22]
hexanal	lung, breast	SPME-GC/FID SPME-GC/MS TD-GC/MS	[ppm] [ppb/ppt] [ppt]	[15, 19-22, 26]
acetone	lung	TD-GC/MS SPME-GC/MS	[ppt] [ppb/ppt]	[20, 24]
2-propanol	breast	TD-GC/MS	[ppt]	[22]
benzene	lung	SPME-GC/MS SPME-GC/FID	[ppb/ppt] [ppm]	[14, 15]
toluene	lung	SPME-GC/MS	[ppb/ppt]	[14]
xylene isomers	lung	SPME-GC/MS TD-GC/MS	[ppb/ppt] [ppt]	[14, 20, 21]
ethylbenzene	lung	SPME-GC/MS	[ppb/ppt]	[14]

Analytical methods for detection of biomarkers

According to the requirements and biochemical properties of analyzed compounds, different analytical methods can be used for volatile biomarkers analyzes (Tab. 1). Some of them such as GC/MS are usually applied for separation and identification of unknown substances present in gaseous samples. Others, ie PTR-MS or SIFT-MS are often used for on-line monitoring of target compounds present in breath air.

Gas chromatography with mass spectrometry (GC/MS)

Gas chromatography is one of the most important analytical methods in organic chemical analysis for determination of individual substances in mixture. Mass spectrometry became an indispensable detector for GC because of selectivity, high sensitivity and identification potential.

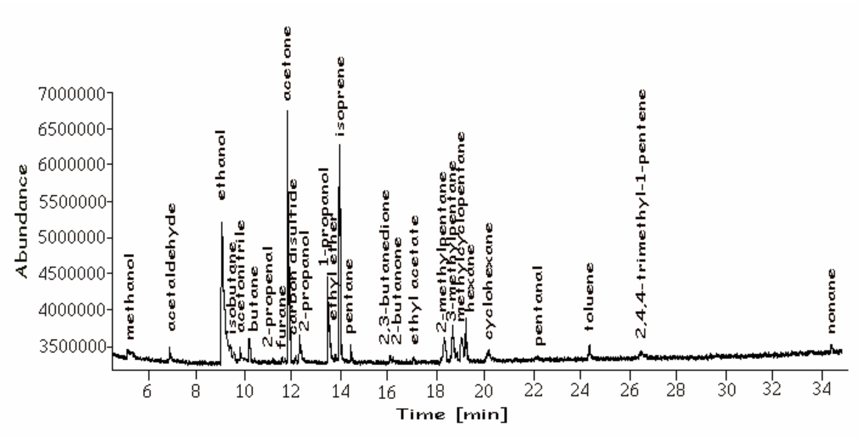

Fig. 4. The exemplary GC/TOF-MS chromatogram of exhaled air from healthy volunteer

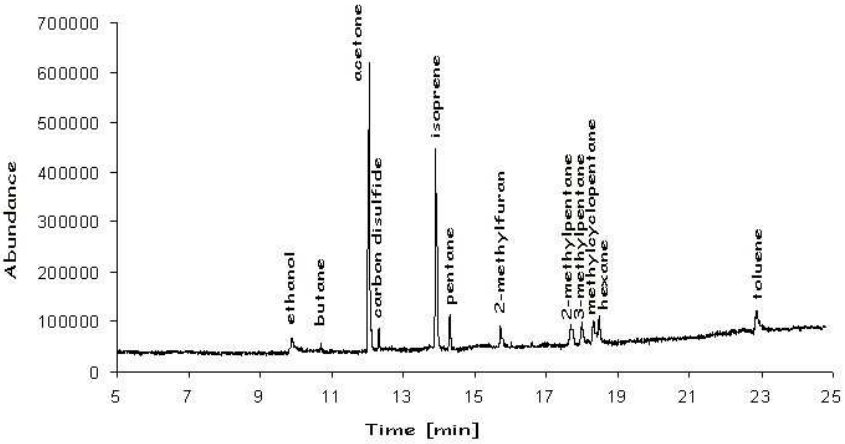

Fig. 5. The exemplary GC/MS chromatogram of exhaled air from healthy volunteer

Therefore, GC/MS technique is still gold standard in breath analyses [25-27] and gaseous emission from lung cancer cell lines at ppb or even ppt level [21]. Nowadays, GC combined with time of flight analyser (GC/TOF-MS) becomes forceful for identification of complicated gaseous mixture. The exemplary GC/TOF-MS and GC/MS chromatograms of exhaled breath of healthy persons are presented in Figure 4 and Figure 5, respectively. Due to very low concentrations of VOCs (ppb-ppt) existing in breath samples the preconcentration techniques such as trapping on solid sorbents followed by thermal desorption (TD) or solid-phase microextraction (SPME) are used prior chromatographic analysis [21, 27].

Gas chromatography with flame ionization detector (GC/FID)

Due to simplicity and high sensitivity the flame ionization detector is often using for breath analysis. The application of GC/FID system allows to detect hydrocarbons and their derivatives at ppm even ppb level (Tab. 1). This detector was applied for determination of acetone, pentane, isoprene, hexanal and others VOCs present in the breath samples [9, 16]. Chen et al applied GC/FID technique for determination of decane, isoprene, benzene, hexanal and heptanal in the headspace of lung cancer cells [16]. Isoprene and sulfur related compounds released by bacteria cultures were analyzed by Schfller et al with using this technique [28].

Proton transfer reaction mass spectrometry (PTR-MS)

The proton transfer reaction mass spectrometry is the technique appropriate for rapid and on-line measurements of VOCs present in human breath [29]. Some of volatile compounds can be detected in ppb concentration level.

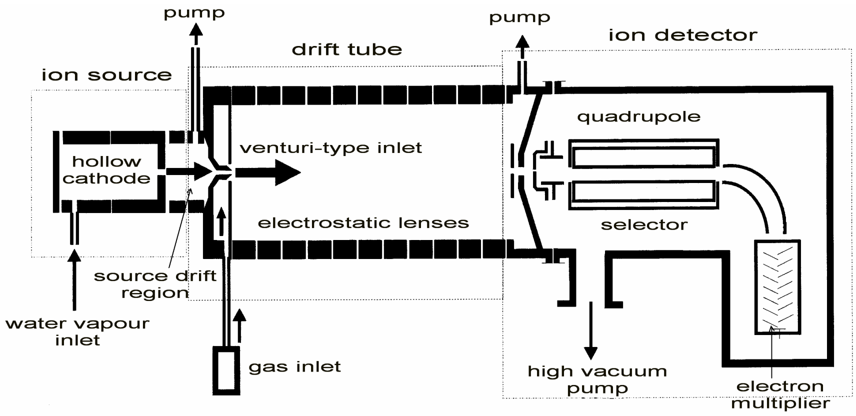

Fig. 6. Scheme of separation system in PTR-MS [30]

The basic principle of PTR-MS involves the mixing of a flowing air sample in a drift tube equipped with a source of H_3O^+. Ionization of vapour water can be done with using corona discharge, electron impact or alpha radiation emitted by ^{241}Am. Analytes rapidly react with reactant ions. During proton transfer reactions protonated cations of analyzed substances are formed which then are separated in electric field according their mass to charge ratio (*m/z*). Identification in PTR-MS is based on molecular mass and thus it is possible interferences from various molecular species to appear. The scheme of PTR-MS separation system has been shown in Figure 6. Therefore, this method should be used for monitoring the concentration of analyte rather than for mixture separation [30]. This method was used to analyze breath isoprene with no preconcentration or preseparation [31] and for determination of acetonitrile and benzene concentration in smokers' breath samples [32].

Selected ion flow tube mass spectrometry (SIFT-MS)

Selected ion flow tube mass spectrometry is used for rapid detection and quantification of trace gases [33]. SIFT-MS involves the chemical ionization of trace gases introduced into flow tube using selected precursor positive ions such as H_3O^+, NO^+ and O_2^+ [34]. However, the most commonly used precursor ion is H_3O^+ which reacts with a wide range of organic species. In this technique sample of VOCs is added to the carrier gas/precursor ion swarm and the trace gases in the sample react with the precursor ions generating characteristic protonated product ions. Then mass spectrometer measures the count rates of the precursor and product ions. The quantification of particular trace gases in the air sample is achieved. Figure 7 presents the scheme of SIFT-MS system. SIFT-MS technique requires minimum sample preparation and no separation of analytes. It was used for on-line analysis of acetone, isoprene, ammonia and acetonitrile present in exhaled breath of smokers and nonsmokers [33, 35] and for analysis of formaldehyde in the headspace of urine from bladder and prostate cancer patients [15].

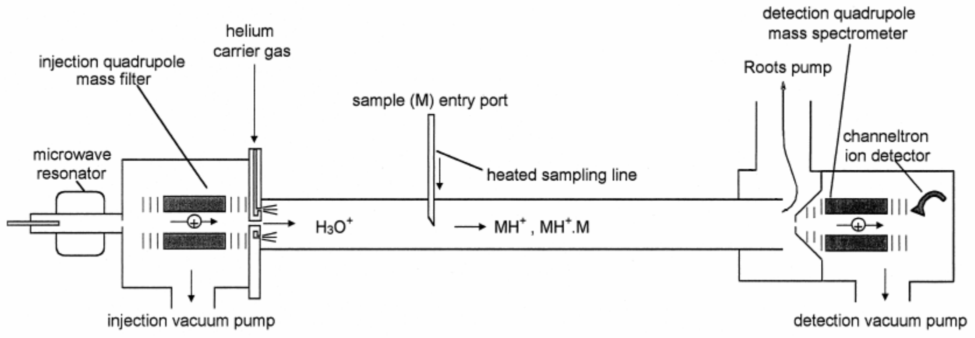

Fig. 7. Scheme of separation system in SIFT-MS [33]

Ion mobility spectrometry (IMS)

Ion mobility spectrometry is very effective and sensitive technique for the determination traces of aldehydes, ketones and esters in low ppb/ppt range [36, 37]. The time required to acquire a single spectrum is in the range of 20÷50 ms. There is no vacuum required for IMS operation and the ambient air can be used as carrier gas. Therefore, the IMS can be miniaturized and applied as an on-line technique for breath detection. Working principles of this device have been described in details by Baumbach [36]. IMS shows low sensitivity in case of alkanes and benzene-related compounds - analytes with the low proton affinity. It is often coupled with standard gas chromatographic columns or multicapillary column (MCC) which enables analysis mixture of gaseous substances on-line and in very short time [38]. The MCC-IMS system was used for on-line breath analysis and compounds such as acetone, ammonia and ethanol were determined [39]. Scheme of typical drift tube used in IMS is presented in Figure 8.

The main advantages of PTR-MS, SIFT-MS and IMS are high sensitivity, possibility of VOCs determination without preconcentration process and on-line analysis.

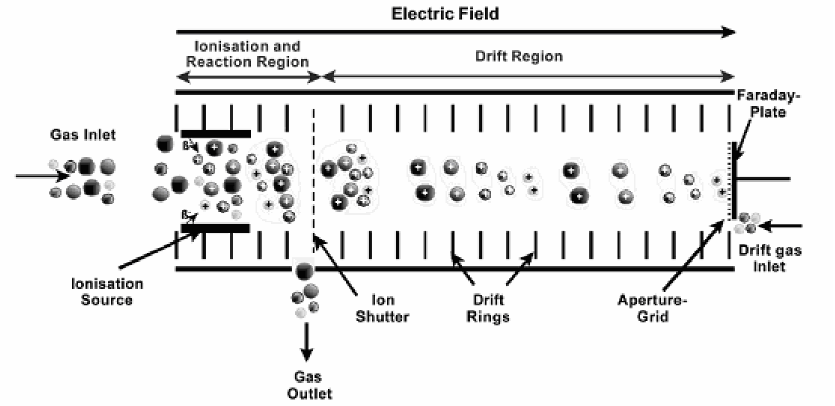

Fig. 8. Scheme of drift tube in ion mobility spectrometer [36]

Sensors

Sensors are become more popular in breath tests such as ammonia, ethanol, NO, CO detection [40]. These systems are relatively cheap, easy to provide and fast response. There is now great interest in the clinical application of an electronic nose; that it is possible for e-nose to diagnose different illness [41, 42]. Different types of sensors are used: bulk acoustic wave sensors [43], surface acoustic wave sensors [44], and multiple conducting polymer sensors [45]. A novel type of gas sensor array was used for quick self-check of breath of malodor components like ammonia, hydrogen sulfide and methanethiol [46]. Another type of sensor is e-nose, named Cyranose C320, based on multiple conducting polymer sensor technology. It has been used to distinguish the breath of smokers from that of non-smokers [47]. The advantage of the sensor is that presence of humidity that not has any influence on the outcome of analysis. Nitrogen oxide/dioxide measurement sensor system is used for on-line recognition of complex VOCs mixtures from patients with asthma [48].

Canine scent

According to Walker et al dog’s sense of smell is estimated to be 10,000 times more sensitive than human, that means they have from 20 to 40 times more nasal receptor cells than human [49]. Figure 9 shows smelling process by dogs. This ability for many years has been used in finding bombs, drugs, and people [14]. From 1989, when William and Pembroke published the letter in *Lancet* [50] and described a case of woman seeking medical attention because of her dogs' continued interest in a skin lesion, scientists decide to use canine scent for cancer detection.

Many analytical investigations confirmed that tumour cells produce volatile chemicals [21] that can be detected in breath [51], urine [52], blood [53] or emitted through the skin [54]. A different smell of probe sampled from healthy and ill persons enable recognizing them by dogs. Nowadays, dogs are trained to detect a various type of cancer by smelling urine [13, 55, 56], breath [57] and skin samples [58]. Five dogs were trained to distinguish, by scent, exhaled breath samples of 55 lung and 31 breast cancer patients from those of 83 healthy controls [57]. Horvath et al taught the dog to distinguish different histopathological types and grades of ovarian carcinomas, including borderline tumours, from healthy control samples [55]. For the experimenters, recruited dogs, should reveal high level of eagerness to sniff objects and respond to commands [57]. Canine scent of six trained dogs has been used to discriminate between urine from patients with bladder cancer and urine from diseased and healthy controls [56]. Willis et al achieved the successful detection of urine samples from patients with bladder cancer 41%. Multivariate analysis suggests that the dogs' capacity to recognize an odour signature characteristic of bladder cancer is independent of other chemical aspects of the urine detectable by urinalysis, such as the presence of blood. Melanoma is the skin cancer which can be first aided by visual inspection by the physician and then confirmed with histopathological research. Another way for its detection is finding the source of chemical markers with using the canine olfaction [59]. Two dogs with satisfactory results were trained to localize melanoma and recognize it from healthy skin.

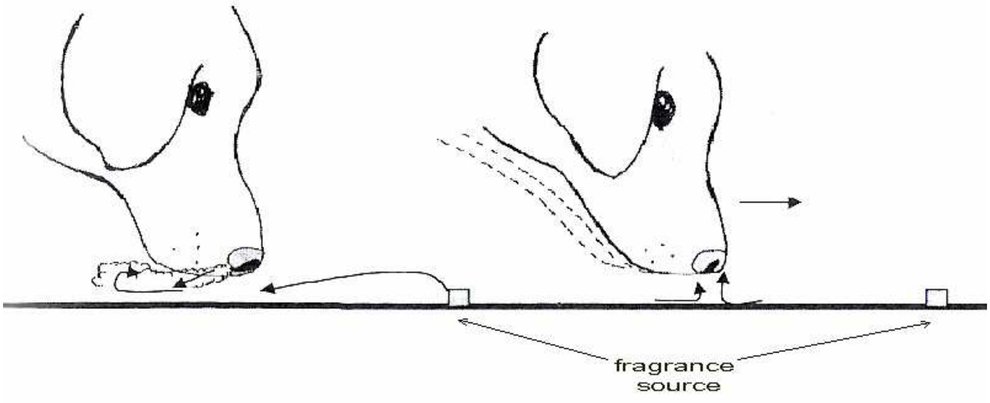

Fig. 9. Smelling process [60]

Quality control in bioanalytical method validation

Selective and sensitive analytical methods for the quantitative evaluation of analytes such as drugs, their metabolites or VOC's are critical for the successful conduct of preclinical, biopharmaceutics and clinical pharmacology studies or therapeutics. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, urine, and breath is reliable and reproducible for the intended use. The fundamental parameters for this validation include accuracy, precision, selectivity, sensitivity, reproducibility and stability. Validation involves documenting, through the use of specific laboratory investigations, that the performance characteristics of the method are suitable and reliable for the intended analytical applications. The acceptability of analytical data corresponds directly to the criteria used to validate the method [61, 62].

Published methods of analysis are often modified to suit the requirements of the laboratory performing the assay. These modifications should be validated to ensure suitable performance of the analytical method (Fig. 10). When changes are made to a previously validated method, the analyst should exercise judgment as to how much additional validation is needed. During the course of a typical drug development program, a defined bioanalytical method undergoes many modifications. The evolutionary changes to support specific studies and different levels of validation demonstrate the validity of an assay's performance.

Analysis of drugs, their metabolites or VOC's in a biological matrix is carried out using samples spiked with calibration (reference) standards and using quality control (QC) samples. The purity of the reference standard used to prepare spiked samples can affect study data. For this reason, an authenticated analytical reference standard of known identity and purity should be used to prepare solutions of known concentrations. If possible, the reference standard should be identical to the analyte. When this is not possible, an established chemical form (free base or acid, salt or ester) of known purity can be used.

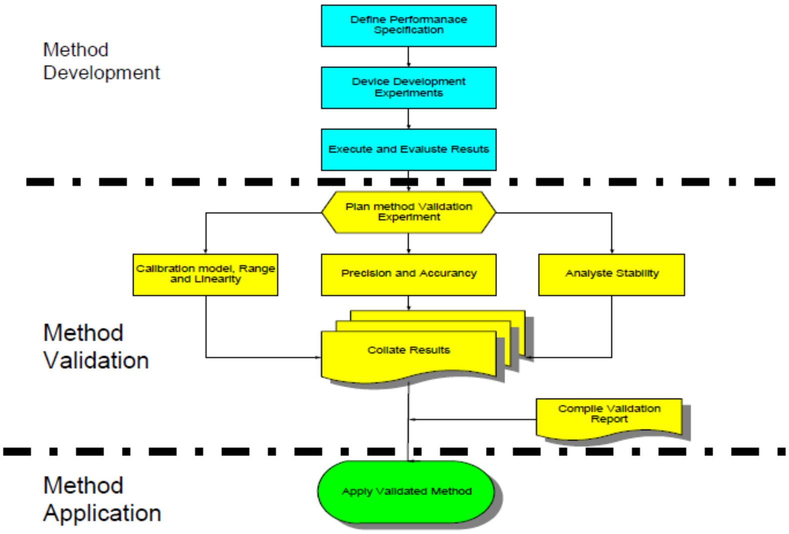

Fig. 10. Method of validation process

Three types of reference standards are usually used: certified reference standards, commercially supplied reference standards obtained from a reputable commercial source, and/or other materials of documented purity custom-synthesized by an analytical laboratory or other non-commercial establishment. The source and lot number, expiration date, certificates of analyses when available, and/or internally or externally generated evidence of identity and purity should be furnished for each reference standard.

The method development and establishment phase defines the chemical assay. The fundamental parameters for a bioanalytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility, and stability. Measurements for each analyte in the biological matrix should be validated. In addition, the stability of the analyte in spiked samples should be determined [63-65]. Assays of all samples of an analyte in a biological matrix should be completed within the time period for which stability data are available. In general, biological samples can be analyzed with a single determination without duplicate or replicate analysis if the assay method has acceptable variability as defined by validation data. This is true for procedures where precision and accuracy variabilities routinely fall within acceptable tolerance limits. For a difficult procedure with a labile analyte where high precision and accuracy specifications may be difficult to achieve, duplicate or even triplicate analyses can be performed for a better estimate of analyte.

A calibration curve should be generated for each analyte to assay samples in each analytical run and should be used to calculate the concentration of the analyte in the unknown samples in the run. The spiked samples can contain more than one analyte. An analytical run can consist of QC samples, calibration standards, and either all the processed samples to be analyzed as one batch or a batch composed of processed unknown samples of one or more volunteers in a study. The calibration (standard) curve should cover the expected unknown sample concentration range in addition to a calibrator sample at LOQ. Estimation of concentration in unknown samples by extrapolation of standard curves below LOQ or above the highest standard is not recommended. Instead, the standard curve should be redefined or samples with higher concentration should be diluted and reassayed. It is preferable to analyze all study samples from a subject in a single run.

Conclusions

Analysis of VOCs produced during metabolic processes supply the information concerning human state of health. Because of fast development of separation techniques, detection and identification of volatile biomarkers at very low level is possible. Therefore, analysis of volatile biomarkers seems to become new, non-invasive method for cancer detection. For on-line monitoring of one or a few VOCs in breath PTR-MS and SIFT-MS methods are used. Obviously, in medical practice more and more popular is sensors technology, because of its high sensitivity, selectivity and short time response. However, GC/MS system is still indispensable for searching new biomarkers.

The newest experiments (from year 2001) showed that canine sense of smell can be also used as natural sensor for early cancer detection. Trained dogs are able to accurately distinguish breath samples of cancer patients from those of controls. However, there is not known, what compounds they smell indeed.

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ŁĄCZONE I NIEKONWENCJONALNE METODY POSZUKIWANIA LOTNYCH BIOMARKERÓW CHORÓB NOWOTWOROWYCH

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Abstrakt: Lotne związki organiczne (VOCs), powstające wewnątrz organizmu ludzkiego dostarczają wielu cennych informacji na temat stanu zdrowia pacjenta i są one identyfikowane w próbkach powietrza wydychanego, krwi i moczu. Wiedzę na ten temat wykorzystuje się podczas analizy lotnych biomarkerów, która w przyszłości może zaowocować opracowaniem nieinwazyjnej metody diagnostyki medycznej stosowanej do wczesnego wykrywania nowotworów. Obecnie znanych jest kilkadziesiąt związków organicznych, które uznane zostały za potencjalne biomarkery chorób nowotworowych. Substancje lotne są analizowane różnymi dostępnymi technikami analitycznymi. Wśród nich najbardziej znana jest chromatografia gazowa sprzężona ze spektrometrią mas (GC/MS). Jednakże do oznaczania biomarkerów wykorzystywana jest również spektrometria mas z jonizacją w strumieniu wybranych jonów (SIFT-MS), spektrometria mas z reakcją przeniesienia protonu (PTR-MS) oraz spektrometria ruchliwości jonów (IMS). Techniki te umożliwiają bezpośrednią analizę np. powietrza wydychanego, która prowadzona jest w czasie rzeczywistym. Oprócz tradycyjnych metod instrumentalnych stosowanych do wykrywania biomarkerów wykorzystywana jest również dość niekonwencjonalna metoda korzystająca z niezwykle czułego powonienia psów. Tresowane psy są w stanie niemal bezbłędnie odróżnić po zapachu próbkę moczu, oddechu oraz skóry objętej czerniakiem, pochodzącą od pacjenta i od osoby zdrowej. Technika ta jest bezbolesna, nieinwazyjna oraz szybka. Ponadto próbki do analizy nie muszą być wcześniej wzbogacone.

Słowa kluczowe: metody analityczne, węch psów, biomarkery, lotne związki organiczne, nowotwór

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ENVIRONMENTAL HETEROGENEOUS CATALYSIS AND WATER PURIFICATION BY ACTIVATED INTERFACES: A SURVEY OF DIFFERENT WAYS OF SURFACE ACTIVATION AND DEMONSTRATION OF A NOVEL, SIMPLE AND EFFICIENT PROCEDURE

HETEROGENNA KATALIZA ŚRODOWISKOWA. OCZYSZCZANIE WODY PRZEZ AKTYWOWANE INTERFEJSY - NOWE PROCEDURY AKTYWACJI POWIERZCHNI

Abstract: Besides the established procedures, a multitude of environmental organic compounds can be mineralized or at least degraded to species like acetate by irradiating iron(III) complexes adsorbed to certain broad-gap semiconductors by visible light. Quantum yields are increased considerably by adsorption with respect to LMCT photochemistry in homogeneous solution, and the semiconductor acts as a electron- (rather, valence band hole) transfer agent which conserves the oxidizing properties of the ligand radical originally produced for longer periods of time (although modulated down to ε_{vb}). The SC colloid particle, not excited itself, transports the hole, then allowing to oxidize co-adsorbed species present in small concentrations but also accumulated by adsorption. Water purification from various kinds of pollutants is feasible, and the reaction which is photocatalytic at turnovers ≥ 100 referring to Fe complex can be kept up by air input into the suspension also tolerating repeated inputs of compounds to be removed. Except for some acetate formed, mineralization of substrates is complete.

Keywords: photooxidation, semiconductor, iron complex, water purification, heterogeneous catalysis, methods of enhanced surface activation

Introduction

The principal end of environmental chemistry is to design or understand chemical reactions which are relevant to the status of our environment or suited to improve it, ie to remove or convert chemical compounds before these get into contact with the environment and do harm to it. Like all other chemical reactions, these can occur in either a homogeneous or a heterogeneous setting, the latter eg involving heterogeneous catalysis

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like in the catalytic car exhaust converter [1]. Heterogeneous systems mainly draw upon adsorption (physi- and chemisorption) inducing polarization, cleavage and rearrangement of chemical bonds in the (potentially hazardous and thus to be removed) substrate.

Apart from eutrophicating pollutants (nitrate, phosphates) and certain toxic cations and anions (eg cyanide, nitrate(III)) most of the compounds which deteriorate the status of ambient and groundwaters are organic compounds, most of them (except for CCl_4, perfluorinated sulfonates and the like) being sensitive towards oxidation. However, most of these compounds are fairly stable even under Earth´s oxidizing atmosphere under ambient conditions, making them capable to pass through sewage treatment plants and persist in the environment for quite some period of time. Thus they can accumulate in both water and - if unpolar - biomagnify in aquatic organisms along the trophic chain. Besides organic compounds wilfully passed into the environment, eg as biocides, there are others with similar properties which get there after passing through and out of human or animal bodies after being applied as pharmaceuticals. Several of these compounds actually reach levels where adverse effects to fishes and other aquatic beings are noticeable, including 17α-ethynylestradiol (EE 2, for oral anticonception) and diclofenac (2´,6´-dichloranilinophenylacetate-2, an analgetic agent, known as Voltaren®), both remaining essentially unchanged by classical procedure of sewage water treatment.

Although oxidation of most environmentally relevant organics is exothermic, it need not occur readily; rather, efficient and complete low-T oxidation of organics requires the use of platinum group metals (PGM) or complexes thereof as catalysts, like in fuel cells. One also has - apart for cost and large-scale availability - to be aware that eg a catalytic car exhaust converter best operates at some 400°C even though it contains both Pt and Rh. This T value of operation otherwise is rather typical for selective air or NO_x oxidations of organics, such as production of nitriles or HCN from amines at Bi_2O_3, but anyway unsuited for water purification (“wet combustion”) essentially. Moreover, certain functional groups are able to produce highly toxic by-products, especially if combined with inorganic catalysts. A classical example is formation of chlorinated dioxines and dibenzofurans by partial air oxidation of halophenols or other haloorganics which occurs when Cu is present. Stepwise oxidation may (when caused by NO_2 and NO_3 radicals) or may not (by OH) decrease subsequent reactivity of intermediates towards mineralization, also [1]. Thus there apparently is a necessity either to achieve complete oxidation by appropriate, far more active catalysts of oxidation (eg enzymes) or to change reaction conditions at interfaces sufficiently. While it is hardly understood what really happens on heterogeneous catalyst interfaces except for direct view in some STM captures of simple cases, like CO oxidation on neat Pt [2], it is more feasible to increase activity by actively altering electronic (binding) conditions at the interface rather than simply looking for more active (and probably more “exotic” and expensive) catalyst formulations. Frankly speaking, even PGM interfaces (including Ru, Rh) will not accomplish organic oxidations near RT (room temperature). The reasons for this are related to the band model which applies to all solids, metals, insulators and semiconductors alike.

It takes additional activation of the interface by passing energy through it - be it electrical, electromagnetic (visible, UV, γ radiation) or even mechanical (tribochemistry) [3] - to achieve reasonable turnover rates near to room temperature, which is essential for water treatment in substantial amounts, like in sewage treatment. An energetized interface then becomes actually capable of complete mineralization of certain sorbates. Such interfaces absorbing and transferring energy (and charge carriers) can be prepared from/on all of metals, semiconductors and isolators, be they solid or liquid (Hg, Ga, or liquid semiconductors as molten iodine or PAHs). The protocol (and reasoning) of stepwise activity increases from "mere" heterogeneous catalysis up to this novel method is outlined below.

All shape, population and energy levels of given bands may be changed, for example by exposure to UV/VIS radiation, inclusion of radionuclides, microwave impact or simply electric polarization. Except for formation of refractory, insoluble oxides with some valve metals (W, Nb, Ta, to some lesser extent Ti and Zr), stability of catalyst all of which will be influenced in both aqueous and other liquid media by such treatments, however. The same holds for semiconductors as the anionic partial lattice is composed of species which all are more reducing than valence band holes - which is inevitable given the origins of the valence band. Conversely, cations in *p*-type materials are more oxidizing than conduction band electrons. Hence the "normal" result of semiconductor excitation across the band gap will be corrosion of the support, unless coupled to protective redox systems such as polychalcogenides. Stability can be improved by either doing simple electrochemistry or "fixing" one kind of charge carrier to the site of its origins, to be cleaved sometime later by a thermochemical transformation.

Catalytic oxidation

Catalytic oxidation [4] - of all gases, fluids and supercritical fluids - is commonplace in environmental technology. As usual in environmental technology, the corresponding techniques and catalyst materials were not tailor-made for this purpose but taken from similar tasks in technical chemistry: eg the 5:1-Pt/Rh catalyst used in car exhaust converters for NO_x redox treatment is simply the classical Ostwald catalyst originally taken for air oxidation of ammonia into NO, eventually producing nitric(V) acid. Likewise, Cu- or Bi oxides are used in similar functions like in technical catalysis [1]. Recently, this approach was extended to biological or biogenic catalysts, enzymes, for similar purposes ("biowashers"). Apart from heating (which is not feasible with enzymes), corresponding interfaces are not "energized", however.

Electrochemistry

Of course, polarization during adsorption depends on relative polarities of sorbent and sorbate plus energy levels and topology of the Fermi surface of the solid and can be altered by "deforming" the latter. The classical way of doing this is by applying a stationary electrical potential, that is, by doing electrochemistry. After transfer of electrons to or from a sorbate, its internal bond energies will change allowing for cleavage of some chemical bonds (dismantling weaker ones, eg M-C bonds), often hitting critical functional groups, while charging of the sorbate (turning it into radical cations or anions) alters its sensitivity towards hydrolysis: eg, radical cations will readily react with and take up OH^- ions [5, 6].

In organic electrochemistry, the material of electrodes is of paramount importance for the product distribution obtained in both reductions (eg of CO_2) and oxidation [5, 7]. Aqueous electrochemistry at metal interfaces actually is that of/at interfacial oxide films, except for gold electrodes. Unless these oxide films display metal conductivity, (eg RuO_2, IrO_2), there actually is a liquid-semiconductor interface, and polarization of the electrode brings about deformation of the Fermi edge. Beneath this, there is another metal-semiconductor interface, acting like a Schottky diode unless the oxide layers are so thin (like on Pt, about 0.4 nm) that tunnelling is relevant. Certain “critical” functional groups are particularly sensitive towards anodic oxidation; eg organotin compounds are readily cleaved [8]. While electrooxidations of hazardous compounds like substituted phenols [9, 10], trialkyltin ions [8], or aromatic amines work well in aqueous media including mud, position and stability of corresponding electrodes remain an issue for often very high potentials are used. Electrochemical (anodic) processes require a sufficient electrical conductivity of the medium to be purified; as a rule, it should be liquid.

Photoelectrochemistry

In photoelectrochemistry, oxidation and reduction entities are produced side by side [1, 2], although usually with either a poor photocatalyst stability (non-oxide semiconductors) or minimal response at most towards visible light irradiation (Ti, Sn, Nb… oxides and ternaries made thereof). On the other hand, a principal advantage of photoelectrochemistry with respect to classical electrochemistry is that there are no preconditions on the conductivity of the medium to be altered; in fact, not only liquids (organic or aqueous, containing salts or acids or not) and solids can be processed but also gases. There are applications of this latter in environmental chemistry, eg air purification removing all CO, VOCs and nitrogen oxides alongside heavy-traffic roads and -tunnels, usually using TiO_2 (rutile, anatas or un-specified). So, given the interference of both CB electrons and catalyst lability, a method would be welcome for oxidation of sorbates (dissolved potential pollutants) which produces oxidation equivalents (valence band holes in semiconductors) selectively while the electrons moving at same time in “classical” PEC would get and remain confined to certain sites. In the optimum case, generation of mobile carriers, ie holes, and trapping of electrons should be directly combined, with holes in semiconductors maintaining rather high oxidation potentials until - the later, the better - recombination occurs or there is attack on some co-sorbent.

Semiconductor-mediated photooxidation by metal complexes

This aim was achieved in our laboratory using sorbate layers of (LMCT [Ligand-Metal-Charge-Transfer]-active) transition metal complexes on either *n*- or *p*-type semiconductors of sufficiently large bandgaps (BG $\geq$ 2.5 eV) [11-14], doing photo- rather than electrochemistry. Radiation absorption is due to these complexes only while the suspended semiconductor particles do just shuttle injected holes to the co-sorbates to be oxidized. The mechanism of selective hole injection was corroborated in subsequent studies [13, 15, 16].

Metal complexes containing oxidizing (redox-active) transition metal or f-group [Eu(III), Yb(III), U(VI), Ce(IV)] elements or Hg^{2+} can undergo photoinduced electron transfer (LMCT-type photochemistry [17, 18]) with appropriate ligands bound to them; however, unless for subsequent irreversible decay of oxidized ligands (azide, oxalate), the quantum yields Φ of such reactions are low as the lifetimes of corresponding excited states are very short (usually, $<<$ 1 ns) and hence ligand-based oxidants will hardly get into a position to react with any external substrates existing as cosolutes [13]. However, recombination (backward electron transfer) can be suppressed most simply by adsorbing the complex (which is the only component to absorb light here) to some broad-gap semiconductor [12-14, 16]. Then, valence band holes are injected, reconstituting the ligand anion while the additional electron is "fixed" to the metal centre. Afterwards these valence band holes diffuse through the semiconductor particle, eventually oxidizing organic co-sorbates somewhere at its surface. This is why this reaction - which was discovered and developed by one of us [11] - can be employed for oxidizing water pollutants also. In other cases, fragments from functional groups or from the C backbone of molecules thus degraded are trapped by either the metal centre (CO, halides, SCN^-) or transferred to the activating ligand, eg to glycinate or anions of other amino acids (see formation of morpholinone) which, however, occurs at small quantum yields and thus poses no problems.

Materials and methods

For the purpose of wastewater treatment, an environmentally acceptable (ie, non-toxic) system was developed which consists of amino acid iron(III) complexes as light absorbers and Bi or Nb oxide colloids (semiconductors; Bi oxides being the basis of versatile heterogeneous catalysts also, although at far more harsh conditions). Reoxidation of Fe after photocharge transfer - leaving behind an amino acid chloro complex of Fe(II) - is done by simply passing air through the illuminated (visible light) suspension; there is no photoexcitation of the SCs themselves.

Except for bismuth oxide (Fluka) and niobia, Nb_2O_5 (Riedel-deHaen, 99.99%, metal-based), the compounds and solvents (THF, methyl acetate, ethyl acetate) were obtained from Sigma-Aldrich (p.a. grade or higher) and used as purchased. Deionized water was obtained from a Millipore system. Diclofenac Na salt, also from Sigma-Aldrich (98.5%) was dissolved in tetrahydrofuran (10 g/dm^3) and kept as a stock solution of which corresponding amounts (3 cm^3) were added to the aqueous suspension (1.5 dm^3) to get a 20 mg/dm^3 solution in water. Fe(III) compounds (10 mg Fe/dm^3 = 180 μM) and amino acids (1 : 2 molar ratio) were mixed shortly before using in distilled water as stock solutions were not stable over long times but produced Fe oxide precipitates. The 1:2 ratio left a fifth and sixth coordination site "vacant" for either other ligands (chloride, water) or chemisorption to an oxide interface; however, the complexes could in fact be removed from either semiconductor by washing with methanol also. Respective stabilities of Fe(II) and Fe(III) glycinato complexes are given in Table 1, showing there should be only weak dissociation of Fe(II) photoreduction product complex before it gets reoxidized by air.

Table 1

Stability of glycinatoferrates (data from [20, 21])

Metal ion (oxidation state of Fe)	**First association constant**	**Second association constant**	**Third association constant**
Fe(II)	4.2		
Fe(III)	10.0	18.3	26

Irradiation (Fig. 1) was done under permanent stirring with admission of air under pressure for periods of 30 min or one hour each; for compounds more refractory than diclofenac and diphenylamine this was extended until overnight periods. Experiments were done with multiple additions of organic pollutant also. Extraction of the suspension was done by ethyl acetate, concentrating the solution in a Turbovap. In addition, tests were run whether hydrolysis of ethyl or methyl acetates might be catalyzed by the oxide semiconductor colloids. No hints for formation of acetate ions in this manner were found; accordingly, all the acetic acid detected can be taken as a metastable degradation product of this photoreaction.

The photoreactor (Fig. 1) is an all-glass airlift reactor, 2 dm^3 volume. Due to the large densities of the suspended oxides, magnetic bar stirring had to augment suspension by air bubbles. Illumination was done by three 15 W neon-discharge bars arranged vertically around the glass vessel (that is, parallel to its long axis). This glassware causes complete UV absorption, precluding direct excitation of either Bi or Nb oxides. The Fe complexes are brownish-coloured; their spectra were obtained by a Lambda 40 spectrophotometer.

Fig. 1. The airlift reactor filled with niobia (2 g/dm^3) and the Fe amino acid complex; suspension by air passage and stirring. Three neon discharge bulbs (15 W each, to the left) provide light. For better viewing, the device is partly opened; in the original system, the bulbs are fitted to the glass vessel directly by wire. Photograph taken by H. Silbernagel

Analysis was done mainly using a Varian GC/MS system, consisting of a GC-3800 gas chromatograph and a Saturn 2000 mass spectrometer fitted to it (EI ionization at 70.1 eV electron energy). The GC device contained a DB-1701 semipolar GC column (30 m×0.25 mm×0.25 µm). Retention times of diclofenac, its proxy diphenylamine and possible fragments or isomerisation products of either such as aniline, carbazol, phenylacetic and 2,6-dichlorophenylacetic acids (all from Sigma-Aldrich) were measured and compared with published mass spectra of these compounds. Among unknown fragmentation products, acetic acid and morpholinone were identified from their mass spectra, compared with retention behaviour of an authenticated CH_3COOH sample. The retrieved sorbent samples were washed with methanol to obtain adsorbed primaries or intermediates to distinguish either from results of "genuine" photodegradation.

Results

Complete removal of 10÷20 mg solute/dm^3 usually takes less than two hours in the irradiated suspension. Small amounts of acetic acid are formed from the degraded organics, besides mineralization products. There are no chloroorganics left over in the product mixture (MS isotopic patterns). The three amino acids combined with Fe(III) [glycine, alanine, tyrosine[1]] in these experiments reacted similarly, bringing all about complete destruction of diclofenac (retention time: 14.78 min) within reasonable periods of time. The efficiency of diclofenac cleavage decreased according to tyr > gly > ala. The morpholinone by-product at 11.60 min retention time did appear with glycine only; with alanine, there was a novel peak at some 14 minutes, instead. Comparing the Fe(III) sources $FeCl_3$ hydrate and $FeSO_4 + H_2O_2$, the latter yielded more active photocatalyst systems after addition of amino acid (afterwards[2]). Accordingly, the white-light irradiation activates aminocarboxylate chelate ligands rather than Cl or OH ligands, then injecting holes. There were no larger amounts of (unidentifiable) additional by-products when replacing glycine with the other amino acids.

There is some conspicuous by-product of this kind of photochemical degradation, namely morpholinone (3-aza-δ-valerolactone), presumably formed by transfer of one ethylene-like C_2 unit to a glycinate ligand subsequent to its photoexcitation. While this reaction is both observed with $FeCl_3$ hydrate and Fe(III)sulphate as iron reducts besides glycine, it does not occur without an external carbon source such as diclofenac or aniline, and, by replacing glycine with other amino acids [alanine, tyrosine], the corresponding signal also vanishes to be replaced by more complicated ones with longer column retention times. There is precedent for ligand exchange at the metal site in such photoreactions [11-13], affording CO ligands and thus metal carbonyls (M = Mo, Os, Ir, Cu) but hitherto not for alkylation of an organic ligand in this kind of photoreactions.

In the niobia system, it is seen that Fe complexes (brownish to orange) are deposited on the white sorbent directly, tanning it accordingly. This is no precipitate of Fe oxides although this sorption brings about a pH decrease (the opposite with Bi_2O_3) but just the adsorbed complex which, unlike Fe oxides, can be readily removed by washing with methanol. By this adsorption of complexes to semiconductors (SCs), quantum yields Φ of LMCT photoreactions increase vastly [13]. Successful degradations, including both removal

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[1] Tyrosine was selected for comparison due to its role in photosynthetic water oxidation which suggests that the tyrosinyl radical, once formed, can also transfer charges to other solid supports. Although the formal redox potential of the tyrosinyl phenoxy radical is but 0.93 V, it is involved in oxidation of water there. In our experiments, tyrosinatoferrates(III) show an enhanced reactivity with respect to the more simple glycinato or alaninato complexes.

[2] This is not a FENTON system; in the catalytic cycle, formation of ferryl $Fe^{IV}O^{2+}$ species as (then likely more efficient) hole injectors is very unlikely.

of functional groups like –CHO, halide, and complete mineralization or degradation to acetate ion, were demonstrated with PAHs (naphthalene up to tetracyclic ones), hetero-PAHs (eg carbazol), aldehydes [11, 15], aliphatic and aromatic (this work) amines, carboxamides, phenols [14] and CH-acids, including pharmaceutical residues such as diclofenac and its environmental decay products which do harm eg, to fishes and vultures. While chlorinated compounds are entirely removed (diclofenac, 4-chlorophenol or 2,6-dichlorophenylacetic acid), carbazol remains stable, except for adsorption, probably because its 1e-oxidation potential is too high to undergo hole attack (Tab. 2).

Table 2

Electron-, hole-, oxidation and reduction potentials of metal oxides and (data from [6] [PAHs]; [24] [aniline, phenols]) organic substrates

Substance	**E_{cb} or 1e-reduction potential (V vs SCE)**	**E_{vb} or 1e-oxidation potential (V vs SCE)**	**Remarks**
Bi_2O_3	About –1.5	About 1.4	E_{cb} calculated from E_{vb}, bandgap (2.9 eV); pH 7
Nb_2O_5	About –1.2	2.2	E_{cb} calculated from E_{vb}, bandgap (3.4 eV); pH 7
diphenylamine		0.83	
carbazol		1.70	
aniline		0.63	Solvent: 2-propanol
phenol		0.63	Solvent: 2-propanol or water
4-cresol		0.54	“
4-chlorophenol		0.65	“
4-nitrophenol		0.92	“
tyrosine		0.93	Forming phenoxy radical
naphthalene		1.31	Solvent: CH_3CN
anthracene		0.84	“
phenanthrene		1.23	“
pyrene		0.86; 1.12	“
biphenyl		1.48	“
glycinate		(1.32)	Refers to onset of oxidation at Pt anodes in a manner of bidentate adsorption

The turnover TON (oxidation yield with respect to Fe catalyst) can be estimated as follows, giving proof this is really a photocatalytic oxidation: [Fe] is 10 mg/dm^3 (some 180 µM/dm^3), while during six repeated additions up to 120 mg (some 590 µM) of C_{14} compound diclofenac are degraded down to CO_2, some acetate plus Cl^-, NH_3…, that is, about 8.2 millimoles of carbon undergo oxidation (> 45 times the amount of Fe in the system). The Fe(III)-induced photooxidation is transferring one electron (or hole, respectively) each time, whereas oxidation of aromatic CH moieties to CO_2 will take three electrons per carbon. Although the yields of acetate by-product are not exactly known (yet small), and degradation of C-Cl sites and of the aliphatic side chains are to be treated differently, with diphenylamine nitrogen apparently undergoing release as NH_3 (pH increase during photoreaction) rather than any oxidation at N (N remaining NH_3 is common in “classical” photoelectrochemistry of organonitrogens also) taking place it is safe to conclude that every iron complex central ion present in the suspension will undergo the cycle of complex formation with amino acid excess, photodissociation, charge transfer and air reoxidation (Fig. 2 a-c) more than a hundred times. Thus it is a catalytic reaction indeed.

Some, thus small, part of glycinate or other amino acid ligand will become alkylated to yield (substituted) morpholinone.

While in removals of aromatic amines including diclofenac, phenols or aldehydes, both niobia and bismuth oxide are completely stable in aqueous suspension even though oxide partial lattices might undergo h^+-induced oxidation, there are problems with long-term stability in decomposing PAHs: while h^+ oxidation potential suffices to remove them all (including naphthalene and biphenyl) at Bi_2O_3, triplet states of some PAHs adsorbed can reduce bismuth oxide to elemental bismuth, causing it to turn first green, then dark-brown and finally black (colloidal Bi). Bleaching by hydration is also observed, forming white BiO(OH). Otherwise, only minute traces of dissolved Bi are passed into solution/suspension by these alterings.

Discussion

The reaction can be done with environmentally acceptable (non-toxic) sensitizer systems, concerning both metal complexes and semiconductors (which, however, was not the only criterion for selecting the two SCs used here); it is photocatalytic (TON > 100 with respect to Fe), allowing for multiple additions and removals of pollutant compound(s) and the by-products are not toxic either. Concerning the “optimum” extent of interaction between a possible catalyst and the substrate(s), consider Sabatier´s rule stating that “intermediate”ly strong binding is best. This also holds for biological catalysis [4, 21]. This is an appropriate description of the sorbent properties of both used oxides also.

It is better suited for combination with sewage water treatment than cleaning up an already polluted open water as the product mixture from ambient photolysis contains substantial amounts of carbazoles (more persistent than the original diclofenac and thus piling up eg in Lake Zurich) formed by photochemical closure of a third ring in the same manner as diphenylmethanes and diaryl ethers, diaryl sulfides convert into fluorenes, dibenzofuranes or dibenzothiophenes, respectively [22], under UV irradiation. As carbazoles readily adsorb to the semiconductors but do not undergo oxidation owing to their high-lying oxidation potentials there, they cannot be removed in a catalytic fashion by this procedure, at least when using these semiconductors and sensitizers.

All the experiments showed that diclofenac was more reactive (degraded faster) than either simple diphenylamine or phenylacetic acid (hypothesized product of aniline group cleavage). Accordingly, oxidations of arylamines or aromatic carboxylic acids conform to the expectation that kinetics are controlled by the Hammett equation [23] with a positive ρ value like is commonly observed in oxidations of benzenoid aromatics, be they induced by free radicals like OH or electrophilic agents such as NO_2^+, SO_3 or by simple oxidants (eg, bromine in glacial acetic acid, Hg^{2+} in water [which latter causes aryl anion transfer from EAr_4, E = Si to Pb, according to $Hg^{2+} + EAr_4 \rightarrow EAr_3^+$ {or Ar_3E-OH} + $ArHg^+$]). The same trend of liability towards this kind of photooxidation vs σ_p was observed with a series of *para*-substituted phenols (and some others, including thymol and salicylic acid) earlier [14]; however, the inertness of 4-nitrophenol here might also be attributed to the fact that its anodic oxidation potential of 0.924 V (in both 2-propanol [24] and neutral water [our measurements by CV; [14]) is very close to ε_{vb} for α-Bi_2O_3. Also see Table 2.

There is a relationship among all σ_p, the 1e-oxidation potential of aromatic organics and k_{OH} (both in air and aqueous; Fränzle 2000, unpublished) while reactions with common 1e-oxidants like Ce(IV), $^1O_2$ [25], follow Hammett-type kinetics also. The same apparently is observed here in photoelectrochemistry, when "blurring" of the data by electron transfer from conduction band is suppressed.

The key mechanism is hole injection to the valence band of the sorbent by photoproduced (LMCT transition) ligand radicals (Figs. 2a-c) [11, 15] and subsequent oxidative quenching of the holes by organic pollutant co-adsorbed to the semiconductor particles, with air reoxidation (Fig. 2c) of Fe(III) and amino acid excess in solution effecting reconstitution of the photosensitizing Fe complex.

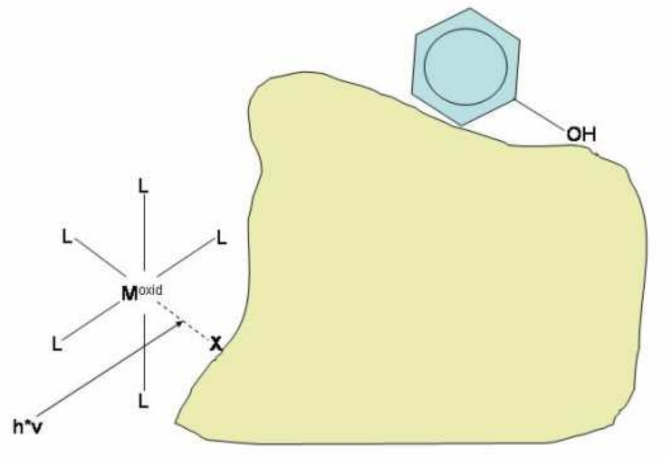

Fig. 2a. Onset of photoreaction, complex still intact, phenol (hole trap) simply adsorbed

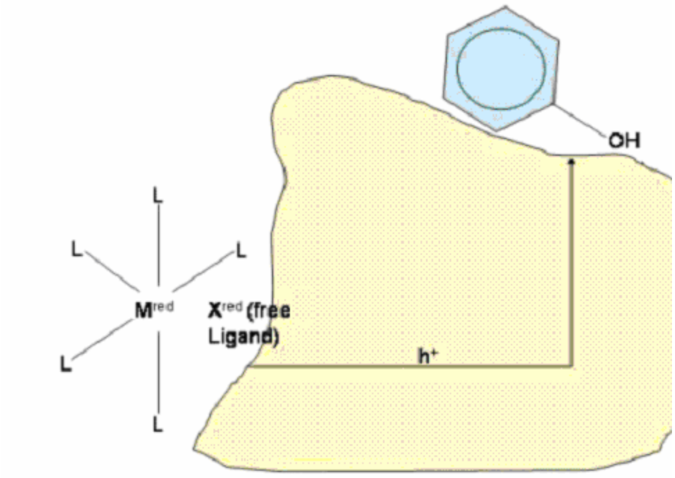

Fig. 2b. Ligand removed and back-reduced, metal center also reduced, hole diffusing to organic co-sorbent

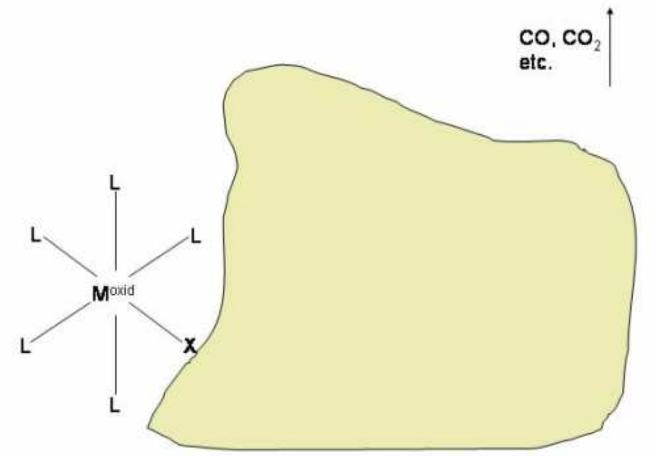

Fig. 2c. Admission of O_2, removal of phenol fragment after h^+ attack by mineralization, Fe gets reoxidized. Complex restores spontaneously at [amino acid] ≈ 0.4 mmol/dm^3

When there is no semiconductor around, Fe(III) α-amino acid complexes undergo photooxidation readily producing CO_2 and aldehydes (or glyoxylic acid) besides NH_4^+ [17, 26], starting in the visible wavelength region at $\lambda \approx$ 450 nm. Adsorption to (either *n*- or *p*-type) semiconductors obviously suppresses this reaction in favour of hole transfer.

The Kolbe electro- and Photo-Kolbe photoelectrooxidations of carboxylate ions from acetate onwards take potentials much above + 2 V vs SCE, on Pt or semiconductors, respectively. Subjecting glycin(at)e to Kolbe electrooxidation conditions does not yield ethylene diamine [7], but trimethylamine [27] as principal product; PEC produces traces of oligopeptides. While photodecarboxylation may take place in presence of diverse oxidizing ions causing formation of some organometal species [28], the oxidation potential of amino acid ligands still remains unknown whereas that of R-COO radical is estimated to be ≈ 2.0 V (also see, photooxidations with NO_2^{2+} [11]), with just psec lifetimes against CO_2 cleavage which still is sufficient for hole injection. Thus glycinate "survives", except for small extents of ring-closing alkylation or apparently of oligopeptide formation (which latter [glycine oligomers] was noticed earlier in classical PEC setups [29]).

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HETEROGENNA KATALIZA ŚRODOWISKOWA. OCZYSZCZANIE WODY PRZEZ AKTYWOWANE INTERFEJSY - NOWE PROCEDURY AKTYWACJI POWIERZCHNI

Abstrakt: Poza ustalonymi procedurami wiele związków organicznych występujących w środowisku może być mineralizowanych lub co najmniej degradowanych do np. octanów przez naświetlanie światłem widzialnym kompleksów z żelazem(III), zaadsorbowanych na niektórych szerokopasmowych półprzewodnikach. Wydajność kwantowa znacznie wzrasta dzięki adsorpcji ze względu na fotochemię LMCT (*Ligand*-to-*Metal Charge Transfer*) w jednorodnym roztworze. Półprzewodnik działa jak przekaźnik elektronu (lub raczej dziury w paśmie przewodnictwa), który utrwala utleniające właściwości rodników ligandu, pierwotnie wytwarzanych w dłuższych okresach czasu. Koloidalna cząstka półprzewodnika, sama niewzbudzona, jest nośnikiem dziury i umożliwia utlenianie współadsorbowanych związków występujących w niewielkich stężeniach, a także zgromadzonych w wyniku adsorpcji. Oczyszczanie wody z różnego rodzaju zanieczyszczeń jest możliwe, a reakcja fotokatalityczna o wydajności ≥ 100 w odniesieniu do kompleksu Fe może zachodzić z udziałem powietrza wprowadzanego do zawiesiny, tolerując ponowne wprowadzenie związków, które mają być usunięte. Z wyjątkiem niektórych octanów mineralizacja substratu zachodzi całkowicie.

Słowa kluczowe: fotoutlenianie, półprzewodniki, kompleks żelaza, uzdatnianie wody, kataliza heterogenna, metoda zwiększonej aktywacji powierzchni

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AIR POLLUTION STUDIES IN BULGARIA USING THE MOSS BIOMONITORING TECHNIQUE

WYKORZYSTANIE MCHÓW W BADANIACH BIOMONITORINGOWYCH ZANIECZYSZCZENIA POWIETRZA W BUŁGARII

Abstract: The moss biomonitoring technique was used to study trace element atmospheric deposition in four areas of Bulgaria (the Western Thracian-Rhodope, the Eastern Thracian-Rhodope, the South-Eastern and the North-Central Bulgaria) during the European moss survey in 2005. A total of 41 elements (Na, Al, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rd, Sr, Mo, Cd, Sb, I, Cs, Ba, La, Ce, Nd, Sm, Tb, Dy, Tm, Yb, Hf, Ta, W, Au, Pb, Th, and U) were determined by instrumental epithermal Neutron Activation Analysis (NAA) and Atomic Absorption Spectrometry (AAS) in 97 samples of terrestrial moss. The moss species used was *Hypnum cupressiforme*. Principal component analysis (factor analysis) was used to identify and characterize different pollution sources and to point out the most polluted areas. The interpretation of the factor analysis findings points to natural crust, marine, and vegetation components as well as to anthropogenic sources: ferrous (Plovdiv, Haskovo) and non-ferrous industries (Plovdiv, Kardzhali, Burgas); oil refining (Burgas), and central heating stations (Plovdiv, Haskovo, Stara Zagora, Burgas). Comparison of the medians of the elemental concentrations in moss samples collected in Bulgaria with those in the Balkan and other European countries reveals that the Balkan countries show considerably higher concentrations of most elements in moss than observed in other European countries where moss sampling has been employed.

Keywords: atmospheric deposition, moss biomonitoring, neutron activation analysis, atomic absorption spectrometry, trace elements, factor analysis, sources

Introduction

The moss technique [1, 2] first introduced in Scandinavia, has shown to be very suitable for studying the atmospheric deposition of trace elements. It is now being used as

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part of monitoring programs for air pollutants in most European countries [3]. Mosses have a rudimentary root system and readily take up elements from the atmosphere. The advantage of the method is in the simplicity of sample collection, although the determination of the species needs an experienced hand. Data from existing surveys of heavy metal concentrations in mosses is an invaluable resource for international negotiations on heavy metal pollution.

The data from moss surveys allow examination of both spatial and temporal trends in heavy metal deposition, and identification of areas where there is high deposition of heavy metals from long-range atmospheric transport and local sources. In the Republic of Bulgaria the study of air pollution from heavy metals and other toxic elements based on moss analysis was undertaken since 2000 within the framework of a Bulgarian-Russian collaboration, in order to assess the general situation regarding heavy metal pollution and to jointly report these results to the European Atlas of Heavy Metal Atmospheric Deposition issued by UNECE ICP Vegetation [4, 5].

The presence of heavy metals in air within the Bulgarian territory had been previously studied only for some geographic regions using neutron activation analysis (NAA) [6] and inductively coupled plasma emission spectrometry (ICP-AES) for determination of a limited number of elements [7-11]. The primary task of the present study was to elucidate the present-day environmental situation in the sampling areas and to compare the results obtained with existing data from the previous moss surveys in Bulgaria. Furthermore the biomonitoring results could serve the purpose of risk assessment of certain endemic diseases such as Balkan nephropathy, arsenosis, etc., which are hypothetically connected with the environmental contamination with toxic substances [12]. This approach is in line with the European trend in establishing correlation of the environmental biomonitoring with human health aspects [13].

Study area

The Republic of Bulgaria is located in the south-eastern part of Europe and in the central-eastern part of the Balkan Peninsula. The area of the country is 110 910 km^2, mostly in mountainous territories. Four regions: the Western Thracian-Rhodope, the Eastern Thracian-Rhodope, the South-Eastern and the Northern Central, comprising around 25% of the whole territory of Bulgaria, were sampled in this study. The climate in most of this area is continental with very hot and dry summers. In areas along the Black Sea coast there is a Mediterranean climate. In summer temperatures in the south of Bulgaria often exceed 40ºC, and the highest temperature ever of 47ºC was recorded at a site near Plovdiv. At such climatic conditions only a limited number of moss species are able to grow in arid areas.

Industry plays a key role in the Bulgarian economy. Bulgaria is among the leading countries in Europe in production of lead, zinc, copper, caustic soda (NaOH), and nuclear energy. It possesses vast reserves of lignite, anthracite coal, and gold. Bulgaria has abundant non-metalliferrous minerals such as rock-salt, gypsum, and marble. Ferrous metallurgy is of major importance. Much of the production of steel and pig iron takes place in Kremikovtsi and Pernik, with a third metallurgical base in Debelt. In production of steel and steel products per capita the country heads the Balkans. The largest refineries for lead and zinc operate in Plovdiv (the biggest refinery between Italy and the Ural Mountains), Kardzhali, and Novi Iskar; for copper in Pirdop and Eliseina; for aluminium in Shumen. Bulgaria ranks first in south-east Europe in production of many metals, measured per capita. About 14% of the total industrial production relates to machine-building, and 24% of the people work in this field. Electronics and electric equipment production have developed to a high degree. The largest industrial centres include Sofia, Plovdiv and the surrounding area, Botevgrad, Stara Zagora, Varna, Pravets and many other cities. These plants produce household appliances, computers, CDs, telephones, medical and scientific equipment. Many factories producing transportation equipment currently do not operate at full capacity. These factories produce trains (Burgas, Dryanovo), trams (Sofia), trolleys (Dupnitsa), buses (Botevgrad), trucks (Shumen), and motor trucks (Plovdiv, Lom, Sofia, Lovech). Lovech has an automotive assembly plant. Rousse serves as the main centre for agricultural machinery. Most Bulgarian shipbuilding takes place in Varna, Burgas and Rousse. Bulgarian arms production mainly operates in central Bulgaria (Kazanlak, Sopot, Karlovo). Foreigners seeking additional homes have recently boosted the Bulgarian properties market. Buyers come from across Europe, but mostly from the United Kingdom, encouraged by relatively low property prices and easy accessibility via air travel.

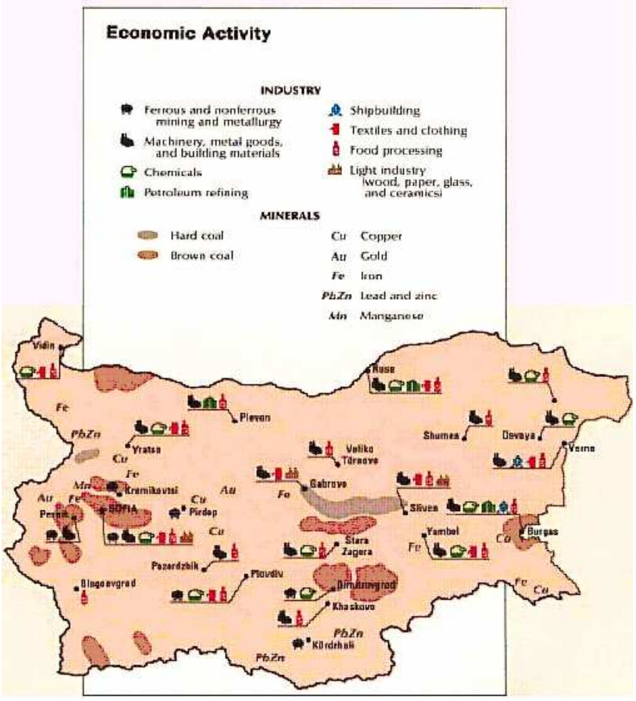


Fig. 1. The economic map of Bulgaria [14]

Experimental

Sampling. Samples of the moss species *Hypnum cupressiforme* were collected at 97 localities covering four geographic regions of the country during the period July-October, 2005. The sampling sites are shown on the map in Figure 2.

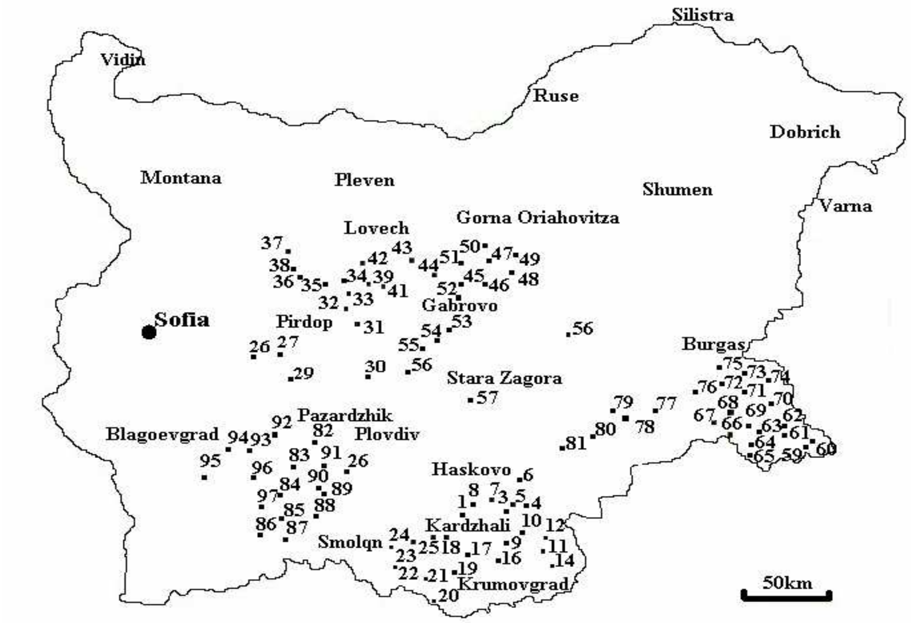


Fig. 2. Study area - Republic of Bulgaria [15]

The sampling was carried out in accordance with the strategy of the European moss survey program [5]. Samples were collected at a distance of at least 300 m from main roads, at least 100 m from roads and at least 200 m from villages, in forest glades or in open heath to reduce through-fall effects from the forest canopy. In order to make the moss samples representative for a reasonably large area, each sample was composed of five to ten sub-samples collected within an area of 10 × 10 m. Collected samples were stored in paper bags. A separate set of disposable polyethylene gloves was used for collection of each sample.

Analysis. **NAA**. The neutron activation analysis (NAA) was performed at the pulsed fast reactor IBR-2 at the Frank Laboratory of Neutron Physics, Dubna, Russia.

At the laboratory the samples were cleaned from extraneous plant material and air-dried to constant weight at 30÷40°C for 48 h. The samples were not washed and not homogenised. Green-brown moss shoots representing the last three years´ growth were subjected to analysis, as they correspond approximately to the deposition over the last three years. Previous experience from the use of NAA in moss biomonitoring employing *Hylocomium splendens* has shown that samples of 0.3 g are sufficiently large to be used without homogenization [16]. The samples were pelletized before irradiation using simple press-forms. For short irradiation unwashed samples of about 0.3 g were heat-sealed in polyethylene bags. For long irradiation samples of the same weight (about 0.3 g) were packed in aluminium cups. Characteristics of neutron flux density in the channels equipped with a pneumatic system are given in [17].

For determinations involving short-lived radionuclides samples were irradiated for 3 min. After irradiation two gamma-spectrometric measurements were performed; the first one for 5 minutes after 2÷3 minutes of decay, and the second for 20 minutes after 9÷10 minutes decay.

Determinations using long-lived radionuclides were performed following an irradiation for 100 h in the cadmium-screened channel 1. After irradiation samples were repacked into clean containers and gamma spectra were registered after 4÷5 and 20÷23 d for 45 min and for 3 h, respectively as described elsewhere [18].

Table 1 lists selected peak energies for NAA and method of analysis. The gamma-spectra of the induced activities were analyzed using software developed at the Frank Laboratory of Neutron Physics [19].

AAS. The environmentally important element lead cannot be determined by INAA, and cadmium and copper are difficult at low concentration levels. These elements were therefore determined by flame atomic absorption spectrophotometry (Perkin Elmer 303) with background correction and acetylene as fuel at the Institute of Botany, BAS, Sofia. About 1 g of the moss material was treated with 15 cm^3 nitric acid (9.67 M) overnight. The wet-ashing procedure was continued by heating on a water bath, following by addition of 2 cm^3 portions of hydrogen peroxide (30%). This treatment was repeated until complete digestion. The filtrate was diluted with doubly distilled deionised water (0.06 µS cm^{-1}) to 25 cm^3. All solutions were stored in plastic flasks.

Table 1

List of selected peak energies for NAA

Element	**Isotope**	**Half-life**	**Gamma-ray peak [keV]**	**Element**	**Isotope**	**Half-life**	**Gamma-ray peak [keV]**
Na	^{24}Na	14.7 h	2753.6	Sr	^{85}Sr	64.8 d	514.0
Al	^{28}Al	2.2 m	1778.9	Mo	^{99}Mo	66.0 h	140.5
Cl	^{38}Cl	37.2 m	2168.8	Cd	^{115}Cd	53.5 h	527.7
K	^{42}K	12.4 h	1524.7	Sb	^{124}Sb	60.2 d	1691.0
Ca	^{49}Ca	8.7 m	3084.4	I	^{128}I	25.0 m	442.9
Sc	^{46}Sc	83.8 d	889.2	Cs	^{134}Cs	2.1 y	795.8
Ti	^{51}Ti	5.8 m	320.1	Ba	^{131}Ba	11.8 d	496.8
V	^{52}V	3.8 m	1434.1	La	^{140}La	40.2 h	1596.5
Cr	^{51}Cr	27.7 d	320.1	Ce	^{141}Ce	32.5 d	145.4
Mn	^{56}Mn	2.6 h	1810.7	Tb	^{160}Tb	72.3 d	879.4
Fe	^{59}Fe	44.5 d	1099.2	Hf	^{181}Hf	42.4 d	482.0
Co	^{60}Co	5.3 y	1173.1	Ta	^{182}Ta	114.4 d	1221.4
Ni	^{58}Co	70.9 d	810.8	W	^{187}W	23.9 h	685.8
Zn	^{65}Zn	244.0 d	1116.0	Au	^{198}Au	2.7 d	411.8
As	^{76}As	26.3 h	559.1	Th	^{233}Pa	27.0 d	312.0
Br	^{82}Br	35.3 h	776.5	U	^{239}Np	2.4 d	228.2
Rb	^{86}Rb	18.7 d	1076.6				

Quality control

The quality of NAA results was ensured by simultaneous analysis of the examined samples and reference materials (RM) Lichen 336 IAEA (International Atomic Energy Agency) and NORD DK-1 (moss reference sample prepared for intercomparison in 1990). The NAA data and recommended/certified values of reference materials one may find in [21]. The quality control (QC) of AAS determinations was based on the standard addition method and it was found that the recovery of the investigated elements ranged between 98.5 and 101.2%. Beside standard addition method, blanks parallel to the decomposition of samples and preparation of sample solutions for analysis were analyzed. Moreover the quality of the determinations was checked by simultaneous analysis of the moss reference materials M2 and M3 prepared in Finland for the European moss surveys. The obtained concentrations of Cu, Pb and Cd were in good agreement with the corresponding recommended values [20]. Deviations between duplicate sample solutions analyzed simultaneously were always below 5%.

Results and discussion

Median values and ranges of the elements studied are presented in Table 2 along with corresponding data from similar studies in the neighbouring Balkan countries: Macedonia, Romania, and Serbia [5, 19-21].

Table 2

Comparison of the results obtained in the present study [mg/kg] with other Balkan countries and a pristine area (Northern Norway)

	Bulgaria		**Bulgaria**		**Macedonia**	
	(Present work)		**(West and South) [6, 8]***		**[21]**	
No. of samples	***99***		***103***		***73***	
Element	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
Na	725	189÷8210	523	155÷5580	419	118÷8670
Al	6930	1532÷43600	3840	1110÷46400	3740	825÷17600
Cl	232	84÷1330	161	59÷1180	149	43÷693
K	6020	2750÷13800	5760	3270÷20500	8620	2860÷18200
Ca	8960	4530÷32200	7280	2270÷19700	5590	1210÷23640
Sc	0.92	0.21÷7.20	0.65	0.2÷6.4	0.81	0.12÷6.79
Ti	340	94÷2590	–	–	163	12÷1370
V	8.7	2.23÷64	8.4	2.2÷113	6.9	1.79÷43
Cr	5.6	1.18÷55	3.2	0.5÷26.9	7.47	2.33÷122
Mn	243	45÷1270	251	32÷986	186	37÷1480
Fe	3000	689÷19400	2310	692÷14700	2460	424÷17380
Co	1.49	0.35÷28	1.08	0.23÷10.6	1.09	0.24÷13.6
Ni	5	1.08÷29	4.1	0.5÷18.6	2.4	0.09÷24
Cu*	6.84	0.1÷63.9	14.5*	5.34÷1860	22	3÷83
Zn	45	23÷774	41	19÷379	39	14÷203
As	0.97	0.27÷8.76	1	0.3÷59.0	0.8	0.12÷8.0
Br	4.4	1.33÷18	3.6	1.1÷11.6	2.16	0.06÷7.7
Rb	15	5.16÷68	12	3.0÷69	10.9	5.0÷87
Sr	36	14÷170	25	7÷106	31	11.8÷136
Mo	0.37	0.01÷1.22	0.99	0.16÷3.36	0.19	0.03÷1.12
Cd*	0.23	<0.1÷5.56	–	–	0.16	0.016÷2.95
Sb	0.29	0.07÷8.7	0.23	0.07÷20.2	0.2	0.039÷1.4
I	2.6	0.85÷6.31	1.4	0.6÷4.4	1.18	0.36÷2.8
Cs	0.52	0.18÷5.71	0.4	0.10÷2.96	0.39	0.097÷1.7
Ba	79	21÷294	68	17÷517	54	14÷256
La	3.3	1÷61.79	2.9	0.8÷23.7	2.32	0.50÷22
Ce	6.8	1.75÷143	–	–	5.6	0.83÷42
Nd	3.15	0.01÷47	–	–	–	–
Sm	0.6	0.19÷8.30	–	–	–	–
Tb	0.076	0.02÷0.98	0.068	0.016÷0.610	0.06	0.01÷0.56
Dy	0.43	0.01÷4.40	–	–	–	–
Tm	0.057	0.02÷0.67	–	–	–	–

Yb	0.22	0.05÷3.32	–	–	–	–
Hf	0.45	0.11÷12.1	0.46	0.11÷4.78	0.26	0.05÷3.8
Ta	0.127	0.03÷1.52	0.076	0.018÷0.563	0.09	0.013÷0.79
W	1.22	0.25÷13	0.193	0.03÷1.39	1.21	0.25÷3.9
Au	0.015	0.0007÷0.043	0.0042	0.0009÷0.047	0.0061	0.001÷0.034
Pb*	11.7	0.5÷368	18.9*	4.55÷887	6	1.5÷37.2
Th	0.86	0.27÷23	0.56	0.11÷4.53	0.67	0.12÷7.6
U	0.3	0.09÷6.28	0.2	0.03÷1.87	0.21	0.03÷1.45

	Romania (Transilvania) [24]		**Northern Serbia [25]**		**Northern Norway [22]**	
No. of samples	***70***		***92***		***100***	
Element	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
Na	902	192÷4330	694	178÷2440	–	–
Al	5550	830÷23000	6800	1280÷22100	200	67÷820
Cl	370	160÷1300	256	105÷1030	–	–
K	7770	4770÷20000	5090	2710÷11800	–	–
Ca	5770	1250÷23500	7720	2890÷18120	2820	1680÷5490
Sc	0.94	0.21÷6.13	1.31	0.27÷4.13	0.052	0.009÷0.220
Ti	–	–	71	11÷297	23.5	12.4÷66.4
V	8.7	1.95÷32	11	2.85÷39	0.92	0.39÷5.1
Cr	13.8	2.72÷51.9	6.51	1.14÷22	0.55	0.10÷4.2
Mn	265	27÷1470	217	30÷2340	256	22÷750
Fe	3290	815÷21340	3110	720÷9230	209	77÷1370
Co	1.41	0.32÷7.0	8.24	1.42÷39	0.202	0.065÷0.654
Ni	5.4	0.6÷32	6.73	1.96÷26	1.14	0.12÷6.6
Cu*	21.5	2.21÷2420	16.9	6.31÷3140	3.6	2.1÷9.2
Zn	135	39÷2950	44	14÷415	26.5	7.9÷173
As	2.2	0.59÷45.1	3.35	0.46÷61	0.093	0.020÷0.505
Br	8.6	2.03÷20.9	5.75	1.83÷18.0	4.5	1.4÷20.3
Rb	15	5.8÷135	13	17200	7.7	1.3÷51.5
Sr	37.4	1.8÷290	22	34900	15.8	3.6÷43.3
Mo	0.65	0.13÷10	0.85	0.12÷23	0.135	0.065÷0.70
Cd*	–	–	0.4	0.4÷6.5	0.058	0.025÷0.171
Sb	0.88	0.16÷51	0.52	0.13÷7	0.033	0.004÷0.240
I	2.17	0.76÷5.55	2.09	0.87÷4	2.5	0.6÷41.7
Cs	0.51	0.12÷3.4	0.76	0.11÷18.2	0.072	0.016÷0.88
Ba	101	20÷658	39	13÷130	17.1	5.6÷50.5
La	2.4	0.4÷15.2	4.66	41518	0.189	0.045÷2.56
Ce	6.1	0.9÷42.5	9.2	1.84÷28	0.342	0.095÷4.61
Nd	–	–	–	–	–	–
Sm	–	–	–	–	–	–
Tb	0.07	0.01÷0.42	0.11	0.02÷0.36	0.003	0.002÷0.030
Dy	–	–	–	–	–	–
Tm	–	–	–	–	–	–
Yb	–	–	–	–	–	–
Hf	0.56	0.12÷4.66	0.78	0.15÷2.6	–	–
Ta	0.1	0.01÷0.66	0.11	0.024÷0.29	–	–
W	1.02	0.12÷8.74	1.34	0.19÷3.3	0.127	0.009÷1.23
Au	0.025	0.003÷0.114	0.0041	0.00029÷0.087	–	–
Pb*	14.3	6.45÷31.5	–	–	1.17	0.64÷6.12
Th	0.81	0.21÷4.16	0.82	0.18÷2.4	0.033	0.004÷0.240
U	0.28	0.04÷1.36	0.32	0.08÷1.03	0.015	0.001÷0.138

* Determined by ICP-AES [8]

Table 3

Factor analysis of NAA and AAS data on moss samples from Bulgaria

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
Na	**0.893**	0.030	0.070	0.111	–0.026
Al	**0.668**	0.138	**0.596**	0.077	0.205
Cl	0.060	0.032	–0.027	**0.782**	0.090
K	**0.526**	–0.027	0.175	0.419	0.322
Ca	0.058	0.089	0.034	**0.695**	0.145
Sc	**0.732**	0.027	**0.626**	0.011	0.115
Ti	**0.605**	0.106	**0.703**	0.074	0.147
V	0.229	0.159	**0.837**	–0.159	0.087
Cr	**0.598**	0.077	**0.737**	0.025	–0.001
Mn	0.215	0.150	0.278	–0.092	**0.573**
Fe	**0.710**	0.030	**0.628**	0.113	0.158
Co	**0.691**	–0.084	0.274	0.173	0.071
Ni	0.424	0.023	**0.824**	–0.011	0.105
Cu*	0.033	0.355	0.176	0.259	0.037
Zn	0.148	**0.954**	0.070	0.021	0.081
As	**0.470**	0.172	**0.580**	0.158	0.225
Br	0.214	0.091	0.097	0.048	**0.779**
Rb	**0.729**	0.136	0.390	0.044	0.292
Sr	**0.737**	0.228	0.057	0.123	0.352
Mo	0.145	**0.479**	0.149	0.075	0.327
Cd*	0.026	**0.956**	0.053	–0.008	–0.014
Sb	0.041	**0.954**	0.068	0.022	0.066
I	0.112	–0.090	0.330	–0.034	**0.750**
Cs	**0.605**	0.180	0.377	–0.006	0.292
Ba	**0.687**	0.097	0.424	0.099	0.255
La	**0.954**	0.074	0.163	–0.028	0.003
Ce	**0.943**	0.088	0.137	–0.066	–0.019
Nd	**0.912**	0.030	0.206	0.013	–0.070
Sm	**0.955**	0.064	0.205	–0.031	0.026
Tb	**0.924**	0.061	0.300	–0.048	0.005
Dy	**0.776**	0.001	0.406	0.011	0.230
Tm	**0.872**	0.053	0.371	0.050	0.163
Yb	**0.924**	0.025	0.294	0.042	0.121
Hf	**0.868**	–0.037	0.168	0.080	0.134
Ta	**0.817**	0.072	0.383	0.109	0.232
W	**0.736**	0.164	0.116	0.077	–0.092
Au	–0.049	–0.032	–0.014	**0.721**	–0.307
Th	**0.975**	0.110	0.049	–0.029	0.052
U	**0.904**	0.106	0.094	0.049	0.191
Pb*	0.131	**0.929**	0.058	–0.009	–0.076
expl.var	*16.665*	*4.313*	*5.582*	*2.181*	*2.615*
Prp.totl	*0.406*	*0.105*	*0.136*	*0.053*	*0.064*

For comparison with a pristine territory corresponding data for northern Norway [22] are shown in the right-hand column. The Norwegian values were obtained by ICP-MS analysis and were based on nitric-acid solutions, potentially leaving out fractions of the elements in moss samples contained in silicate minerals (attached soil particles). The same argument may apply to the present AAS results.

Based on the data in Table 2, countries can be ranged according to the median values for each element. This presents a generally favourable picture for Bulgaria in comparison with the other three Balkan countries concerning elements predominantly of industrial origin. In comparison with the pristine northern Norway however the Bulgarian data are substantially higher for typical air pollution elements (V, Cr, As, Ag, Cd, Sb and Pb). The opposite is the case for halogens of presumed marine origin where the Norwegian data are higher. The Norwegian data are also much lower for elements that have been previously ascribed mainly to soil particles attached to the moss (Sc, Ti, Fe, REE [rare earth elements], Th and U). In this case, the fact that INAA determines the whole content could be one factor contributing to this difference. For elements where the local microenvironment is known to be a more dominant source to the moss than atmospheric deposition (Mg, Ca, Mn, Rb, Sr, Cs, Ba and partly Zn) the Norwegian data are of the same order of magnitude as the Balkan values.

A similar comparison (cfr. Fig. 3) with data from the European Moss Atlas [4] reveals that the Balkan countries show considerably higher concentrations of most elements in moss than observed in other European countries where moss sampling has been employed. This simple comparison confirms that the East and the West of Europe differ greatly by the levels of many contaminants in the atmospheric deposition, and indicates that some eastern European countries still have a long way to go in order to bring their emissions down to a satisfactory level. The only exception in the west is Belgium where high levels of Cu, Cr, V and Zn in moss are explained by smelter emissions in this industrialized country.

The unfavourable air pollution situation may adversely affect not only the natural environment, but also human health. An environmental hypothesis suggests that endemic distributions of diseases directly correlate with the geographic patterns of soil deficiencies or excess of essential elements, which could be of natural or anthropogenic origin. Examples of adverse health in the Balkans associated with trace element deficiencies and excesses have been described in the literature [23]. For example environmental chronic exposure to non-essential elements such as arsenic has been considered to possibly affect human health, given the fact that elevated concentrations of arsenic were observed in drinking water in some areas of Serbia (Zrenjanin), Romania (Baia Mare), and other Balkan countries.

Considering heavy metal atmospheric deposition in the Balkans specific geographical distribution patterns of several elements are evident. The highest median value for As (present data) were observed in Bulgaria, mainly connected mostly with the mining and smelting of copper ores and coal combustion. Still the most severe metal pollution in Bulgaria comes from old iron mines and metallurgic plants (Cu, Fe, Cr, As, V and Zn).

Multivariate statistical analysis (factor analysis) was used to identify and characterize different pollution sources and to point out the most polluted areas. Factor analysis is a multivariate technique for reducing matrices of data to their lowest dimensionality by the use of orthogonal factor space and transformations that yield predictions and/or recognizable factor [27]. Values of the five factors are given in Table 3. Factor scores representing the contributions of individual sampling sites to the relevant factor are given in Figure 4. Five identified factors are interpreted as follows:

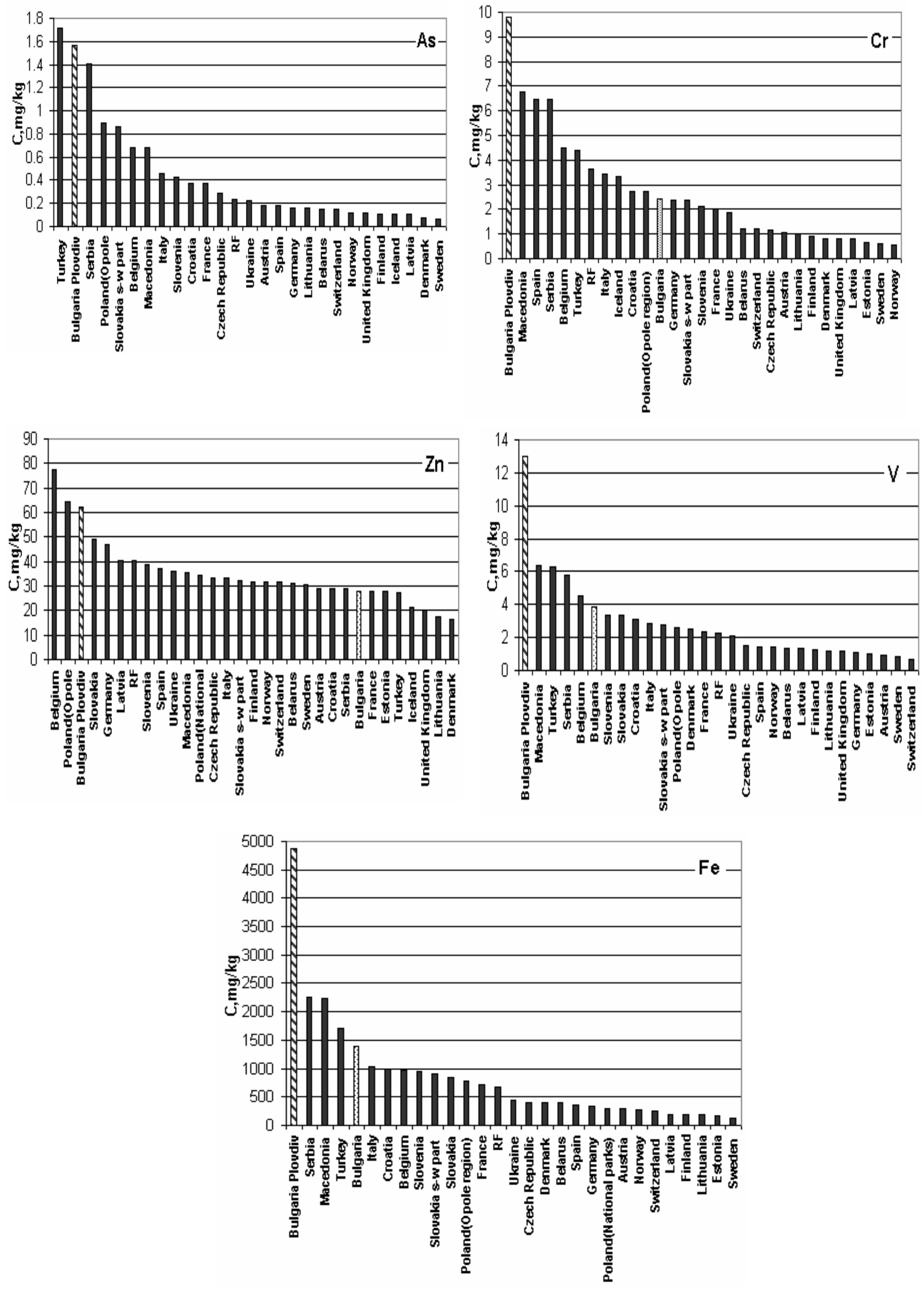
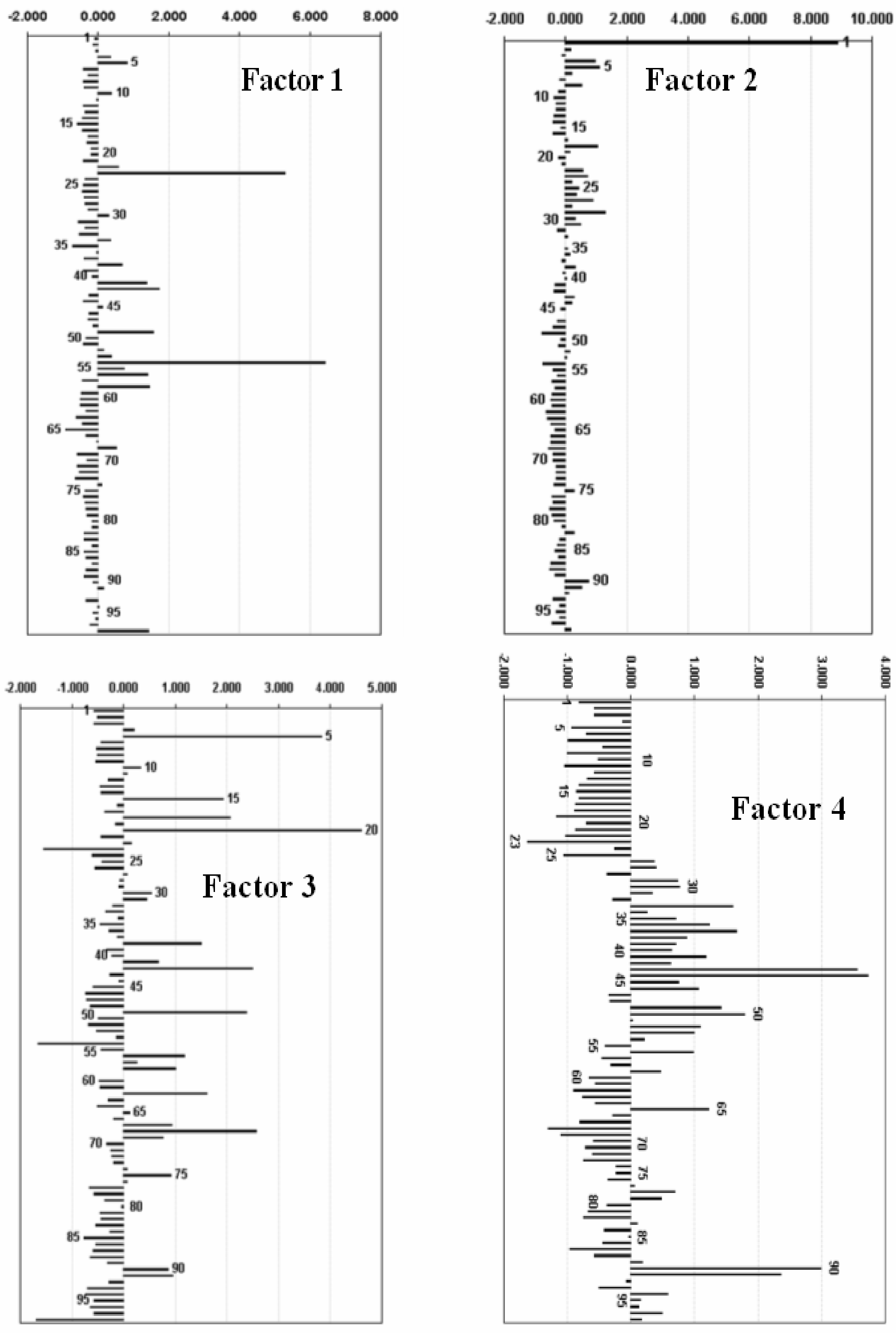


Fig. 3. Range of median values for selected elements in moss according to simultaneous moss surveys in different European countries [4]. Data for Turkey are from [26] and represent the European part of the country


Factor 1
-2.000
0.000
2.000
4.000
6.000
8.000
Factor 2
-2.000
0.000
2.000
4.000
6.000
8.000
10.000
Factor 3
-2.000
-1.000
0.000
1.000
2.000
3.000
4.000
5.000
Factor 4
-2.000
-1.000
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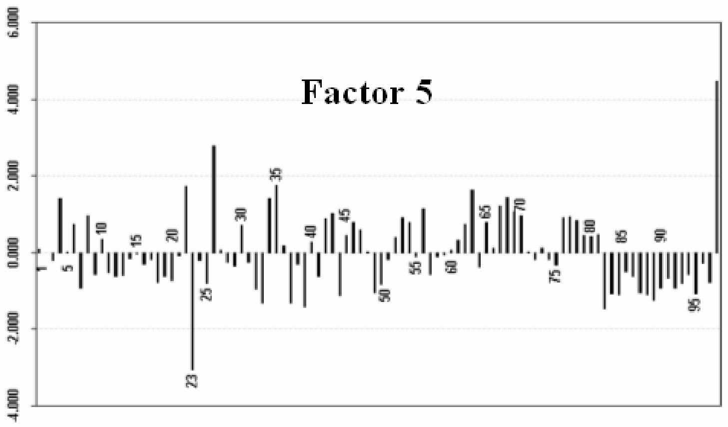

Fig . 4. Plots of factor scores for moss samples from Bulgaria

Factor 1 has particularly high values of Na, Al, Sc, Ti, Cr, Fe, Co, Ni, As, Rb, Sr, Cs, Ba, W, U, and REEs (rare-earth elements). Most of these elements are typical for crustal material, and most probably this component at least partly reflects the contamination of moss samples with soil particles. Another possible source could be fly-ash particles resulting from high-temperature processes such as coal burning, which may have a major element composition similar to crustal material.

Factor 2 is an exceptionally clear-cut industrial component with high values for Zn, Mo, Cd, Sb, and Pb. These elements show particularly high concentrations at a limited number of sites located within the region of Kardzhali. The main source of high pollution in this region is extraction of lead-zinc ores. Elements such as Cd and Sb are used in chemical industry, which may explain their high concentration in the regions of Burgas, Gabrovo, Troyan, Panagiurishte, and Pazardzhik.

Factor 3 has high values for Ti, V, Cr, Fe, Ni and As. These elements may be associated with metallurgical and chemical industries. The main contribution to the concentration of V and Ni is likely to come from oil-fired power plants. For the other elements the main source may be mining of iron and chromium ores in areas around Medet, Teteven and Burgas, and Ivailovgrad, respectively.

Factor 4 has high levels of Cl, Ca, and Au. These elements may be associated with emissions from metallurgical industry in Balkan mountain region and from gold mining in Zlatitza.

Factor 5 has high levels for Br and I, which are associated with atmospheric deposition of aerosols influenced by processes in the marine environment. In our case this is strongly supported by high Br and I concentrations in samples collected near the Black Sea.

Arsenic. The elevated concentrations of this toxic and carcinogenic element are mainly related to the copper and gold mines, as well as to coal combustion. The relatively high values at sites 67, 68, 74 might be from the copper mining in the «Rosen basin» and coal combustion at Burgas region, which is another plausible source of air pollution. A high correlation of As with Fe (R = 0.79) and Ni (R = 0.67) is noted.

Chromium. Cr is mainly associated with the crustal component. The median value of Cr in the investigated region is similar to the corresponding values in the neighboring countries. Cr correlates most strongly with Sc (R = 0.93), Ti (R = 0.86), and V (R = 0.74). The highest factor scores are observed at sites 38, 42, 49, 54, 58, 67, and 75, where high concentrations of V are also evident. In the case of 38, chromium may originate from a nearby metallurgical plant situated in Pirdop. Combustion of coal may also lead to an increased chromium load on the ecosystem.

Iron. The concentration in moss is accounted for by the soil factor as indicated by the high Fe-Sc correlation (R = 0.96) and the principal component analysis (sites 53, 56, 58, 68 and 91), pointing to iron ores in region near Troyan. However, high concentration of iron was also observed within the Burgas region (sites 67, 68), which is likely to be due to local emission sources such as the metallurgic plant in Debelt.

Vanadium. The median value of V in Southern Bulgaria is similar to the corresponding values in the neighbouring areas in Romania and Serbia [24, 25]. V in the present samples is most strongly correlated with Al, Se, Ti, and Ni. Vanadium and nickel are usually found in relatively high concentrations in crude oil and these elements are therefore often used as markers of fuel oil combustion in air pollution studies (including moss surveys). The over-all correlation between V and Ni in this material is R = 0.78, and high factor scores are evident at sites 75, 74, 76, and 73 in the Burgas region. The reason for high vanadium deposition is associated with the oil refinery and chemical industry in the town of Burgas and with the nearby metallurgic plant.

Nickel. The element distribution pattern of nickel corresponds closely to that of vanadium. The slightly elevated Ni concentrations in sites 67, 68, 69 may thus also be related to the Burgas oil refinery and to the nearby metallurgic plant.

Zinc. The median value of zinc in the investigated region is similar to the corresponding values in other continental regions of Europe. Only a few sampling sites (1, 5, 8, 18, 22, 23, 91) showed high Zn values. Apparently the extraction of lead-zinc ores is mainly responsible for these values.

Bromine and Iodine. These elements are strongly correlated and show high loadings in Factor 5. The highest scores of this factor are observed at sites near the Black Sea coast, confirming the marine origin of these elements.

Thorium and Uranium. The concentrations of these elements in the present samples are of the same order as those found in neighbouring areas in other Balkan countries. The highest values were observed at two sampling sites (58, 68) located in the vicinity of a uranium mine, which may be a source of windblown soil material.

Aluminum. The regional distribution of Al is typical for the group of crustal elements predominantly supplied to the moss by windblown soil dust, showing relative uniform mean values for different regions. However, it is also a typical representative of ferrous industry and cement plants.

Calcium. The level of Ca in moss is high and uniform, probably reflecting a contribution from higher vegetation. Calcium sulphate is used as construction material. Quicklime is calcium oxide (CaO) and may be responsible for high concentrations at the other sampling sites.

Manganese. The concentration of this element shows a high, relatively uniform level in the interior areas of the country, and decreases rapidly towards the coast. This is probably due to the low retention capacity of Mn in moss, where Mn may be lost by exchange with Na and Mg ions from marine aerosols.

Antimony. The association with Factor 2 suggests a predominantly industrial origin of this element.

Gold. The concentration of this element shows a high, relatively uniform level in the interior areas of the country. Bulgaria is rich in gold ores. Mines such as «Chelopech» in the Central Balkan Mountains and the nearby Krumovgrad mine «Ada Tepe» in the Eastern Rodopi may be responsible for high concentrations of gold and other related elements such as As used for gold extraction along with cyanide.

Conclusions

This study confirms that the moss method is suitable for detecting temporal and spatial trends in heavy metal deposition. The effects of newly introduced measures in order to decrease heavy metal emissions are clearly visible The method is thus a valuable tool for the evaluation of atmospheric input of metals to the environment. Although deposition levels in Bulgaria decreased, we still found very high levels of some heavy metals (Pb, Cd) in mosses due to still heavily contaminated soils around ferrous industry, polymetal works and old mines, appeared local emissions, and site-specific characteristics as serpentine spots. It thus appears to be important that new technologies and preferably also new environmental legislation and controls are implemented and that the monitoring of deposition of heavy metals is continued.

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WYKORZYSTANIE MCHÓW W BADANIACH BIOMONITORINGOWYCH ZANIECZYSZCZENIA POWIETRZA W BUŁGARII

Abstrakt: Za pomocą biomonitoringu z wykorzystaniem mchów zbadano zawartość pierwiastków śladowych w opadzie atmosferycznym na czterech obszarach Bułgarii (Zachodnia Tracja - Rodopy, Wschodnia Tracja - Rodopy, południowo-wschodnia i północno-środkowa Bułgaria). Badania prowadzono w ramach europejskiego programu badania mchów w 2005 roku. Oznaczono 41 pierwiastków (Na, Al, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rd, Sr, Mo, Cd, Sb, I, Cs, Ba, La, Ce, Nd, Sm, Tb, Dy, Tm, Yb, Hf, Ta, W, Au, Pb, Th i U) w 97 próbkach naziemnych mchów, z wykorzystaniem neutronowej analizy aktywacyjnej (NAA) i absorpcyjnej spektrometrii atomowej (AAS). Do badań wykorzystano mech *Hypnum cupressiforme*. Do identyfikacji i charakterystyki różnych źródeł zanieczyszczeń oraz wyznaczenia obszarów najbardziej zanieczyszczonych wykorzystano analizę głównych składowych (analiza czynnikowa). Interpretacja wyników analizy czynnikowej pozwala wskazać naturalne źródła tych pierwiastków: skorupę ziemską, składniki morskie oraz elementy roślinne, a także źródła antropogenne: przemysł żelaza (Plovdiv i Haskovo), przemysł metali nieżelaznych (Plovdiv, Kyrdżali i Burgas), przetwórstwo ropy naftowej (Burgas) oraz ciepłownie (Plovdiv, Haskovo, Stara Zagora i Burgas). Porównanie wartości mediany stężeń oznaczanych analitów w próbkach mchów pobranych w Bułgarii z pobranymi na Bałkanach i w innych krajach europejskich wskazuje, że mchy z Bałkanów wykazują znacznie większe stężenia w przeważającej części tych pierwiastków w porównaniu z mchami w innych krajach europejskich.

Słowa kluczowe: opad atmosferyczny, biomonitoring z wykorzystaniem mchów, neutronowa analiza aktywacyjna, absorpcyjna spektrometria atomowa, pierwiastki śladowe, analiza czynnikowa, źródła zanieczyszczeń

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EFFECTS OF A PERMANENT MAGNETIC FIELD TOGETHER WITH THE SHIELDING OF AN ALTERNATING ELECTRIC FIELD ON CARROT SEED VIGOUR AND GERMINATION

WPŁYW STAŁEGO POLA MAGNETYCZNEGO Z RÓWNOCZESNYM EKRANOWANIEM PRZEMIENNEGO POLA ELEKTRYCZNEGO NA WIGOR I KIEŁKOWANIE NASION MARCHWI

Abstract: The purpose of the study was to determine the effect of a permanent magnetic field together with the shielding of an alternating electric field on seed vigour and germination of carrot cultivars 'Perfekcja' and ‘Nantejska’. An ADR-4 (**A**dvanced **D**ielectric **R**adiation Trap) apparatus constructed of the porous ceramic with embedded water in a crystalline form was used in the experiment. This material has a very strong shielding effect on alternating electric fields in the low frequency range from 10^{-2} Hz to 10^6 Hz. The maximum dielectric loss is reached at about 50 Hz. Ten ferrobaryte permanent magnets were placed on the dielectric in a well-defined configuration. During germination seeds were exposed to the action of the ADR-4 apparatus, the ceramic material without magnets and magnets alone. Seed vigour and germination were evaluated at 20°C in the dark. Seed vigour was determined by means of the rate and uniformity of seed germination. The percentage of germinating seeds, germination capacity, the percentages of diseased and deformed seedlings and dead seeds were evaluated. ADR-4 did not affect seed vigour, but improved the germination capacity of seeds and decreased the percentage of diseased seedlings. In cv. ‘Nantejska’ a lower percentage of dead seeds was observed compared with the control. The ceramic dielectric without magnets accelerated germination of ‘Perfekcja’ seeds at the initial phase, improved germination capacity and decreased the number of dead seeds in cv. ‘Nantejska’. ‘Perfekcja’ seeds exposed to magnetic field alone germinated faster than untreated seeds, whereas in case of cv. ‘Nantejska’ a delay in seed germination was found. In cv. ‘Perfekcja’ a lower percentage of diseased seedlings and in cv. ‘Nantejska’ an improved germination capacity were observed.

Keywords: stationary magnetic field, alternating electric field, carrot, seed vigour, germination

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Organic crop production demands even higher quality seeds than conventional farming. Preventive and control measures using chemicals are prohibited and competition with weeds requires high vigour propagation material. Moreover, for organic farmers, seed health is very important. Hence, physical methods of improving seed quality have been the subject of intense research. First successful attempts to improve quality of seeds by their exposure to magnetic or electromagnetic fields were carried out in the 1930s. At present research concerning this area is still being continued, but with a better understanding of the mechanism of action of magnetic fields on plants. Enhancement of seed vigour and germination of different species by treating seeds with magnetic or electromagnetic fields has been confirmed by many scientists [1-6]. Vashisth and Nagarajan [7] reported a 46÷71% increase in chickpea seed vigour, a 58÷90% improvement in seedling root length and a 25÷47% increase in seedling dry weight. However, not all results are so spectacular. They depend to a large extent on the conditions of experiments.

The purpose of the study was to determine the effect of a permanent magnetic field together with the shielding of an alternating electric field on seed vigour and germination in carrot cultivars 'Perfekcja' and 'Nantejska'.

Methods and materials

In the experiment a ADR-4 plate (**A**dvanced **D**ielectric **R**adiation Trap) constructed of a porous ceramic with embedded water in a crystalline form and ten permanent magnets was used [8]. Magnets made of ferrobaryte of magnetic field induction ca 40 mT were placed on the ceramic in a well-defined configuration. The ceramic dielectric shows a strong, characteristic, low-frequency absorption of an electric component of the electromagnetic wave in the frequency range from 10^{-2} Hz to 10^6 Hz at room temperature. The ceramic exhibits a broad low-frequency dielectric anomaly around the temperature of 273 K. The size and shape distribution of pores in the ceramic element is responsible for a broadening of the dielectric anomaly related to freezing of the confined water. The effect is a strong absorption of an electric field from the surrounding medium. The maximum dielectric loss is reached at about 50 Hz (Figures 1-3).

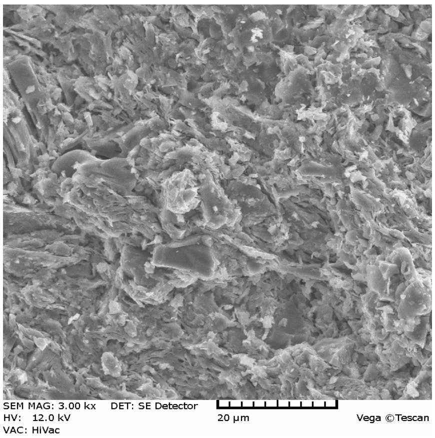

Fig. 1. Topography of surface of ceramic fracture at a compressing pressure of 20 MPa and a temperature of 1100ºC shown on a SEM image

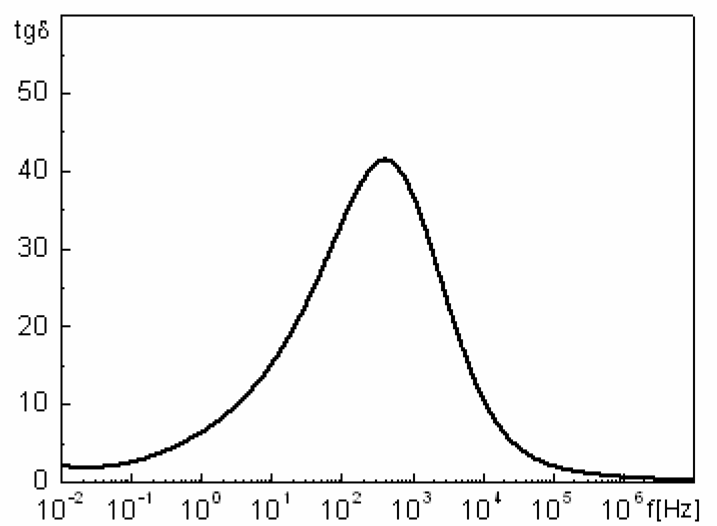

Fig. 2. Results of electric testing dielectric loss. Measurement taken with an Impedance Analyzer HP4192A, at 16ºC and relative humidity of 65%

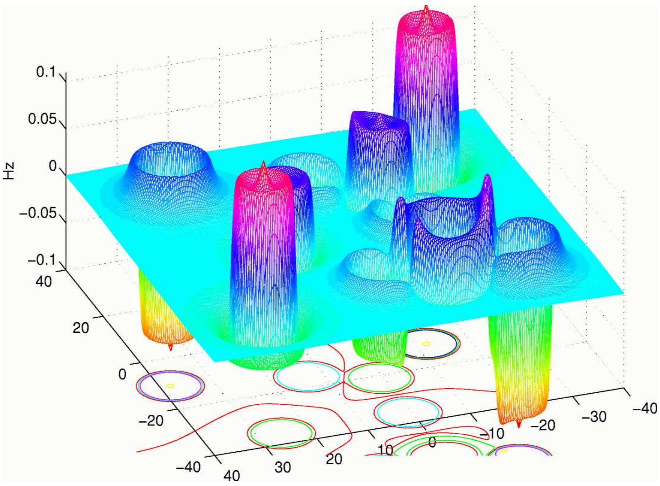

Fig. 3. Spatial distribution of [Hz] magnetic field intensity over ADR-4™

The effect of the ADR-4 plate, the ceramic dielectric without magnets and magnetic elements without the ceramic dielectric on vigour and germination of carrot ‘Perfekcja’ and ‘Nantejska’ seeds was examined in the experiment.

Seed vigour was evaluated at 20°C in the dark on 8 replicates of 50 seeds from each treatment. Seeds were placed in 9 cm diameter Petri dishes containing 6 layers of blotting paper moistened with distilled water. The Petri dishes with seeds were placed directly on the top of ADR-4 plates, ceramic dielectrics or on the top of plates which contained only magnetic elements. Germinating seeds, i.e. showing a visible root protrusion through the pericarp, were counted daily until no new germs appeared and they were removed from Petri dishes. Values of T_{10} (time to 10% of the total number of germinating seeds) and T_{50} (time to 50% of the total number of germinating seeds) were calculated. These parameters describe the rate of seed germination. The uniformity of seed germination was evaluated by means of U_{75-25} (time between 75 and 25% of the total number of germinating seeds). Untreated seeds constituted the control.

Germination tests were conducted on 8 replicates of 50 seeds from each treatment. Seeds were incubated under the same conditions as described above. After 14 days of incubation, the percentages of normal seedlings (germination capacity), diseased seedlings, deformed seedlings and dead seeds were evaluated according to the ISTA Rules [9]. Moreover, G_{max} (the percentage of germinating seeds) was determined.

The SeedCalculator version 2.1 software [10] was applied to calculate T_{10}, T_{50}, U_{75-25} and G_{max} parameters. All vigour and germination results were evaluated by means of variance analysis followed by the Duncan test.

Results

ADR-4 did not affect seed vigour, but improved germination capacity of seeds and decreased the percentage of diseased seedlings in both cultivars. In cv. 'Nantejska' a lower percentage of dead seeds was observed after their exposure to the action of ADR-4 compared with the control (Tables 1-3).

Table 1

Effects of treatment on seed vigour

Seed treatment	'Perfekcja'			'Nantejska'		
	T_{10} [days]	T_{50} [days]	U_{75-25} [days]	T_{10} [days]	T_{50} [days]	U_{75-25} [days]
Untreated seeds	2.35 a	3.01 a	0.83 a	2.27 ab	3.11 b	0.99 a
ADR-4	2.29 ab	2.98 ab	0.86 a	2.24 ab	3.07 b	1.07 a
M1	2.18 b	2.90 ab	0.93 a	2.21 b	3.08 b	1.08 a
M2	2.15 b	2.86 b	0.91 a	2.42 a	3.26 a	1.02 a

Means in columns followed by the same letters are not significantly different at $\propto = 0.05$ level according to Duncan's test.

ADR-4 - **A**dvanced **D**ielectric **R**adiation Trap.
M1 - ceramic dielectric without magnetic elements.
M2 - ten magnets in the same configuration as in ADR-4 without ceramic dielectric.
T_{10} - time to 10% of the total number of germinating seeds.
T_{50} - time to 50% of the total number of germinating seeds.
U_{75-25} - time between 75 and 25% of the total number of germinating seeds.

Ceramic dielectric without magnets increased germination rate of 'Perfekcja' seeds at the initial phase. The T_{10} value was significantly lower than that for untreated seeds. In cv. 'Nantejska' this treatment improved seed germination capacity and decreased the percentage of dead seeds (Tables 1-3).

Magnets without the ceramic dielectric accelerated germination of 'Perfekcja' seeds. The T_{10} and T_{50} values were significantly lower than those for untreated seeds. On the other hand, this treatment had an adverse effect on seed germination rate in cv. 'Nantejska'. The T_{50} value was significantly higher than that in the control (Table 1). Exposure to magnetic field improved germination capacity of 'Nantejska' seeds and decreased the percentage of dead seeds. In cv. 'Perfekcja' a lower percentage of diseased seedlings and higher percentage of deformed seedlings were observed after this treatment compared with the control (Tables 2 and 3).

Table 2

Effects of treatment on germination of 'Perfekcja' seeds

Seed treatment	**G_{max} [%]**	**Germination capacity [%]**	**Diseased seedlings [%]**	**Deformed seedlings [%]**	**Dead seeds [%]**
Untreated seeds	93.0 a	56.5 b	35.3 a	1.3 b	7.0 a
ADR-4	93.0 a	64.3 a	26.5 bc	2.8 ab	6.5 a
M1	91.8 a	56.5 b	31.5 ab	2.8 ab	9.3 a
M2	91.0 a	62.3 ab	24.0 c	4.8 a	9.0 a

Means in columns followed by the same letters are not significantly different at $\propto = 0.05$ level according to Duncan's test.

ADR-4 - **A**dvanced **D**ielectric **R**adiation Trap.
M1 - ceramic dielectric without magnetic elements.
M2 - ten magnets in the same configuration as in ADR-4 without ceramic dielectric.
G_{max} - the percentage of germinating seeds.

Table 3

Effects of treatment on germination of 'Nantejska' seeds

Seed treatment	**G_{max} [%]**	**Germination capacity [%]**	**Diseased seedlings [%]**	**Deformed seedlings [%]**	**Dead seeds [%]**
Untreated seeds	91.3 a	50.5 b	34.5 a	0	15.0 a
ADR-4	90.8 a	67.8 a	21.5 b	0	9.3 bc
M1	90.0 a	69.0 a	23.3 a	0	6.3 bc
M2	88.8 a	64.0 a	28.0 a	0	7.3 bc

Means in columns followed by the same letters are not significantly different at $\propto = 0.05$ level according to Duncan's test.

ADR-4 - **A**dvanced **D**ielectric **R**adiation Trap.
M1 - ceramic dielectric without magnetic elements.
M2 - ten magnets in the same configuration as in ADR-4 without ceramic dielectric.
G_{max} - the percentage of germinating seeds.

None of the treatments applied influenced the uniformity of germination or the percentage of germinating seeds (Tables 1-3).

Discussion

Both seed samples used in the study were of poor quality. Germination capacity of untreated seeds was low and a high percentage of diseased seedlings was observed. Seed health evaluation showed that seeds were infected to a large extent by two pathogenic fungi *Alternaria dauci* (J.G. Kühn) J.W. Groves & Skolko and *A. radicina* Meier, Drechsler & E.D. Eddy, which cause seedling damping-off (unpublished data).

This study showed that exposure of seeds from both samples to ADR-4 plates or the ceramic dielectric in general did not affect their vigour. The influence of magnets alone on seed vigour was differentiated. Magnetic field accelerated seed germination in cv.

‘Perfekcja’, but delayed it in cv. ‘Nantejska’. However, ADR-4 improved seed germination to a larger extent than the ceramic dielectric or the magnetic field alone. It increased seed germination capacity and decreased the percentage of diseased seedlings in both samples. All treatments decreased the percentage of dead seeds in cv. ’Nantejska’.

The range of permanent magnetic fields applied to induce seed germination is from several to 500 mT [11], while for alternating magnetic fields it is 0.1 μT÷30 mT, 1 Hz to 100 Hz, respectively [11-14]. Soltani et al [15] found that in a magnetic field of 7 mT asparagus seeds imbibed and germinated more rapidly. Seed germination percentage and epicotyl and hypocotyls lengths were also significantly higher than without the influence of a magnetic field. Carbonnel et al [6] reported that mean germination time of *Festuca arundinacea* Schreb. and *Lolium perenne* L. seeds was reduced by more than 10%, compared with controls, when the seeds were exposed to magnetic fields of 125 mT or 250 mT. The roots of treated grass seedlings were significantly longer than those of untreated ones when seeds were permanently exposed. Florez et al [2] found that the higher germination rate of rice seeds treated with static magnetic fields of 125 mT or 250 mT was in agreement with the higher lengths and weights of rice plants exposed to a magnetic field.

Mano et al [3] found that an extremely low frequency magnetic field (60 Hz, 5 mT) applied to *Arabidopsis thaliana* (L.) Heynh., *Lactuca sativa* L. and *Zinnia elegans* Jacq. seeds suppressed the reduction in germination, caused by seed incubation at high relative humidity (90%) and temperature (37ºC). The authors suggested that the magnetic field suppressed the irreversible deterioration of seeds, preventing water absorption.

The main phenomenon investigated by different authors, found to be responsible for enhanced seed germination capacity and acceleration of morphogenetic processes, is increased water absorption by seeds [14-16]. Water subjected to the action of a magnetic field is altered in a certain way so that it not only penetrates inside seeds faster, but also affects the rate of enzymatic reactions. In the first phase accelerated imbibition is observed in seeds subjected to the action of a magnetic field and their weight increases. Additionally, a plant weight increase may also be connected, apart from effects related with faster metabolism, with higher water content in plants, including fruits [16].

The influence of a magnetic field on seeds may be short-term, e.g. 10 min, followed by the effect in the form of increased water absorption occurring after several hours and lasting for at least several days [14]. This fact is confirmed by the variant, in which an effective action of a magnetic field on plants, including germination, may be replaced by the action on water or substrate which is used to water plants [11, 17]. In this respect it is important to remember that studies on water exposed to the action of a magnetic field indicate an altered, usually reduced, surface tension, viscosity [18], as well as heat of evaporation, manifested in a faster vaporization of such water [11, 19]. These three phenomena are interrelated by the power of actions defined as hydrogen bonds. Changes in these parameters result in a faster penetration of water inside seeds and thus also faster and more effective germination [11, 15]. This facilitated penetration of water solutions is confirmed by a more efficient extraction of chemicals from plant material [20]. Moreover, changes in activity are observed in these enzymes, for which water is involved in the notation of their reaction. Such an enhanced enzymatic activity under the influence of a permanent or alternating magnetic field is observed in case of catalase [21], horseradish peroxidase (the participation of H_3O^+ ion), esterase [11], amylase, protease and lipase [22]. Some of these enzymes participate in seed germination.

The above-mentioned extended action after water or seeds cease to be exposed to the action of a magnetic or electromagnetic field is called water memory. Probably the primary role is played here by the reversal of nuclear spins of hydrogen atoms in the water molecule [23], leading to the parallel or antiparallel orientation of hydrogen nuclear spins in the water molecule and determining the occurrence of two isomers of water - *ortho* and *para*. These isomers are of limited stability and in case of distilled water they spontaneously reach the state of dynamic balance in a matter of several dozen minutes [24]. These isomers affect the formation and stabilization of specific structures of water bound by hydrogen bonds, with an opposite effect also occurring - structures of water strengthened e.g. by the presence of ions may increase many-fold the time, over which water properties changed by the magnetic field are retained [25]. Baran and Degtyarev [26], even before reports on the separation of water into *ortho*- and *para*-isomers was published, had interpreted the influence of a magnetic field on water as its effect on the magnetic moment of protons participating in the hydrogen bond, resulting in a loosening of this bond. In reference to *ortho*- and *para*-hydrogen they also reported that clusters formed by water are controlled by the orientation of proton spins, by which the energy of intermolecular interactions of water in clusters is weakened and ion hydratation in the solution increases [26].

Sensitivity to the action of a magnetic or electromagnetic field covers especially the period from the dry kernel phase before sowing to approx. 17÷24 h swelling period (in case of wheat) [12]. The time of the action of information introduced by the magnetic field is limited, although it lasts for many days. An increase in plant weight at the vegetative stage is much more marked than at the generative stage. For example, after the activation of seeds by a field of 90 mT and 60 Hz for 10 min, the leaf area increment in tomato at the vegetative stage was 58% and at the generative stage it was 22% [4]. Moreover, it was also significant that an advantageous effect of the exposure of seeds to the action of a magnetic field is found mainly in seeds with low viability [1].

Królicka et al [27] found a two-fold higher accumulation of umbelliferone in transformed *Ammi majus* L. callus grown on a medium exposed to ADR-4. Further research on the accumulation of secondary metabolites in *Ammi majus* L. callus treated with ADR-4 or its components separately, as in our experiment, showed the effect of the interaction of the magnetic field and the ceramic dielectric (unpublished data).

The decrease in the number of diseased seedlings or dead seeds observed in our study suggests that the treatments controlled to some extent the growth of fungi. Nagy and Fischl [28] investigated the effect of a static magnetic field of 0.1, 0.5 and 1 mT on the growth of selected phytopathogenic microscopic fungi: *Alternaria alternata* (Fr.) Keissler, *Curvularia inaequalis* (Shear) Boedijn and *Fusarium oxysporum* Schlecht. The magnetic field decreased the growth of colonies by 10%. Lipiec et al [29] found that oscillating magnetic field pulses reduced the number of bacteria *Erwinia carotovora* (Jones) Bergey et al and *Streptomyces scabies* (Thaxter) Waksman et Henrici in a liquid nutrient medium 4 000 times and the number of the fungus *Alternaria solani* (Ellis & Martin) Jones & Grout 30 times.

Conclusions

1. The ADR-4 plate (**A**dvanced **D**ielectric **R**adiation Trap) did not affect the rate and uniformity of germination of carrot seeds.

2. Exposure to ADR-4 improved germination capacity of carrot seeds and decreased the percentage of diseased seedlings in both cultivars. In cv. ‘Nantejska’ a lower percentage of dead seeds was observed.
3. The ceramic dielectric without magnets accelerated germination of ‘Perfekcja’ seeds at the initial phase and improved germination capacity and decreased the number of dead seeds in cv. ‘Nantejska’.
4. ‘Perfekcja’ seeds exposed to magnetic field alone germinated faster than untreated seeds, whereas in case of cv. ‘Nantejska’ a delay in seed germination was found. In cv. ‘Perfekcja’ a lower percentage of diseased seedlings and in cv. ‘Nantejska’ an improved germination capacity were observed.

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WPŁYW STAŁEGO POLA MAGNETYCZNEGO Z RÓWNOCZESNYM EKRANOWANIEM PRZEMIENNEGO POLA ELEKTRYCZNEGO NA WIGOR I KIEŁKOWANIE NASION MARCHWI

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Abstrakt: Celem prowadzonych badań było określenie wpływu stałego pola magnetycznego z równoczesnym ekranowaniem przemiennego pola elektrycznego na wigor i kiełkowanie nasion marchwi odmiany ‘Perfekcja’ i ‘Nantejska’. W doświadczeniu wykorzystano urządzenie o nazwie ADR-4 (**A**dvanced **D**ielectric **R**adiation Trap) zbudowane z porowatej ceramiki, w której znajduje się woda w postaci krystalicznej. Tak wytworzony materiał ma silną zdolność do ekranowania przemiennego pola elektrycznego na niskich częstotliwościach, to jest w granicach od 10^{-2} do 10^{6} Hz, przy czym maksimum tangensa delta (stratność pola elektrycznego) poprzez proces technologiczny zostało wyznaczone dla około 50 Hz. Na takiej bazie dielektrycznej umieszczono 10 elementów magnetycznych wykonanych z ferrytobaru o określonej konfiguracji. Przez cały okres kiełkowania nasiona poddano działaniu urządzenia ADR-4, a także samego dielektryku ceramicznego bez elementów magnetycznych oraz samego pola magnetycznego (wyłącznie elementy magnetyczne). Ocenę wigoru i kiełkowania nasion przeprowadzono w temperaturze 20ºC w ciemności. Wigor nasion określono za pomocą parametrów opisujących szybkość i wyrównanie kiełkowania. Przy ocenie kiełkowania nasion określano ogólną liczbę kiełkujących nasion, zdolność kiełkowania, odsetek siewek z objawami chorobowymi i zniekształconych oraz nasion martwych. Poddanie nasion działaniu urządzenia ADR-4 nie wpłynęło na ich wigor, ale stwierdzono poprawę zdolności kiełkowania i zmniejszenie liczby siewek z objawami chorobowymi. U odmiany ‘Nantejska’ obserwowano również mniej nasion martwych niż w kontroli. Zastosowanie samego dielektryka ceramicznego przyspieszyło kiełkowanie nasion odmiany ‘Perfekcja’ w początkowej fazie oraz poprawiło zdolność kiełkowania i zmniejszyło liczbę nasion martwych u odmiany ‘Nantejska’. Nasiona odmiany ‘Perfekcja’ poddane działaniu samego pola magnetycznego kiełkowały szybciej niż nasiona kontrolne, natomiast w przypadku nasion odmiany ‘Nantejska’ stwierdzono opóźnienie kiełkowania. U odmiany ‘Perfekcja’ notowano mniej siewek z objawami chorobowymi, a u odmiany ‘Nantejska’ obserwowano poprawę zdolności kiełkowania nasion.

Słowa kluczowe: stałe pole magnetyczne, zmienne pole elektryczne, marchew, wigor nasion, kiełkowanie

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CALORIMETRIC AND KINETIC STUDIES OF THE EFFECT OF NITROGENOUS FERTILIZERS ON ORGANIC MATTER DECOMPOSITION IN SOILS

KALORYMETRYCZNE I KINETYCZNE BADANIA WPŁYWU NAWOZÓW AZOTOWYCH NA ROZKŁAD MATERII ORGANICZNEJ W GLEBIE

Abstract: The course of changes for the rate of heat production (RHP) provides information on kinetics of biodegradation processes of organic matter in soil. The following publication aims at reviewing the literature and presenting on its background the results of own research on applying calorimetric method for kinetic analysis of glucose decomposition in soil in presence of nitrogenous fertilizers and nitrificaton inhibitor - N-Serve. Additionally, a process of biodegradation of pig liquid manure in different soils has been examined. The kinetics of glucose decomposition has been determined by charting change curves for the rates of heat production (RHP), graphs for total heat effect (Qt), and based on those - the time (PT) of reaching the maximum value of heat power (maxRHP) and the value of apparent constant rate (k) of the examined changes. Using the calorimetric coefficient (%MHP), the percentage of produced metabolic heat in relation to the enthalpy of organic combustion of the substrate introduced to the soil has been presented. The results of the research have shown the increase of the rate of glucose decomposition in soil in presence of $(NH_4)_2SO_4$, NH_4NO_3 and urea. Diversification of thermokinetics of glucose and liquid manure biodegradation in various soils has been identified.

Keywords: calorimetry, kinetics, soil, organic matter, nitrogenous fertilizers

To obtain high crops of cultivated plants, nitrogen is introduced to soils in a form of mineral or organic fertilizers. Nitrogen is partially used by the plants, migrates to ground and surface waters, escapes to the atmosphere and participates in a cycle of processes, including ones that stimulate metabolic activity of soil [1-3]. Changes of nitrogen and carbon compounds in soils are widely described in scientific literature [4-7]. Kinetics of those processes depends on numerous factors: temperature, oxygen concentration, soil moisture, availability of the organic substrate, and many more that are difficult for prediction and submitted to conscious or unconscious human activities [8-10].

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Microbial decomposition of organic substances in soils entails heat effects measured with a calorimetric method. The application of the soil, microbiological and ecological calorimetry have been discussed in several reviews papers [11-15].

The heat output from the soils unamended with organic substrate shows a wide range of values between 2÷35 μW g^{-1} for mineral soils, and the higher values for grassland, organic and forest soils and peat. The freshly collected soils have higher heat output than stored. [16-19]. Values of specific heat rate production of the soils collected in Galicia (Spain) and Brazilian Amazon were in the range 0.49÷6.59 J/g day [20].

Microcalorimetric measurements and other methods were used to assess biomass content and activity of Scotland soils. The rate of heat production of soil biomass was remarkably constant for the most soils but showed differences for freshly-collected, stored or amended with glucose [17-19]. Correlation between arginine ammonification, N-mineralization biomass carbon, heat output, ATP content, protease activity and microbial numbers for twenty-two agricultural and grassland soils was assessed by Alef et al [21].

Calorimetry has found an application for the thermokinetics determination during decomposition of organic substrate at the presence of nitrogen fertilizers. Mortensen et al [22] and Ljungholm et al [23] investigated processes of glucose and cellulose biodegradation in soil in presence of ammonium sulphate and other salts. The effect of the water content, the effect of nitrogen in the form ammonium sulphate and the effect of amount of substrate added to soil in pre-culture on the shift of growth thermograms were investigated by Konno [24]. Kinetic analysis of the growth thermograms showed that soil microbial growth can be expressed quantitatively by using specific growth rate constant, doubling time and the calorimetric coefficient of digestibility of organic matter in soil [25]. The first Konno’s paper [24] and review paper [25] were published only in Japanese. In excellent work Yamano and Takahashi [26] presented decomposition thermograms of D-glucose, D-fructose, D-galactose, D-mannose, sucrose and lactose in presence $(NH_4)_2SO_4$ over the incubation temperature range of 295.2 to 320.9 K in alluvial soil The apparent activation energies and the apparent Gibbs energies of activation for various sugars were determined during this study, too. Kimura and Takahashi showed [27] that the changes in the number of soil viable microbial cells and the amount of glucose degraded revealed linear relationships both between heat production and the viable cell counts.

Soil microbial calorimetry can be preferentially used as a tool to assess side-effects of chemicals on the soil microbial activity. Kawabata et al [28] used mercury, cadmium, selenium, copper compounds, iodoacetic acid were used as pollutants of soil and the course of thermograms of glucose decomposition in alluvial soil showed different kinds of inhibition. Effects pesticides such as: Ioxinil, Tiuram and $HgCl_2$ on actual and potential heat output (for glucose amended soil) were compared to arginine ammonification, fluorescein diacetate hydrolysis, Fe(III)-reduction [29, 30] and DMSO reduction method [31]. Calorimetry applied to testing of the effect Paraquat, Diquat, Posphamidon [32], 2,4-D [33, 34] and Diuron [35] on glucose biodegradation in Brazilian soils.

The heat evolution and N-mineralization were determined and compared with other methods estimation of soil microbial biomass [21] and also for soils which contained insecticides [36]. Barros and Feijóo [37] proposed a new method to quantify the efficiency of carbon utilization by soil microbes. In another work Barros et al [38] applied calorimetry for testing the sensitivity of calorimetric indices in comparative study between the basal respiration and the degradation of an external carbon sources in soils. A combination of flow-microcalorimetry and gas chromatography was used for measuring the heat production, CO_2 and N_2O emission for soils incubated with glucose under aerobic and anaerobic (N_2) conditions [39]. Additionally, a range of valuable microcalorimetric studies were carried out examining the microbial activity of soils that included nitrogenous fertilizers mainly in the form of $(NH_4)_2SO_4$ [40-44].

Most of the calorimetric examinations of the soil discussed in this paper have been performed using a Calvet microcalorimeter (Setaram) [17-19], a Bio Activity Monitor (LKB) [16, 21, 24], a Thermal Activity Monitor (Thermometric) [38, 39] and Takahashi's multi-channel calorimeters [28, 41].

Based on calorimetric measurements, the examinations aimed at identifying kinetics the following soil microbial processes:

- glucose biodegradation in presence of various nitrogenous fertilizers and N-Serve,
- biodegradation of liquid manure with high content of nitrogen.

Experimental

Materials and methods

Soils: The soils were sampled from the Agricultural Stations of the University of Warmia and Mazury in Tomaszkowo and Balcyny. They were collected from the upper surface layer (0÷20 cm) and after sieving (2 mm^2), 1-2 months stored in polyethylene bags at room temperature with air admission and after that at temperature of 277 K. The soils classification performed according to the Polish Soil Society standards.

Table 1

Characteristics of the experimental soils

No.	Soil	Texture	pH_{KCl}	N	C_{org}	P_2O_5	MgO	K_2O	MWHC
				[%]		[mg/100 g]			[g/100 g]
1	Cambisol	loamy sand	6.30	0.057	0.42	24.05	5.97	15.78	31.8
2	Luvisol	sandy loam	5.40	0.086	0.97	8.93	5.47	10.12	32.5

MWHC - Maximum Water Holding Capacity

Soils before calorimetric measurements were preincubated 24 hours at 295 K. Then, soil samples were introduced to a stainless measuring vessel (0.5 dm^3), a measured amount of water was added and the whole was left closed for ca 4÷5 hours for preliminary thermostating. After that, the thermostated solution with the examined organic substance was placed on the surface of the soil and the vessel was moved to a measuring chamber of the calorimeter. In a reference chamber there was a vessel with thermally passive and microbiologically inactive soil. Microbiological deactivation of the soil was performed by heating it for ca 15 hours in 105°C, additionally sterilising it and introducing double-distilled water in the amount equivalent to 60% MWHC.

Calorimetric measurements: A KRM-type calorimeter, produced by the Institute of Physical Chemistry of Polish Academy of Sciences in Warsaw was been used for calorimetric measurements. A temperature regulator, type 660, produced by UNIPAN Warsaw, with a Pt100 sensor, ensured a stable temperature of 25°C for the measurement system, to an accuracy of > 0.005°C. The calorimetric signal $\Theta(t)$ was recorded by a system including a microvolt amplifier by UNIPAN and a recorder by VEB Messgeratewerk, Magdeburg.

Calorimetric calculations: Heat effects of the studied changes were calculated on the basis of the course of $\Theta(t)$ changes, read every 0.5 hour. Before starting the examinations, the calorimeter was characterized by determining the heat loss coefficient (α) and value of $\Theta(t)$ for the baseline. Time constant (T) was determined in separate measurements, based on the curves for cooling the measuring vessel containing the microbiologically inactive soil of identical origin, mass, and water content, when compared with the examined sample of soil. Just after inserting the measuring vessel containing the soil into the calorimeter, its temperature may vary from the temperature of the measurement system. That situation results from different environment temperature and other factors. Therefore, initial fragments of the thermograms were corrected with a ballistic method using the curves for cooling or heating the vessels containing the thermally inactive soil [45]. Applying that method seems to be justified in the case of the KRM calorimeter with a high value of time constant (T) and examining low-energy processes of microbiological decomposition of organic substances in soil. The time of introducing the organic substrate into the soil was accepted as the beginning of the examined chances and it was assumed that the initial rate of the heat production from the soil equals zero.

Tian's equation [45-47] was the basis for calculating the rate of heat production (RHP) and total heat effect (Qt), based on the changes of the calorimetric signal $\Theta(t)$ and the defined value of the time constant T

$$f(t) = \Theta(t) + Td\Theta(t)/dt$$

Methods for calculating RHP, Qt and apparent constant rate (k) of the microbiological increase were described in the author's previous publication [48]. Based on the RHP change curves, a peak time (PT) was defined, after which the RHP value is at its maximum (maxRHP). When discussing the results of the research, the ($\%MHP_{(t)}$) indicator was used, which represents a percentage of metabolically produced heat ($Q_{(t)}$) after time (t) and in relation to enthalpy of combustion of the glucose (EGC) introduced to the soil. For 1 mol of glucose, the EGC is equal to 2803 kJ [49]

$$\%MHP_{(t)} = Q(t)/EGC$$

Results and discussion

The results of calorimetric examinations of decomposition of 75 mg of glucose in 75 g of brown soil (No. 1) in presence of nitrogenous fertilizers have been presented in Figure 1. The fertilizers were introduced to 75 g of the soil in a dose equivalent to 15.9 mg $N\text{-}NH_4$ in ammonium fertilizers and $N\text{-}NH_2$ in urea. Basing on the carried out research, it may be conducted that the thermograms of glucose decomposition in presence of $(NH_4)_2SO_4$ and NH_4NO_3 manifest similar course and close values of maxRHP and PT.

In the case of urea presence, the maxRHP value was lowered in comparison with the remaining nitrogenous fertilizers. The analysed values for the glucose decomposition were at their lowest with no nitrogenous fertilizers. The change curves Qt were of similar shape and their courses do not indicate so clearly as the thermograms the differences in the kinetics of glucose decomposition. Table 2 presents the values that characterize the course of glucose biodegradation. After 30 hours, the heat equivalent to ca 39÷41% MHP was produced from the examined samples of soils containing $(NH_4)_2SO_4$, NH_4NO_3 and $CO(NH_2)_2$. After 50 hours of decomposition, those values were at ca 52÷53% MHP, which indicates that after that time more than half of the chemical energy provided with the glucose was released as the heat, and the rest remained in the soil in a form of metabolites and soil biomass. For the soil with no fertilizers, those values were at 37.41 and 44.51% MHP, respectively.

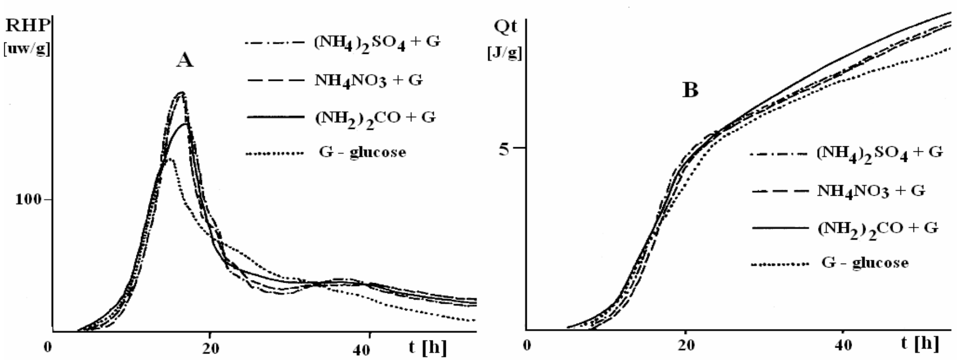

Fig. 1. Changes of RHP (A) and Qt (B) in soil during glucose decomposition in presence of nitrogenous fertilizers

Table 2

Calorimetric and kinetic characteristics of glucose decomposition in soil

Soil Additives	PT	maxRHP	Qt(30h)		Qt(50h)		k±SD	Δt	R
	[h]	[μW g^{-1}]	[J g^{-1}]	[%MHP]	[J g^{-1}]	[%MHP]	[h^{-1}]	[h]	
G	16	148	5.82	37.41	6.94	44.61	0.221±0.034	5÷13	0.90
G + $(NH_4)_2SO_4$	16	185	6.02	38.69	8.08	51.95	0.409±0.075	6÷15	0.87
G + NH_4NO_3	18	185	5.94	38.18	7.96	51.16	0.359±0.064	7÷14	0.81
G + $(NH_2)_2CO$	17	160	6.33	40.68	8.31	53.41	0.231± 0.039	6÷14	0.86

G - Glucose

Higher values of maxRHP and apparent constant rate (k) confirm the increase of glucose metabolism rate in the soil with of nitrogenous fertilizers present, when compared with the soil with no fertilizers. The nitrogen originating from mineral fertilizers stimulates synthesis of soil microbial biomass. The differences in the course of changes of RHP and Qt (Fig. 1) indicate a periodical lower availability of nitrogen from urea for microorganisms, when compared with sulphate and ammonium nitrate. That fact may be linked to the rate of urea hydrolysis - a reaction leading to creation of ammonium ions that later are used for the synthesis of soil biomass and other products. The urea hydrolysis is a first-order reaction and its rate it influenced by environmental factors and other, soil-connected ones [50-52].

Nitrification inhibitors are introduced to soils to increase the level of nitrogen usage by cultivated plants, particularly in acid soils [53]. An emulsion preparation, known as N-Serve (by Dow AgroSciences LLC, USA), contains 2-chloro-6-(trichloromethyl)pyridine (nitrapyrin). Nitrapyrin limits the process of nitrogen oxidization in the soil environment, which favours presence of nitrogen ions and compounds that are more easily accessible for plants and soil microorganisms, and which decreases the possibility of transforming nitrogen into gas products of nitrification. The efficiency of N-Serve as and soil nitrification inhibitor results from physical and chemical properties of nitrapyrin, used dose of this compound, and numerous environmental factors [53-59]. The aim of the research was to define the influence of the N-Serve nitrification inhibitor on the process of decomposition of 75 mg of glucose in 75 g of soil (No. 2) containing 75 mg of $(NH_4)_2SO_4$. The inhibitor was introduced into the soil in doses of 1, 2 and 3 µg g^{-1}. The characteristic of those examinations has been presented in Table 3. The course of RHP and Qt changes during the glucose biodegradation was similar to the research results presented in the Figure 1.

Table 3

Characteristics of glucose decomposition in soil (No. 2) in presence of nitrapyrin

Soil additives	Nitrapyrin	maxRHP	PT	$Qt_{(50h)}$		$Qt_{(70h)}$	
	[µg g^{-1}]	[µW g^{-1}]	[h]	[J g^{-1}]	[%MHP]	[J g^{-1}]	[%MHP]
G	0	135	21	9.94	63.8	-	-
G + $(NH_4)_2SO_4$	0	153	18	9.53	61.2	10.73	68.9
G + $(NH_4)_2SO_4$	1	152	19	9.26	59.5	10.53	67.7
G + $(NH_4)_2SO_4$	2	175	18	9.03	58.0	10.21	65.6
G + $(NH_4)_2SO_4$	3	159	18	8.36	53.7	9.73	62.5

G - Glucose

The data from Table 3 confirm that N-Serve, even when used in the highest dose, did not have any significant influence on the kinetics of the glucose decomposition in presence of $(NH_4)_2SO_4$. The changes of %MHP indicator show a higher accumulation of chemical energy in the soil in presence of N-Serve. To fully understand that interesting result of calorimetric measurements, the scope of the research should be significantly broadened and include the changes of nitrogen forms as well as examinations of the content and the activity of the soil biomass.

Decomposition of Organic Fertilizers in Soils

Liquid manure is a waste product of non-litter-bed pig or cattle farming. Particularly high content of nitrogen in liquid manure makes it be treated as an organic nitrogenous fertilizer of high content of other macro- and microelements [60]. The aim of the so far research was to characterize calorimetrically and kinetically the decomposition of organic fertilizers in soil [25, 48, 61]. The results presented in this paper are concerned with calorimetric research on the decomposition of pig liquid manure (PLM) in soil (No. 1 and 2). The pig liquid manure was collected directly from a sewage channel in a pig farm. The PLM contained 5416 mg of N dm^{-3}, 5.63% of dry mass, and its pH was 6.92. The PLM dose was 20 mg for 100 g of soil.

The thermograms in Figure 2 and the data in Table 4 indicate a faster decomposition of the fertilizer in the brown soil (No. 1). In case of the soil (No. 2), lengthening of the lag phase and a lower value of maxRHP were found, however, the determined value of the apparent constant rate of microbiological increase was higher.

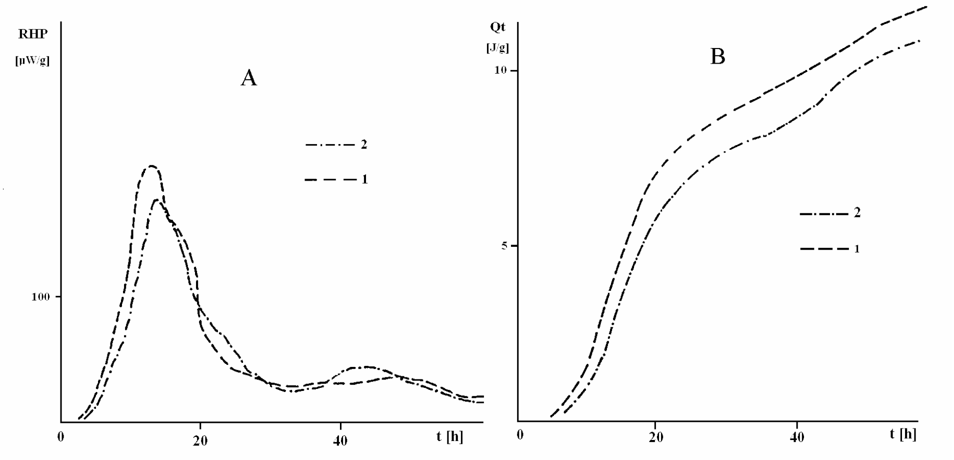

Fig. 2. Changes of RHP (A) and Q(t) (B) during the pig liquid manure decomposition in different soils

Table 4

Characteristics of pig liquid manure decomposition in different soils

Soil	Organic substrate	Dose	PT	max RHP	Qt(50h)	k±SD	Δt	R
		[mg N/g]	[h]	[μWg^{-1}]	[Jg^{-1}]	[h^{-1}]	[h]	
1	pig liquid manure	20	13	206	11.3	0.282±0.054	2÷9	0.84
2	pig liquid manure	20	14	172	10.4	0.344±0.109	4÷9	0.89

The obtained results indicate diversification of thermokinetics of decompositions for the examined organic fertilizer in soils of various types. The course of that process depends on physical and chemical properties of the soils that the liquid manure was introduced into, as well as on others conditions of microbiological growth.

Conclusions

The high-capacity KRM calorimeter and the applied methods of calorimetric research make it possible to monitor the kinetics of processes of organic matter decomposition in soils. Based of the carried out examinations, the following conclusions has been found:

1. The kinetics of organic substances decomposition (glucose and liquid manure) depends on the type of the soil.
2. Nitrogenous fertilizers intensify the decomposition of glucose in soils, and the kinetics depends on the type of the fertilizer.
3. The N-Serve nitrification inhibitor, dosed in 1÷3 μg g^{-1}, was of insignificant influence on the kinetic parameters of glucose decomposition in soil in presence of $(NH_4)_2SO_4$.

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KALORYMETRYCZNE I KINETYCZNE BADANIA WPŁYWU NAWOZÓW AZOTOWYCH NA ROZKŁAD MATERII ORGANICZNEJ W GLEBIE

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Abstrakt: Przebieg zmian szybkości wydzielania ciepła dostarcza informacji o kinetyce procesów biodegradacji materii organicznej w glebie. Celem niniejszej publikacji jest przegląd literatury i przedstawienie na jego tle wyników badań własnych dotyczących zastosowania metody kalorymetrycznej do kinetycznej analizy rozkładu glukozy w glebie w obecności nawozów azotowych i inhibitora nitryfikacji - N-Serve. Dodatkowo zbadano proces biodegradacji gnojowicy trzody chlewnej w różnych glebach. Kinetykę rozkładu glukozy określano, wyznaczając krzywe zmian szybkości wydzielania ciepła (RHP), wykresy całkowitego efektu cieplnego (Qt), a na ich podstawie czas (PT) osiągnięcia największej wartości mocy cieplnej (maxRHP) oraz wartość pozornej stałej szybkości (k) badanych przemian. Za pomocą współczynnika kalorymetrycznego (%MHP) wyrażano w procentach metabolicznie wydzielone ciepło w stosunku do entalpii spalania organicznego substratu wprowadzonego do gleby. Wyniki badań wykazały wzrost szybkości rozkładu glukozy w glebie w obecności $(NH_4)_2SO_4$, NH_4NO_3 oraz mocznika. Stwierdzono zróżnicowanie termokinetyki biodegradacji glukozy i gnojowicy w różnych typach gleb.

Słowa kluczowe: kalorymetria, kinetyka, gleba, materia organiczna, nawozy azotowe

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TOXICITY OF PETROLEUM SUBSTANCES TO MICROORGANISMS AND PLANTS

TOKSYCZNOŚĆ SUBSTANCJI ROPOPOCHODNYCH DLA DROBNOUSTROJÓW I ROŚLIN

Abstract: The paper discusses sources of petroleum substances in environment and threats caused by petroleum contamination of water and soil. The physicochemical and biological properties of petroleum substances have been determined along with their toxicity, particularly that of aromatic hydrocarbons, including the so-called *BTEX* group (benzene, toluene, ethyl benzene and xylenes) to microorganisms and plants. Much emphasis has been laid on the properties which shape toxicity. The effect (typically degrading one) on live organisms present in water and soil environment, including biological equilibrium of soil (abundance of microorganisms, enzymatic activity), has been discussed as well as the growth and development of plants growing on soil polluted with these substances. In addition, factors (soil or water properties and condition, C:N ratio, pH reaction, soil moisture, content of organic substance, fertilization, type and species of aquatic or land organisms) which influence the effect produced by petroleum substances on live organisms in environment have been mentioned.

Keywords: toxicity, petroleum substances, sources of contamination, microorganisms, soil, plants

Sources of petroleum substances in environment

Man has long been interfering with and frequently disturbing the natural balance in ecosystems, thus modifying and re-shaping the surrounding nature [1]. The development of civilization, accompanied by industrialization, has caused severe contamination of natural environment over extensive parts of many countries. The whole biosphere is being degraded due to man's destructive activity, and live organisms are poisoned by noxious substances from various sources [2, 3].

Recently, the advancing industrialization of the world economy has led to a large increase in the consumption of petrochemical compounds [4, 5]. Likewise, in Poland the dynamically growing number of motor vehicles leads to increased demand for fuel, which in turn means purchasing and processing more crude oil. It also stimulates fuel transport, storage and distribution. As a result, the risk that petroleum substances will permeate into

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environment has increased [6]. Thus, contamination of natural environment with petroleum substances has become a major issue in ecology. Migration of petroleum substances to open water reservoirs, groundwater and soil can have serious consequences due to the highly toxic and cancerogenic nature of such chemicals [7, 8].

Petroleum substances when introduced to environment affect both its abiotic (non-live) and biotic elements, including microorganisms, plants, animals and people. Thus, soil contamination with these chemicals is a grave problem as the components of petroleum products affect all live organisms [9].

Petroleum products consist of over 1,200 different hydrocarbons [10], of which around 230 contain from 3 to 12 carbon atoms, which means that they include aliphatic, aromatic and cyclic hydrocarbons [11]. Paraffin (aliphatic) hydrocarbons are the basic hydrocarbon group of the oldest types of petrol and dominate in the petrol fraction. They are chemically inert, therefore are of no risk to environment [12]. However, the most abundant in petroleum (over 50% of its mass) are naphthene (cycloparaffin) hydrocarbons, whose amount increases as the boiling point is higher. Within this group, methylcyclopentane and methylcyclohexene are the most numerous, in some cases constituting 2% of petroleum mass [13]. As for aromatic hydrocarbons, petroleum rarely contains more than 15% of these compounds. Noticeable are mono- and polycyclic aromatic hydrocarbons. The former include the so-called BTEX group: benzene, toluene, ethyl benzene and xylenes, which are highly toxic and common pollutants of soil and groundwater due to petrol leaks from leaking tanks [14]. Polycyclic hydrocarbons are not very mobile in soil, hardly soluble in water and resistant to physical, chemical or biological degradation [15].

Products obtained from petroleum have different physical, chemical or toxic properties; they are characterized by different refinement additives and produce various effects to environment [8]. It is unquestionable that use of petroleum and petroleum-based products is very dangerous to nature. Therefore, identification of toxicity of particular petroleum components is extremely important as it will enable people to recognize potential ecological threats connected with exploitation of petroleum products [16].

The actual toxicity of petroleum and petroleum products can vary depending on the chemical composition and structure of hydrocarbons. Large concentrations of such substances in nature are sources of severe contamination and an immediate threat to all live organisms dwelling in a polluted habitat. Petroleum pollution also influences plant production, threatens groundwater and creates a risk of poisoning animals and people, in extreme cases causing death [17]. It needs to be emphasised that petroleum substances can accumulate in food and this is the most common way in which they enter live organisms [16].

Besides, hydrocarbons and their derivatives are the most numerous and widespread group of organic compounds which occur in surface and groundwaters. It is so because they can penetrate the hydrosphere as a result of transport accidents or industrial failures, in surface effluents or with sewage [18]. Contamination of water reservoirs with petroleum substances is extremely dangerous as such pollutants create on the surface of a water body a membrane, which cuts off oxygen supply and hinders the self-cleaning of water [19]. Moreover, decomposition of petrol in water causes oxygen consumption from water and is determined by the water temperature, salinity, chemical composition and reaction [20].

Seas are mainly polluted by crude petroleum, which can be spilt due to accidents involving tankers, faulty operations on oil rigs and leaking tanks stored in port warehouses.

Such spills are typically single events, but volumes of spilt oil are usually large enough to cause enormous ecological disasters. Much smaller amounts of petroleum enter seawaters with waste discharged from ships and as natural leaks from oil deposits under seabed [21]. However, the amounts of petroleum reaching seas and oceans due to tectonic motions of the earth's crust are almost negligible when compared with the oil spills occurring as a result of man's activity [22]. Evidently, the highest proportion of the pollutants found in seawater, over 1/3 of crude oil permeating into sea, is made up by petroleum and paraffin derivatives released during regular exploitation of ships. One of the major sources is ballast water discharged from cargo holds of tankers, which contains about 0.3÷1.0% of petroleum [18].

A small amount of petrol spilt on sea makes a spill which spreads with waves and wind at a speed of about 3÷4% of the wind speed. The spill is obviously the thickest at the source of spill and thinner at the edges, and typically equals 0.01 to 1 μm. An oil spill disappears in 12-24 hours owing to evaporation, photolysis and absorption by sea plants and animals [6]. Nevertheless, petroleum and its products are very harmful to water bodies as they can float on water surface for a long time [20], thus limiting oxygen and sunlight access to deep water. Besides, under the influence of gravitational forces and surface tension, a petroleum spill spreads and stratifies itself over the sea surface. In between one to three weeks, some petroleum (about 1/3) evaporates and, airborne, travels over large distances. With rainwater, it can re-enter sea or reach land [18].

Effect of petroleum substances on microorganisms

Both small and large oil spills affect organisms inhabiting aquatic habitats, causing their diseases and death [23]. Petroleum compounds often accumulate in organisms of animals dwelling at seabed, thus entering a food chain in which man is the final link. Aromatic hydrocarbons of a low boiling point are highly toxic to nearly all water organisms, unlike paraffin hydrocarbons, which do not reveal toxic properties. Furthermore, petroleum lying on feeding grounds of birds may accelerate their extinction [22].

Among the components of petroleum and petroleum products, the most toxic are aromatic compounds, particularly polycyclic aromatic hydrocarbons, which are highly mutagenic and cancerogenic. It has been found out that presence of petroleum pollutants in water facilitates solubility of PAHs and increases contamination of water with toxic combinations of these compounds. According to the Ordinance of the Minister for the Environment of 20th August 2008 [24], the permissible quantity of petroleum derived compounds, the oil index for inland waters, should not exceed 0.2 mg dm^{-3}, including 0.1 μg dm^{-3} of benzo[a]pyrene, 0.03 μg dm^{-3} of total benzo[b]fluoranthene and benzo[k]fluoranthene, and 0.002 μg dm^{-3} of total benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene.

Petroleum compounds can enter soil environment with falling dust, after which they strongly absorb to soil surface [22]. Benzene, toluene and other aromatic hydrocarbons make up 82% of the total hydrocarbon pollutants which permeate from the aeration sphere into groundwater [25]. Moreover, the uptake of these hydrocarbons by edible and fodder plants can have serious ecological consequences, both sanitary and health-related ones [15].

Due to their very slow migration, which is associated with the persistence and insolubility of their particles, PAHs accumulate in soil and can persist in it for a very long time. It is important to add that these compounds are hardly biodegradable. They can undergo biotransformation via adduction to other organic compounds and entering biochemical cycles. Benzo[a]pyrene is considered to be the strongest cancerogenic polycyclic hydrocarbon [22]. The list of mutagenic and cancerogenic substances includes a large number of indirect products obtained while processing petroleum [26]. The Ordinance of the Minister for the Environment of 9th September 2007 [27] states that the permissible amount of benzo[a]pyrene in soil, depending on soil usage, ranges from 0.02 to 5 mg kg^{-1} d.m., that of the total PAHs is from 1 to 2 mg kg^{-1} d.m. and for benzene from 0.05 to 3 mg kg^{-1} d.m. of soil.

It is also important that aromatic hydrocarbons are capable to join DNA and RNA, thus revealing mutagenic activity by disturbing the proper structure of genetic material of multiplying cells in a living organism, typically in a way that is not seen in a given individual but in its progeny, resulting in physical or mental retardation or in genetic diseases. Aromatic hydrocarbons act as initiators of mutation and cancer processes, which cause transformation of healthy cells, which means that they do not always directly cause mutation in DNA but are the so-called premutagenes, ie having entered an organism they undergo metabolic activation, stimulated by appropriate enzymes, and this activated form of PAHs has a degenerative influence on the cell DNA [28].

Oils, like other petroleum products entering soil, can produce varied effects on soil microorganisms [29, 30], including saprophytic fungi [31, 32], causing changes in quantitative and qualitative composition of soil microorganisms. Moreover, petroleum products can affect other soil properties. In a study conducted by Wyszkowski and Ziolkowska [33], soil pollution with diesel oil raised the acidity of soil, expressed as lowered soil pH, total exchangeable cations, total exchangeable capacity and degree of alkaline cation saturation as well as tendency towards increasing hydrolytic acidity. The influence of petrol on physicochemical properties of soil was evidently weaker than that of diesel oil. Petroleum substances can also influence the content of organic carbon and available forms of micronutrients in soil [34]. Such modifications of the physicochemical soil properties cause changes in the biological composition of soil habitats, particularly mass mortality among animals inhabiting topmost layers of soil and rapidly increasing content of organic matter [18]; resultant is the disruption of the biological equilibrium of soil [35]. This is due to the decreasing number of aerobic microorganisms [30]. Small amounts of petroleum substances can stimulate abundance of soil microbes. With this respect, diesel oil produces better effects than petrol, which is more toxic [36]. Many studies [37-43, 45, 46] confirm that petroleum substances can considerably modify the natural biological activity of soils. In most cases, they are highly toxic, strongly inhibiting the development of microorganisms and, consequently, depressing the enzymatic activity of soils. Oleic components derived from petroleum coat soil particles and crops with thin film which retards proper course of live processes and disturb microbiological processes in soil [19].

Moderate soil pollution with petroleum substances can stimulate the enzymatic activity of soil [41], including the activities of dehydrogenases, urease, alkaline phosphatase and nitrification activity of soil [35]. Galas et al [47] as well as Xu and Johnson [48] found out that the level of the activity of dehydrogenases is largely a function of the concentration of hydrocarbons in soil. Higher biological activity of most of the examined parameters of soil contaminated with diesel oil is most likely caused by enhanced multiplication of microorganisms and their raised activity, as diesel oil is a good potential nutrient substrate to some microorganisms [45, 49-51]. Soil contamination with petroleum substances for the first few weeks caused increased activity of amylases [9], proteases [9], dehydrogenases [9, 35], urease [35], alkaline phosphatase [35] and soil nitrifying activity [35], whereas later it typically depressed the activity of these enzymes [9, 35].

However, the enzymatic activity of soil depends on many other factors, such as soil pH reaction or presence of organic matter. Amending soil with organic substance, for example compost, or modifying the soil reaction by liming can stimulate the activity of urease, alkaline phosphatase and soil nitrifying activity [35]. Raised activity of dehydrogenases, urease, alkaline phosphatase and soil nitrifying activity in a limed series can most probably be attributed to the increased pH value, as in the soil of a reaction close to neutral the growth of bacteria and *Actinomyces* is more intensive whereas that of fungi slows down [47]. Moreover, amounts of plant available nutrients increase, which stimulates the growth and development of plants [5, 52]. Activities of particular soil enzymes are mutually correlated. In the study of Wyszkowska and Wyszkowski [35], the activity of dehydrogenases, urease, alkaline phosphatase in soil contaminated with diesel oil was positively correlated while that of acidic phospahatse - negatively correlated with soil nitrifying activity.

Effect of petroleum substances on plants

Small amounts of petroleum or petroleum-derived substances are not harmful to soil. However, more than 1 kg petroleum per m^2 of soil surface can lead to necrosis of plants. This effect is due to the anaerobic, hydrophobic conditions created by the pollutants, which cause disorder in the soil-plant-water relationships [53]. Strong contamination of soil with petroleum substances can lead to extensive degradation of plant cover up to its complete disappearance [54-56]. Surface contamination of soil not only limits or deteriorates the conditions for plant growth, but also depresses the value as well as the technological and nutritional usability of plant yields [57]. Wlodkowic and Tomaszewska [4] prove that plants respond variously to soil contamination with petroleum substances. It has been demonstrated that spring oilseed rape is highly sensitive to hydrocarbons leached from soil. Wyszkowski and Ziolkowska [58, 59], who completed experiments with spring barley, spring oilseed rape and yellow lupine as main crop, and maize and oat as aftercrop, demonstrated that depressed growth of crops attributable to soil contamination with petroleum substances depended on plant species and their sensitivity to contamination. Petroleum substances retarded the growth of plants proportionately to the contamination rate. They affected main crops more strongly than aftercrops. Rates of pollutants even as small as 2.5 $cm^3\ kg^{-1}$ of soil depressed the growth and development of crops. In general, diesel oil produced stronger effect than petrol. Regarding spring barley, the highest rates (10 $cm^3\ kg^{-1}$ soil) of petrol completely stopped plant germination whereas identical rates of diesel oil strongly inhibited the process. Toxic effect of diesel oil on growth and development of spring barley has also been evidenced in a study by Przybulewska [60], in which soil contamination with diesel oil at a rate equal 10% w/w soil caused complete inhibition of germination of spring barley kernels. Also Wyszkowska and Kucharski [61] confirmed toxic influence of diesel oil on plants, as they demonstrated that it was highly toxic to yellow lupine on light soil (plant mass decreased by half) when it was introduced to soil in an amount as low as 0.5% of the maximum water capacity. In heavy soil, its negative effect was much weaker. Toxicity of petroleum substances towards yellow lupine has also been verified in a study by Wyszkowski et al [5], in which the extent of the effect produced by soil contamination with diesel oil on the test crop depended on the type of soil, fertilization and plant organ.

The sanitary and nutritional quality of yields can also be questionable when contamination of soil with petroleum substances does not weaken the vegetative development of crops but can cause accumulation of hydrocarbons in plants [57]. Hydrocarbons, derived from petroleum products, apart from producing an indirect effect on plants via soil [62, 63], can have a direct influence by producing oily film on aerial parts of plants, thus reducing plant transpiration and respiration, decreasing permeability of plant membranes, causing disorders in metabolic processes which result in modifications in the chemical composition of plants and, finally, due to the toxic effect of some hydrocarbons in plants [5, 64].

According to Siuta [57], soil can be degraded to the following degrees:

- small degree, when contamination does not produce adverse effect on plant vegetation but the occurrence of hydrocarbons in the soil topmost layer is high;
- moderate, when point necrosis of grass plants is observable and the growth of arable crops is strongly weakened;
- large, when patches of grass plants die out and yields of crops decline by about 50%;
- very large, when grass plants disappear or completely die out and crop cultivation is impossible without soil rehabilitation treatments.

The actual effect of such pollutants on plants depends on many factors, including the concentration of petroleum substances in soil, soil properties and condition, the C:N ratio, pH reaction, moisture and oxygen content, content of organic substance, fertilization and plant species [65]. Plants which are grown on soils polluted with petroleum substances are often characterized by delayed germination; in some, the meristemal and root hair zones can be damaged. Furthermore, such plants have thin leaves of small assimilation surface and thin, short and poorly branched stems [18, 66]. The growth and development of plants is affected not only by the toxic influence of petroleum substances but also due to the lack of conditions needful for existence of plants [15] caused by the negative influence of petroleum substances, and in particular deficiency of phosphorus and potassium [67] as well as that of nitrogen, oxygen and water [68]. Because of their indirect toxicity, petroleum leaks are harmful to plants, whose resistance or tolerance to contamination with petroleum substances is connected with the depth of root systems, efficiency of replacing leaves that have been shed due to the contamination and whether or not the plants are equipped with storage organs and underground stalks, particularly rhizomes. Biodegradation of hydrocarbons in soil leads to the appearance of anaerobic conditions in the plant rhizosphere. Lack of oxygen and consequent production of hydrogen sulphide destroy roots of most plants, including large trees, which have a well-developed root system [53]. Petroleum contaminants, apart from increasing the amount of hydrocarbons in soil [56, 69], considerably raise the predominance of organic carbon over the content of nitrogen in the humus horizon, and when its availability is very high with a consequent large supply of energy and proportionately depressed availability of nitrogen, strong competition for nitrogen beings between microorganisms decomposing hydrocarbons versus plant root systems. This competition results in an acute shortage of nitrogen for plants, minimizing and sometimes completely inhibiting their growth [48].

The influence of petroleum substances on the growth and development of plants depends on their type and degree of contamination. Negative impact of small pollution with diesel oil on plants can be stronger than that produced by petrol. The effect produced by these pollutants on aftercrops is typically small, although it can be negative as well [58]. At the same time, petroleum substances affect the uptake of many macro- and micronutrients. Their influence on the content of macronutrients in plants depends on the type and rate of a petroleum substance involved on the species of plants. In an experiment reported by Wyszkowski and Wyszkowska [52], small rates of diesel oil (up to 3 g kg^{-1} soil) raised the content of nitrogen, potassium and sodium in oat, whereas higher ones depressed these values. The negative influence of diesel oil on nitrogen concentration in plants has also been confirmed by Wyszkowski et al [5] testing yellow lupine. In other experiments, carried out by Wyszkowski and Ziolkowska [58, 70], it has been found out that evidently negative correlations occur between the degree of soil contamination with petrol or diesel oil and the content of N-total and N-protein in yellow lupine and, partly, in maize (only when polluted with diesel oil) [58]. They also depressed the content of most macronutrients in spring oilseed rape and, to a smaller extent, in oat [70] except calcium and magnesium in spring oilseed rape and potassium in oat, whose concentrations in plants increased under the influence of petroleum substances. Smaller modifications were observed in treatments polluted with petrol and larger ones when diesel oil was introduced to soil [70]. Diesel oil favoured accumulation of most macronutrients in aerial parts of maize, particularly the content of sodium, potassium and calcium [52]. Dimitrov and Mitova [71] found out that three out of the seven species they tested contained elevated amounts of macronutrients whereas the content of phosphorus and calcium did not change significantly under the influence of diesel oil. Petrol and diesel oil contamination favoured accumulation of potassium in grain and phosphorus in straw (only petrol) and roots of spring barley, but depressed the concentration of other macronutrients [59].

Thus, petroleum products polluting environment are an increasingly more serious ecological problem as contamination of water and soil with these chemicals has adverse effect on the microbiological and biochemical properties of soil and on plant production. They also have negative impact on animal and human health due to the fact that most of them are toxic to live organisms.

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TOKSYCZNOŚĆ SUBSTANCJI ROPOPOCHODNYCH DLA DROBNOUSTROJÓW I ROŚLIN

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Abstrakt: Przedstawiono źródła obecności substancji ropopochodnych w środowisku i zagrożenia związane z zanieczyszczeniem nimi wód i gruntów. Określono ich właściwości fizykochemiczne i biologiczne z uwzględnieniem toksyczności (zwłaszcza węglowodorów aromatycznych, w tym tzw. grupy BTEX - benzenu, toluenu, etylobenzenu i ksylenów) dla mikroorganizmów i roślin. Szczególny nacisk położono na właściwości decydujące o ich toksyczności. Opisano oddziaływanie (najczęściej degradujące) na organizmy żywe występujące w środowisku wodnym i glebowym, w tym na biologiczną równowagę gleby (liczebność drobnoustrojów, aktywność enzymatyczną) oraz wzrost i rozwój roślin rosnących na terenach zanieczyszczonych tymi substancjami. Przedstawiono także czynniki (właściwości i stan wód lub gleby, stosunek C:N, odczyn pH, uwilgotnienie gruntów, natlenienie, zawartość substancji organicznej, nawożenie, rodzaj lub gatunek organizmów wodnych i lądowych), które wpływają na oddziaływanie substancji ropopochodnych na organizmy żywe występujące w środowisku.

Słowa kluczowe: toksyczność, substancje ropopochodne, źródła zanieczyszczenia, drobnoustroje, gleba, rośliny

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ACCUMULATION OF NITROGEN AND PHOSPHORUS BY MAIZE AS THE RESULT OF A REDUCTION IN THE POTASSIUM FERTILIZATION RATE

AKUMULACJA AZOTU I FOSFORU PRZEZ KUKURYDZĘ POD WPŁYWEM OGRANICZONEGO NAWOŻENIA POTASEM

Abstract: The field experiment was carried out to evaluate the effect of K fertilization rates on uptake and distribution of macroelements (N and P) in maize grown in a soil with very high soil-test K levels (>250 mg K · kg^{-1}). Plant samples were taken at 37, 48, 58, 69, 79, 89, 100, 109, 118, 132 and 140 days after maize sowing and analyzed for nitrogen and phosphorus contents. Data was subjected to analysis of variance with significant differences among means determined by LSD at $p < 0.05$. It was found that fertilization was not a factor that significantly differentiated nutrient uptake by maize. Additionally, under conditions of soil with very high soil-test K levels, potassium fertilization had a minimal effect on nutritional status (optimal N and P contents) of maize plants.

Keywords: maize, nitrogen, phosphorus, uptake, K fertilization

Recommendations of balanced fertilization with potassium, one of the principle plant nutrients underpinning crop yield production and quality determination, should take into account a soil K fertility test as a chemical method for estimating the nutrient-supplying power of a soil [1]. Over-fertilization with potassium can lead to an increase of this element in soil, its luxury consumption, and as a consequence induce a nutrient imbalance in plants (eg nitrogen and phosphorus). Non-application of K fertilizer under conditions of soil with very high soil-test K levels may constrain environmentally and economically undesirable potassium flow [2, 3]. The aim of the research was to evaluate the effect of K fertilization rates on uptake and distribution of macroelements (N and P) in maize grown in a soil with very high soil-test K levels (> 250 mg K · kg^{-1}).

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Material and methods

The two-year field experiment was conducted at an agricultural farm situated in the south-eastern part of the Lubelin province, where maize (*Turini* variety) was grown. Soil at the experimental site was classified as Cambisols derived from loess, characterized by a neutral reaction, middle soil-test levels of P and Mg and very high soil-test K levels (> 250 mg K kg^{-1}). Taking into account soil K concentration, NPK and NP fertilization was supplied.

Fertilizers were applied prior to planting at rates per hectare of 140 kg ammonium phosphate (16.8 kg N and 31.9 kg P) and 280 kg Kemira Corn fertilizer (16.8 kg N, 24.6 kg P and 65 kg K). Additional N fertilizer as nitrate(V) ammonium (81.6 kg N ha^{-1}) was side-dressed at the 5-6 leaf growth stage.

Plant samples were taken at 37 (I term), 48 (II term), 58 (III term), 69 (IV term), 79 (V term), 89 (VI term), 100 (VII term), 109 (VIII term), 118 (IX term), 132 (X term) and 140 (XI term) days after maize sowing and analyzed for nitrogen and phosphorus contents. Nitrogen concentrations were determined by using the Kjeldahl method and the phosphorus concentrations, in the samples digested in sulfuric acid and hydrogen peroxide, by colorimetric analysis using the phosphovanadomolybdic complex. Data was subjected to analysis of variance with significant differences among means determined by LSD at $p < 0.05$.

Results and discussion

The uptake and content of nitrogen and potassium in maize plants were not significantly influenced by potassium fertilization (Figs. 1 and 2, Tab. 1). However, the analyzed nutrient accumulation rate for maize was different at critical phases. During an intensive growth of new tissues (from the 7th leaf stage up to the 9th leaf stage) the nitrogen uptake rate was respectively 66.7% greater, and at tasseling 35.7% lower under soil without additional potassium supply (Fig. 1).

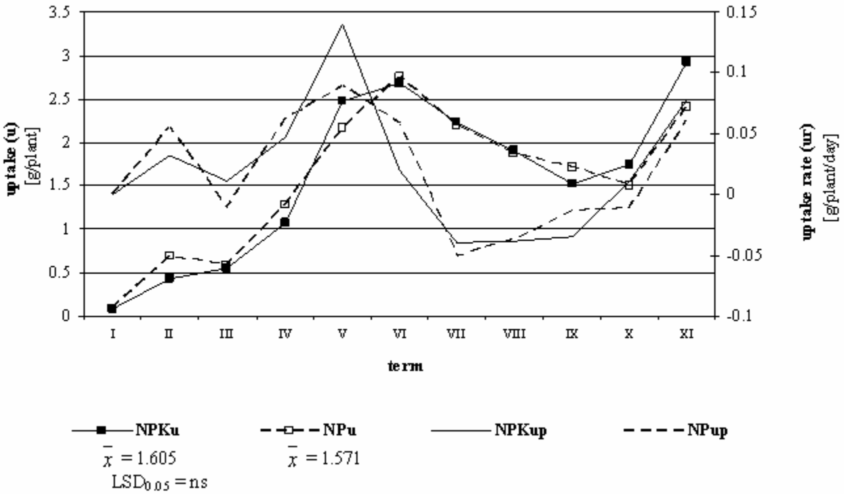

Fig. 1. Nitrogen uptake by maize during vegetation

The highest phosphorus accumulation in maize was found at reproductive stages (at tasseling and early dough). It should be underlined that fertilization with potassium intensified this process slightly during tasseling and slowed it down at early dough (Fig. 2). According to data from Niu et al [4] concentrations of N and P transferred in the phloem and xylem sap did not change in the case of a better potassium supply for plants. Some authors reported that increase in the available soil-potassium levels above the threshold value of 150 mg kg^{-1}, does not influence nitrogen and phosphorus accumulation by maize [3, 5].

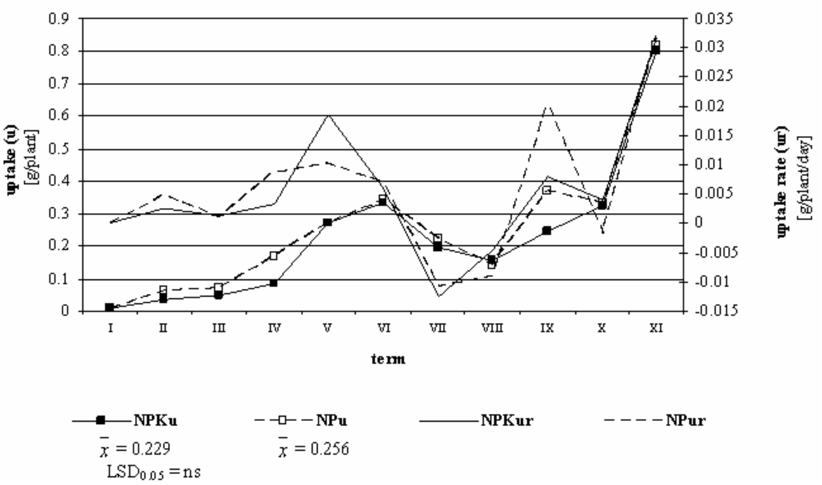

Fig. 2. Phosphorus uptake by maize during vegetation

Nutrient distribution pattern within plant organs was dependant on the intensity of metabolism processes. Therefore, the leaves and grain were the main accumulation pool for elements such as N and P (Tab. 1). Regardless of the fertilization applied, the higher translocation efficiency for P (phosphorus harvest index (PHI) - 0.80÷0.81) than N (nitrogen harvest index (NHI) - 0.64÷0.65) was observed.

Maize treated with K fertilizers tended to increase the amount of nitrogen accumulated in grain, while an adverse trend was found with P (Tab. 1). However, from the nutritional point of view, the concentrations of N and P agreed with data from other authors for the optimal growth of maize [2, 6]. Changes in the concentrations of phosphorus and nitrogen in NPK-treated maize were probably due to the reduction in soil pH. Potassium fertilizer induced acidification of the soil (the physiological and the exchange acidity) could indirectly influence the bioavailability of nutrients [7]. The relatively high replacing power of H$^+$ is an important factor in mobilizing non-exchangeable NH_4^+. Protons leave the crystal lattice in an expanded state and thus render the non-exchangeable NH_4^+ more accessible to replacing cations. On the other hand, with a decrease in the rhizosphere pH, phosphate activity depletes as a result of the specific and non-specific anion adsorption by soil [5, 8]. Furthermore, K fertilizer shows beneficial effects in the absorption, the translocation rate and the reduction of nitrates in plants [9, 10].

Table 1

N, P concentration in maize organs [g kg^{-1}]

Fertilization	**Organ**	**N**	**P**
NPK	leaves	7.84	1.3
	stalks	4.48	0.6
	grain	15.96	5.4
	cobs	3.22	0.5
	roots	3.92	0.4
$\overline{x}$		7.08	1.64
NP	leaves	8.68	2.0
	stalks	3.78	0.6
	grain	14.42	6.2
	cobs	3.08	0.4
	roots	3.92	0.4
$\overline{x}$		6.78	1.91
$LSD_{0.05}$ fertilization		n.s.	n.s.
$\overline{x}$	leaves	8.26	1.63
	stalks	4.13	0.58
	grain	15.19	5.80
	cobs	3.15	0.45
	roots	3.92	0.40
$LSD_{0.05}$ organ		n.s.	n.s.

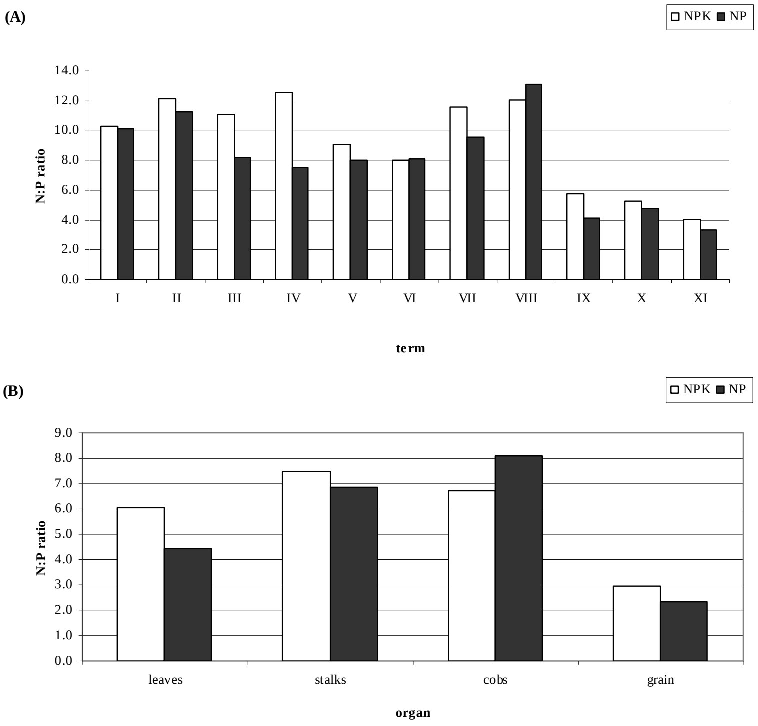

Fig. 3. The N:P ratio in maize during vegetation (A) and in the final yield (B)

The dynamics of phosphorus and nitrogen accumulation in maize were reflected in the N:P ratios of plant biomass (Fig. 3). The N:P ratios were lower (by about 14 %) in the treatments without K application in comparison with the treatments with K application during the vegetative period. Analysis of the data indicated a greater influence of P uptake than of N uptake on the N:P ratios, ie $R^2 = 0.59$ for phosphorus versus $R^2 = 0.16$ for nitrogen. This was probably due to the higher translocation efficiency for P than N. According to some authors the greater importance of phosphorus as a source of variation in crop N:P ratio is connected with a molecular component, since the P content of major biomolecules is more variable than their N content [6, 11]. The narrowing of the N:P ratios with plant age (Fig. 3) may be attributed to the dilution effect (Fig. 4), and additionally, in the case of final yield (grain), to the intensification of phosphorus accumulation during the reproductive stage when seeds were formed.

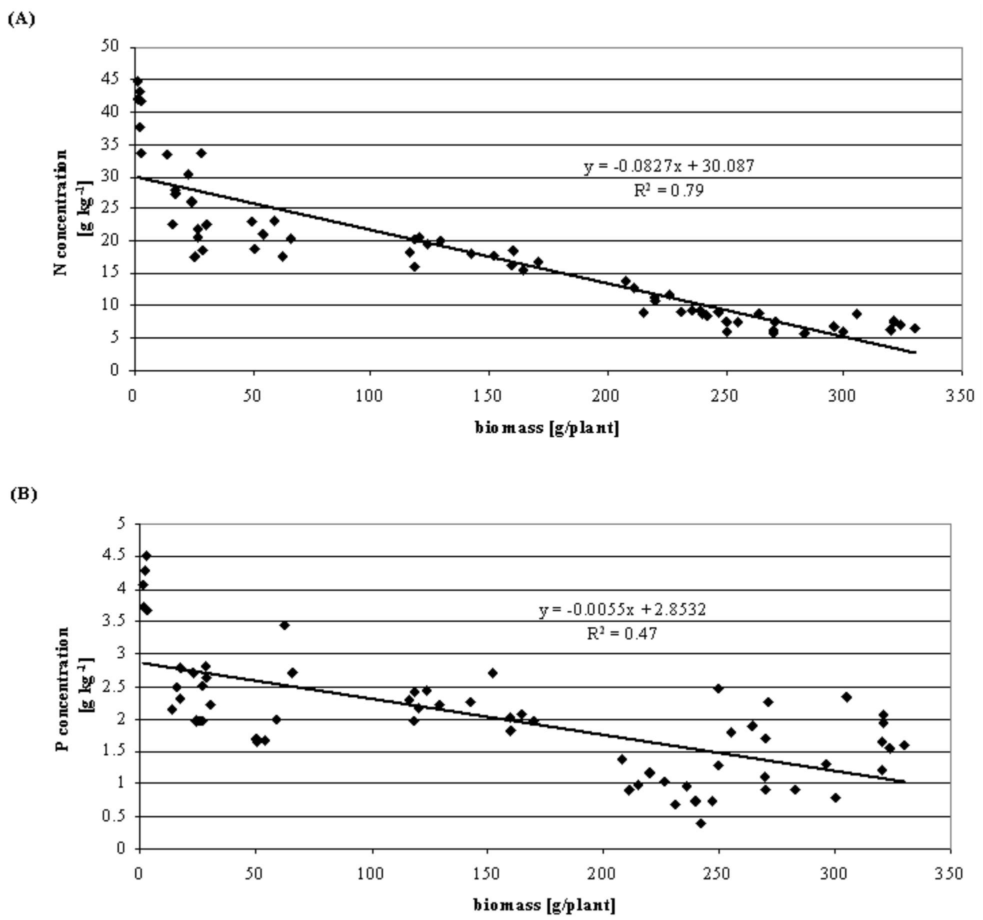

Fig. 4. Relationship between nitrogen (A), phosphorus (B) concentration and maize biomass

Conclusions

1. Under conditions of the very high soil-test K levels, fertilization with potassium had little effect on the uptake and concentrations of nitrogen and phosphorus in maize.
2. Regardless of the potassium supply, the macronutrient accumulation (N and P) was intensified during the highest metabolic activity of maize plants, ie at leaf development (N), at tasseling (N, P), and during early dough (P).
3. In spite of the fertilization applied, the process of remobilization of N and P from vegetative organs (mainly leaves) appeared to be an important pool for these nutrients in generative organs (grain).
4. Maize treated with K fertilizers tended to increase the N:P ratio, which was attributed to a greater degree to changes in P uptake than N uptake.

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AKUMULACJA AZOTU I FOSFORU PRZEZ KUKURYDZĘ POD WPŁYWEM OGRANICZONEGO NAWOŻENIA POTASEM

Katedra Chemii Rolnej i Środowiskowej, Uniwersytet Przyrodniczy w Lublinie

Abstrakt: Celem przeprowadzonego doświadczenia polowego było określenie wpływu nawożenia potasem na pobranie i rozmieszczenie makroelementów (N i P) w kukurydzy uprawianej na glebie o bardzo dużej zasobności w potas (> 250 mg K kg^{-1}). Próbki roślinne pobierano 37, 48, 58, 69, 79, 89, 100, 109, 118, 132 i 140 dnia po wysiewie kukurydzy i oznaczano w nich zawartość azotu i fosforu. Dane opracowano statystycznie za pomocą analizy wariancji przy poziomie istotności $\alpha = 0,05$. Stwierdzono, że nawożenie nie było czynnikiem, który statystycznie istotnie różnicował pobranie składników pokarmowych przez kukurydzę. Ponadto, w warunkach gleby o dużej zasobności w potas nawożenie K miało niewielki wpływ na stan odżywienia (optymalną zawartość N i P) roślin kukurydzy.

Słowa kluczowe: kukurydza, azot, fosfor, pobranie, nawożenie potasem

VARIA

CENTRAL EUROPEAN CONFERENCE ECOpole'09
Short Conference Report

The Conference ECOpole'09 was held in hotel Uroczysko (near Szklarska Poreba, Poland), from Oct. 14th, 2009 to Oct. 17th, 2009. It was the eighteenth ecological conference of the series of meetings organised by the Society of Ecological Chemistry and Engineering, Opole, PL.

126 participants, including delegates representing 10 countries (from Austria, Czech Republic, Germany, Greece, Great Britain, Latvia, Poland, Russian Federation, Slovakia and Ukraine) took part in the event and presented 16 oral contributions and 118 posters.

The Abstracts of the Conference contributions were available on the Conference website

ecopole.uni.opole.pl

before and during the conference.

The Conference issue of the quarterly *Ecological Chemistry and Engineering S* containing among others keynote speakers papers, was distributed at the Conference Reception desk together with a CD-ROM (containing short info on Keynote Speakers, Abstracts of the Conference presentations as well as ECOpole'09 Conference Programme).

The Conference Agenda was divided into 5 sections:

- SI Ecological Chemistry and Engineering - 15.10.2009 (Thursday)
- SII Risk, Crisis and Security Management - 15.10.2009 (Thursday)
- SIII Environment Friendly Production and Use of Energy - 16.10.2009 (Friday)
- SIV Forum of Young Scientists and Environmental Education - 16.10.2009 (Friday)
- SV Health, Ecology and Agriculture - 17.10.2009 (Saturday).

The Conference was opened by prof. Maria Wacławek, Chairperson of the Organising Committee and prof. Witold Wacławek, Chairman of the Scientific Board and President of the Society of Ecological Chemistry and Engineering.

Prof. Magnuss VIRCAVS **(***University of Latvia, Rīga, LT)* initiated the Plenary Session with the invited lecture: "Chemical composition and assessment of Latvia drinking water quality". During the conference the plenary lectures were delivered also by other invited lecturers: prof. Waldemar WARDENCKI (*Gdansk University of Technology, Gdańsk, PL*): "Application of gas chromatography, mass spectrometry and olfactometry for quality assessment of selected food products", prof. Edward BORODZICZ (*Portsmouth Business School, UK*): "Risk, crisis and resilience", prof. Elena MASAROVIČOVÁ (*Comenius University Bratislava, SK*): "Cost and benefit of energetic plants - challenges for environment friendly management", prof. Marina V. FRONTASYEVA (*Joint Institute for Nuclear Research, Dubna, Moscow, RF*): "Accumulation of trace elements by biological matrice of *Spirulina platensis"* and dr Hagen SCHERB (*Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, DE*): "Increased reproductive health risks after Chernobyl across Europe".

Very interesting presentations were also given by prof. S. FRÄNZLE (*IHI Zittau, DE*): "Environmental chemistry on activated interfaces", prof. R. Tsitouridou (*University* *of Thessaloniki, Thessaloniki, GR*): “Nanoparticle air pollution a case study of Thessaloniki, Greece”, dr Z. A. SZYDŁO (*Highgate School, London, UK*): “Environmental chemistry in English schools” and prof. Manfred SAGER (*Austrian Agency for Health and Food Safety, Wien, AT*): „Potassium chlorate – nitric acid reagent for the analysis of trace metals and selected non-metals in food and agricultural products”.

Thursday, a day of hard work, was finished by the Poster Session of the Section Chemical Pollution of Natural Environment and its Monitoring. Many of the discussions begun at the posters, lasted until the evening hours.

The next point of Thursday Conference Programme was the one-hour-long concert of „Tadeusz Pabisiak Trio”. Classical and contemporary compositions were played. The audience applauded the musiciens and claimed for bis.

At 19.30 the Conference participants were invited for a Conference Dinner.

On Friday morning, the day started with an ecological excursion to Kostrzyca Forest Gene Bank in Miłków. This is one of the five existing gene banks around the world, the first to set up in Central Europe.

As usually during the ECOpole Conference, the second day included the Session of the Young Scientists (a forum of young scientists that present and discuss local ecological problems of their countries). During the Young Scientists’, Environmental Education and Renewable Energy Poster Session 37 posters were presented. The Scientific Board (consisting of prof. Elena Masarovičová (*Comenius University Bratislava, SK*), prof. Bohumil Vybiral (*University of Hradec Králové, Hradec Králové, CZ*) and prof. Witold Wacławek (*Society of Ecological Chemistry and Engineering, Opole, PL*) granted awards (sponsored by the Society of Ecological Chemistry and Engineering) for the best presentations. The awards for poster presentations were given to **N. Baljinnyam** for the poster: N. Baljinnyam, Sh. Gerbish, G. Ganbold, S. Lodoysamba, M.V. Frontasyeva and S.S. Pavlov (*Joint Institute for Nuclear Research, Dubna, RU*): “Heavy metals in the environmental objects of non-ferrous industrial region of Mongolia, the town of Erdenet”, to **P. Ostrowski** for the poster: E. Radziemska, P. Ostrowski, A. Cenian and M. Sawczak (*Gdansk University of Technology, Gdańsk, PL*): “Chemical, thermal and laser treatment in recycling of photovoltaic solar cells and modules from crystalline silicon” and to **L. Trnková** for the poster: L. Trnková, I. Boušová, I. Ortová and J. Dršata (*University of Hradec Kralove, Hradec Králové, CZ*): “Damage to proteins caused by free radicals from environment: effect of natural antioxidants”. The Scientific Board awarded also honourable mentions for two oral presentations and two posters presented during Forum of Young Scientists. They were given to **K. Nawrotek** for the oral: K. Nawrotek, R. Zarzycki and Z. Modrzejewska (*Lodz University of Technology, Łódź, PL*): “Kinetics of adsorption and release from chitosan hydrogels” and to **M. Worłowska** for the oral: M. Worłowska and M. Marko-Worłowska (*Jagiellonian University, Kraków, PL*): “Can works of art shape respectful attitude towards nature? - ecological art in Poland” as well as to **F. Donigiewicz** and **M. Wadowski** for the poster: F. Donigiewicz, M. Wadowski, K. Hoffmann and J. Hoffmann (*Wroclaw University of Technology, Wrocław, PL*): “Study on the concentration of extractive phosphoric acid and for fodder phosphates receiving requirements” and to **G. Łagód** and **Z. Suchorab** for the poster: G. Łagód, Z. Suchorab, H. Sobczuk, J. Malicki, M. Chomczyńska and A. Montusiewicz (*Lublin University of Technology, Lublin, PL*): “Methodical aspects of bioreactor modeling in water and wastewater management”.

After the conclusion of the Friday scientific agenda, the Conference participants were invited for a Grill Party.

The third day was dedicated to the impact of environment food and pollution on human health.

After very interesting plenary lectures delivered among others by Keynote Speakers Marina V. Frontasyeva and Hagen Scherb the last Conference Poster Session came up.

Closing the conference, prof., prof. Maria and Witold Wacławek made short recapitulation. In general, ECOpole'09 was focused on monitoring the quality of natural environment, its effects on human life, environmental education as well as application of renewable sources of energy.

They expressed gratitude to all participants for coming and taking active part in the Conference and thanked all Chairpersons of Sessions.

They announced, that full text of the presented papers will be published (after obtaining reviewers' positive opinions) in the successive issues of the journals *Ecological Chemistry and Engineering A* and *S* and they will be distributed to all participants.

The Extended Abstracts of the presentations will be published in two subsequent issues of semi-annual *Proceedings of the ECOpole*.

Thanks to the financial support of the Polish Ministry of Scientific Research and Higher Education, it was possible, among others to publish Extended Abstracts and full Conference contributions as well as to help to pay the fees of the young scientists taking part in the Conference.

At the end they invited all Colleagues to attend the ECOpole'10 conference, which will be held also in Piechowice in next October.

Maria Wacławek

SPRAWOZDANIE
ze Środkowoeuropejskiej Konferencji ECOpole'09

W dniach 14-17 października 2009 r. w hotelu „Uroczysko" w Piechowicach odbyła się osiemnasta proekologiczna konferencja ECOpole. Organizatorem konferencji było Towarzystwo Chemii i Inżynierii Ekologicznej (TChIE) w Opolu.

Uczestniczyło w niej 126 osób, reprezentujących Austrię, Czechy, Federację Rosyjską, Grecję, Łotwę, Niemcy, Polskę, Słowację, Ukrainę i Wielką Brytanię.

Każdy z uczestników otrzymał w teczce konferencyjnej zeszyt kwartalnika *Ecological Chemistry and Engineering S*, zawierający referaty zaproszonych wykładowców, oraz CD-ROM z krótkimi informacjami o zaproszonych wykładowcach, abstraktami wystąpień oraz programem konferencji. Abstrakty wystąpień były także dostępne na stronie webowej konferencji

ecopole.uni.opole.pl

przed i w czasie jej trwania.

Podobnie jak w latach poprzednich, konferencja ECOpole'09 była poświęcona różnym aspektom ochrony środowiska przyrodniczego. W czasie jej trwania zostało wygłoszonych 16 referatów oraz przedstawiono 118 posterów. Obrady konferencji ECOpole'09 były zgrupowane w pięciu Sekcjach:

- SI Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring
- SII Odnawialne źródła energii i jej oszczędne pozyskiwanie oraz użytkowanie
- SIII Zarządzanie środowiskiem w warunkach kryzysowych
- SIV Forum Młodych (FM) i Edukacja prośrodowiskowa
- SV Wpływ zanieczyszczeń środowiska oraz żywności na zdrowie ludzi

Konferencję otworzyli prof. Maria Wacławek (*Uniwersytet Opolski*) - przewodnicząca Komitetu Organizacyjnego i prof. Witold Wacławek (*Towarzystwo Chemii i Inżynierii Ekologicznej, Opole*) - przewodniczący Rady Naukowej Konferencji i prezes TChIE.

Sesję plenarną rozpoczął prof. Magnuss VIRCAVS (*University of Latvia*, *Rīga*, *LT*) referatem: "Chemical composition and assessment of Latvia drinking water quality".

Zaproszeni wykładowcy wygłosili w czasie konferencji jeszcze pięć referatów plenarnych, a mianowicie: prof. Waldemar WARDENCKI (*Politechnika Gdańska, Gdańsk*): "Application of gas chromatography, mass spectrometry and olfactometry for quality assessment of selected food products", prof. Edward BORODZICZ (*Portsmouth Business School, UK*): "Risk, crisis and resilience", prof. Elena MASAROVIČOVÁ (*Comenius University Bratislava, SK*): "Cost and benefit of energetic plants - challenges for environment friendly management", prof. Marina V. FRONTASYEVA (*Joint Institute for Nuclear Research, Dubna, Moscow, RF*): "Accumulation of trace elements by biological matrice of *Spirulina platensis"* i dr Hagen SCHERB (*Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, DE*): "Increased reproductive health risks after Chernobyl across Europe".

Ponadto bardzo interesujące referaty przedstawili: prof. S. FRÄNZLE (*IHI Zittau, DE*): "Environmental chemistry on activated interfaces", prof. R. TSITOURIDOU (*University of Thessaloniki, Thessaloniki, GR*): "Nanoparticle air pollution a case study of Thessaloniki, Greece", dr Z.A. SZYDŁO (*Highgate School, London, UK*): "Environmental chemistry in English schools" and prof. Manfred SAGER (*Austrian Agency for Health and Food Safety,* Wien, AT): „Potassium chlorate - nitric acid reagent for the analysis of trace metals and selected non-metals in food and agricultural products".

Naukowy czwartkowy program zakończyła Sesja Posterowa Sekcji Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring. Łącznie zaprezentowano na niej 57 plakatów.

O 18.30 rozpoczął się godzinny koncert zespołu „Tadeusz Pabisiak Trio". W programie znalazły się utwory z różnych epok i różnych stylów muzycznych. Publiczność dziękowała artystom za występ hucznymi oklaskami i skutecznie domagała się bisów.

W piątek, w czasie wycieczki proekologicznej, zwiedzaliśmy Leśny Bank Genów (LBG) Kostrzyca w Mikołowie. LBG Kostrzyca to jedyny bank genów, nie tylko w kraju, ale i w Europie, skupiający się przede wszystkim na ochronie i zachowaniu zasobów genowych leśnej roślinności drzewiastej. W ubiegłym roku Leśny Bank Genów Kostrzyca został laureatem dziesiątej edycji Konkursu Ministra Środowiska „Lider Polskiej Ekologii" za ochronę bioróżnorodności i leśnych zasobów genowych.

W piątek po południu tradycyjnie odbywało się Forum Młodych (FM).

Jury w składzie prof. Elena Masarovičová (*Uniwersytet Comeniusa Bratislava, SK*), prof. Bohumil Vybiral (*Uniwersytet w Hradec Králové, Hradec Králové, CZ*) oraz prof. Witold Wacławek (*Towarzystwo Chemii i Inżynierii Ekologicznej, Opole*) przyznało nagrody i wyróżnienia młodym pracownikom naukowym w nim uczestniczącym. Zostały przyznane trzy nagrody, które otrzymali: **N. Baljinnyam** za poster: N. Baljinnyam, Sh. Gerbish, G. Ganbold, S. Lodoysamba, M.V. Frontasyeva and S.S. Pavlov (*Joint Institute for Nuclear Research, Dubna, RF*): "Heavy metals in the environmental objects of non-ferrous industrial region of Mongolia, the town of Erdenet", **P. Ostrowski** za poster: E. Radziemska, P. Ostrowski, A. Cenian and M. Sawczak (*Politechnika Gdańska, Gdańsk*): "Chemical, thermal and laser treatment in recycling of photovoltaic solar cells and modules from crystalline silicon" oraz **L. Trnková** za poster: L. Trnková, I. Boušová, I. Ortová and J. Dršata (*University of Hradec Kralove, Hradec Králové, CZ*): "Damage to proteins caused by free radicals from environment: effect of natural antioxidants".

Ponadto przyznano wyróżnienia **F. Donigiewiczowi i M. Wadowskiemu** za plakat: F. Donigiewicz, M. Wadowski, K. Hoffmann and J. Hoffmann (*Politechnika Wrocławska, Wrocław*): "Study on the concentration of extractive phosphoric acid and for fodder phosphates" oraz **G. Łagódowi** i **Z. Suchorabowi** za plakat: G. Łagód, Z. Suchorab, H. Sobczuk, J. Malicki, M. Chomczyńska and A. Montusiewicz (*Politechnika Lubelska, Lublin*): "Methodical aspects of bioreactor modeling in water and wastewater management".

Przyznano także wyróżnienia dla **K. Nawrotek** za referat: K. Nawrotek, R. Zarzycki and Z. Modrzejewska (*Politechnika Łódzka, Łódź*): "Kinetics of adsorption and release from chitosan hydrogels" oraz dla mgr **M. Worłowskiej** za referat: M. Worłowska and M. Marko-Worłowska (*Uniwersytet Jagielloński, Kraków*): "Can works of art shape respectful attitude towards nature? - ecological art in Poland".

Przed kolacją odbyła się prezentacja i wystawa produktów naturalnych firmy Calio.

Trzeciego dnia motywem przewodnim obrad było rolnictwo, zdrowa żywność i zdrowie. Sesję plenarną rozpoczęły 2 referaty wygłoszone przez prof. Marinę V. Frontasyevą i Hagena Scherba. Program naukowy konferencji zamykała Sesja Posterowa Sekcji Rolnictwo - Zdrowie - Ekologia.

Dużym zainteresowaniem uczestników konferencji cieszyła się wystawa książek Wydawnictw Naukowo-Technicznych (WNT), Warszawa oraz czasopism i książek wydanych przez Towarzystwo Chemii i Inżynierii Ekologicznej w Opolu.

Na zakończenie konferencji Maria i Witold Wacławek podziękowali wszystkim uczestnikom za udział, a szczególnie gorąco osobom mającym wystąpienia oraz przewodniczącym obradom. Słowa gorących podziękowań zostały także skierowane do członków Komitetu Organizacyjnego konferencji za duży, wielomiesięczny wkład ich pracy. Do publikacji swoich prac zostali zaproszeni wszyscy uczestnicy konferencji. Organizatorzy zapewnili, że pełne artykuły z wystąpień konferencyjnych będą sukcesywnie publikowane w *Ecological Chemistry and Engineering A* oraz *S*. Poinformowali oni, że rozszerzone streszczenia ukażą się w półroczniku *Proceedings of ECOpole*.

Organizatorzy konferencji zaprosili wszystkich do udziału w dziewiętnastej konferencji ECOpole. Zapowiedzieli oni, że na konferencji ECOpole'10 aktywni uczestnicy Forum Młodych mogą liczyć znowu na znaczne obniżenie opłaty konferencyjnej. Do uprzywilejowanych w tym względzie należą także członkowie Towarzystwa Chemii i Inżynierii Ekologicznej.

Konferencja ECOpole'09 uzyskała wsparcie finansowe Ministerstwa Nauki i Szkolnictwa Wyższego w Warszawie, co umożliwiło częściowe sfinansowanie druku *Proceedings of ECOpole* oraz opłat konferencyjnych młodych uczestników konferencji.

Maria Wacławek

15th ICHMET

15th INTERNATIONAL CONFERENCE
ON HEAVY METALS IN THE ENVIRONMENT

SEPTEMBER 19-23, 2010
GDAŃSK, POLAND

ORGANIZED BY
CHEMICAL FACULTY, GDANSK UNIVERSITY OF TECHNOLOGY (GUT)
TOGETHER WITH
COMMITTEE ON ANALYTICAL CHEMISTRY
OF THE POLISH ACADEMY OF SCIENCES (PAS)

15th ICHMET- is a continuation of a series of highly successful conferences that have been held in major cities of the world since 1975. These conferences typically draw 500-1000 participants from countries in many parts of the world. Well over 5000 scientists have taken part in this series of conferences including most leaders in the field. Apart from the city's natural beauty, Gdańsk is logical choice for the 15th Conference to highlight the outstanding work that is being done on heavy metals in Central Europe. The venue for the meeting will be the Gdansk University of Technology (GUT) which features many tourist attractions.

The Conference will include a number of invited lectures treating frontier topics prepared by specialist with international reputation, oral presentation and poster sessions. ICHMET welcomes contributions on all aspects of any heavy metal in the environment. All presentation will be connected with such topics as:

- Risk assessment and risk management pertaining to toxic metals in the environment
- Susceptibility and protection of children from toxic metals in their environment
- Measurement and exposure assessment
- Biomarkers of exposure and effects of heavy metals
- Gene-environment-metal interactions
- Trend tracking/analysis of heavy metal data - spatial and temporal
- Risk communication pertaining to heavy metals
- Life cycle analysis for metalliferous consumer products
- Soil quality criteria
- Remediation technologies
- Control strategies for heavy metal emissions and deposition
- Metal mixtures - mechanistic and epidemiological studies
- Nutrient-metal interactions
- Advancements in analytical tools (procedures and measurement devices)
- Toxicology of heavy metals, from cellular and genomic to ecosystem levels

- Heavy metals in foods
- Impact of global change on heavy metal cycle

For further information on the conference, please contact:
Professor Jacek Namieśnik (Conference Chairman)
Gdansk University of Technology, Chemical Faculty
Department of Analytical Chemistry
G. Narutowicza 11/12, 80-233 Gdańsk (Poland)
email: chemanal@pg.gda.pl
homepage: http://www.pg.gda.pl/chem/ichmet/

INVITATION FOR ECOpole'10 CONFERENCE

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 19th annual Central European Conference ECOpole'10, which will be held in **13-16 X 2010** (Thursday-Saturday) on Wilhelms Hill at Uroczysko in Piechowice, the Sudety Mts., Lower Silesia, PL.

The Conference Programme includes oral presentations and posters and will be divided into five sections:

- **SI Chemical Pollution of Natural Environment and its Monitoring**
- **SII Environment Friendly Production and Use of Energy**
- **SIII Risk, Crisis and Security Management**
- **SIV Forum of Young Scientists and Environmental Education**
- **SV Impact of Environment Pollution on Food and Human Health**

The Conference language is English.

Contributions to the Conference will be published as:

- abstracts on the CD-ROM (0.5 page of A4 paper sheet format)
- extended Abstracts (4-6 pages) in the semi-annual journal *Proceedings of ECOpole*
- full papers will be published in successive issues of the *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (Ecol. Chem. Eng.) ser. A and S.

Additional information one could find on the Conference website:

ecopole.uni.opole.pl

The deadline for sending the Abstracts is **15.07.2010** and for the Extended Abstracts: **1.10.2010**. The actualised list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one can find (starting from 15.07.2010) on the Conference website.

The papers must be prepared according to the Guide for Authors on Submission of Manuscripts to the Journals.

At the Reception Desk each participant will obtain a CD-ROM with abstracts of the Conference contributions as well as Conference Programme (the Programme will be also published on the Conference website).

Maria Wacławek

Further information is available from:
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Chairperson of the Organising Committee
of ECOpole'10 Conference
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17. 2008 CEC ECOpole'08 Piechowice
18. 2009 CEC ECOpole'09 Piechowice

ZAPRASZAMY
DO UDZIAŁU W ŚRODKOWOEUROPEJSKIEJ KONFERENCJI
ECOpole'10
W DNIACH 13-16 X 2010

SUBSTANCJE CHEMICZNE W ŚRODOWISKU PRZYRODNICZYM

Będzie to dziewiętnasta z rzędu konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotycząca różnych aspektów ochrony środowiska przyrodniczego. Odbędzie się ona w ośrodku „Uroczysko" na Wzgórzu Wilhelma w Piechowicach koło Szklarskiej Poręby. Doroczne konferencje ECOpole mają charakter międzynarodowy i za takie są uznane przez Ministerstwo Nauki i Szkolnictwa Wyższego. Obrady konferencji ECOpole'10 będą zgrupowane w pięciu Sekcjach:

- **SI Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring**
- **SII Odnawialne źródła energii i jej oszczędne pozyskiwanie oraz użytkowanie**
- **SIII Zarządzanie środowiskiem w warunkach kryzysowych**
- **SIV Forum Młodych (FM) i Edukacja prośrodowiskowa**
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