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COST AND BENEFIT OF ENERGETIC PLANTS - CHALLENGES FOR ENVIRONMENT FRIENDLY MANAGEMENT

KOSZTY I KORZYŚCI WYKORZYSTANIA ROŚLIN ENERGETYCZNYCH - WYZWANIA DLA PRZYJAZNEGO ZARZĄDZANIA ŚRODOWISKIEM

Abstract: Biomass energy has been recognized as one of the most promising and most important renewable energy sources in near future. It was emphasized that besides of woody plant species as energetic plants can be also used both crops (mainly maize, rapeseed, sunflower, soybean, sorghum, sugarcane) and non-food plants (e.g. switchgrass, jatropha, algae). **Energetic plant** was characterized as a plant grown as a low cost and low maintenance harvest used to make biofuels, or directly exploited for its energy content (heating or electric power production). Moreover, by-products (green waste) of crops and non-food plants can be also used to produce biofuels. It was stressed that European production of biodiesel from energy crops has grown steadily in the last decade, principally focused on rapeseed used for oil as a substance in FAME (*fatty acid methyl ester*) production. Similar tendency was observed for bioethanol (as a biocomponent in gasoline) prepared mainly from maize or cereals. At present bioethanol and biodiesel primarily produced from the crops (maize and rapeseed) are used in the traffic. However, in the past these crops were used only as a food. Consequently, a new ethical problem appeared: discrepancy between utilization of maize and rapeseed as a food or as an alternative source of energy. New biotechnological approach showed that energetic plants have also significant application for environment friendly management, mainly in phytoremediation technology. Phytoremediation was presented as a cleanup technology belonging to the cost-effective and environment-friendly biotechnology. Thus several types of phytoremediation technologies being used today were briefly outlined.

Keywords: alternative energy source, bioethics, biofuels, energetic plants, environment, phytoremediation

Introduction

In the worldwide scale biomass is the greatest source of renewable energy (in detail see [1]). The amount of energy stored in the biomass is approximately 7.5-times greater than is global share (46%) responded to biomass [2]. Under condition of Slovakia it is actual to use for energetic purposes forest biomass including energetic wastes from wood-processing industry as well as food industry and waste biomass from industrial and communal field. The use of forest biomass for energetic purposes is relatively favourable. It is mainly residual wood and wood mass which could not be used for other purposes (residua after timber production, smallwood of trees, salvage felling timbre, etc.). For combustion are suitable wood pieces, wood chips, briquettes or pellets made from forest biomass. It was shown that very perspective is mainly cultivation of energetic forest coppices (willow, poplar, and black locust tree). Wood-working industry represents app. 40% portion from total technically utilizable potential of biomass (wastes originated from mechanical processing of wood, filings, bark). Biomass from the agriculture (straw, plant residues) arised either from cultivation of crops (maize, cereals, rapeseed) or from food industry (pressing of oilseeds and fruits, cutting of fruit trees or vine) (in details see [3]).

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Biomass as a source of renewable energy

According to some authors (eg [4]) biofuels are likely more ecological than conventional fossil fuels what could be a substantial argument mainly from the aspect of world-wide concentration increase of greenhouse gases, mainly CO_2 [5]. Further arguments supporting the use of biofuels are: continually increasing price of liquid fossil fuels, the use of soils with lower bonita for cultivation of technical crops, overproduction of crops with lower quality which could not be used as a food. At present extraordinary attention is devoted to the study of exploitation of both, second generation biofuels (produced from technical crops, which could not be used as a food, as well as from biomass wastes) [6, 7] and third generation biofuels (produced from transgenic - GM - energetic plants or from algae). However, the most important biomass in Europe as a source of renewable energy is presented by fast-growing trees like willow, poplar and to some extent alders (cf. [8]).

Energetic plants

In general, energetic plants - EP (energy crops) are the plants grown as a low cost and low maintenance harvest used to make biofuels, or directly exploited for its energy content (heating or electric power production). According to Weger [9] for the choice of suitable energetic plants following criteria could be considered: a) high biomass production (mass, volume, energy content, b) manageability of cultivation (effective cultivation techniques), c) biomass suitability for biofuel production (with respect to different criteria for solid, liquid and gaseous fuels, respectively), d) economy of biomass production (at a given economic conditions and financial subvention); e) environmental aspects (eg greenhouse gases balance, invasive plant species, etc).

There are many species used as EP (eg [10]). Some of them are herbs (eg *Zea mays*, *Brassica napus*, *Triticum aestivum*, *Helianthus annuus*, *Helianthus tuberosus*, *Sorghum bicolour*, *Miscanthus* sp., *Jatropha curcas*), shrubs or trees (eg *Populus* sp., *Salix* sp., *Alnus glutinosa*, *Ailanthus altissima*, *Ulmus montana*). Since cultivation of the most of above mentioned herbs is in general very well known, therefore in the following text our attention will be paid to cultivation of energetic trees - energy forestry. Basis for this approach is sustainable tree biomass production presented eg by Andersson [11].

Energy forestry

Energy forestry is a form of forestry in which a fast-growing shrubs or trees are grown specifically to provide biomass or biofuel for heating or power generation [cf. 12]. There are two forms of energy forestry: **short rotation forestry (SRF)** and **short rotation coppice (SRC)** (in detail see [10, 13]). The first one are species like alder, ash, birch and poplar grown for 8 to 20 years before the first harvest. SRC uses high yield varieties of poplar and willow grown for 2 to 5 years before the first harvest. This woody solid biomass can be used in applications such as district heating, electric power generating stations, alone or in combination with other fuels [8].

Energetic plants and climatic changes

Anthropogenic factors continue to elevate atmospheric CO_2 concentration, which on average has already exceeded 377 ppm in the year 2006 [14] which shows a substantial increase from 280 ppm in the year 1750 (IPCC 2001). The change in atmospheric CO_2 is correlated to the 0.8°C increase in global average surface temperature in the past century, and the warming rate of about 0.2°C per decade [15]. Biomass can be used to produce C-neutral fuels to power for transportation industry [16]. Biomass fuels are C-neutral because they release recently-fixed CO_2, which does not shift the C-cycle. Biomass may generate the same amount of CO_2 as fossil fuels per unit C, but every time a new plant grows it removes that same CO_2 from the atmosphere [10].

Causes of both short-term and long-term climatic changes on the earth are discussed for many years (eg Kyoto Protocol 1997, summit OSN, Bali, 2007). Nowadays 9 milliards tonnes of carbon are emitted from anthropogenic sources into atmosphere [17]. We suppose that high greenhouse gases concentration in atmosphere will increase temperature of our planet, mainly in the north part of hemisphere.

Besides the most important greenhouse gas - CO_2 the further greenhouse gas - N_2O outcoming from fertilization (especially rapeseed) is intensively discussed [18]. This gas was classified as a third most important greenhouse at all. Its global warming potential (GWP) is 296x higher than GWP of CO_2. [5]. It could be supposed that N_2O emission will increase in connection with higher cultivation area of rapeseed.

Invasive and genetically modified energetic plants - potential risk for the environment?

Several biofuel crops, which many countries are promoting as an alternative to fossil fuels, have many traits in common with invasive species [19, 20]. These species fulfil characteristics of an ideal biomass crop: low energy into maintenance relative to the production of energy-rich biomass; efficient use of irradiance, water and nutrients; C_4 photosynthesis; nutrient translocation into storage organs during the non-growing season; and perennial growth. Domestication of non-native crops, in fact, is considered one of the main pathways of biological invasions [21]. In particular, according to Barney and DiTomaso [20], biofuel feedstock can survive in conditions that mimic natural habitat.

The enhancement of environmental tolerance in GM energetic plants likely will increase the risk of invasion into surrounding environments. Similarly, enhancement of aboveground biomass production via biotechnology could allow such cultivars to be more competitive with native vegetation or other cultivated crops. Genetic modification can change the phenotype or physiology of a plant species sufficiently to lead to alterations in plant-plant interactions and ecological functions. Thus, it is important to recognize that, like non-native species, even native plants, if modified would pose an unknown risk of becoming invasive [22]. Based on above-mentioned facts it should be beneficial to perform genotype-specific pre-introduction screening for a target region, which consists of risk analysis, climate-matching modelling, and ecological studies of fitness responses to various environmental scenarios. Such screening procedure will provide reasonable assurance that economically beneficial biofuel crops will pose a minimal risk of damaging native and managed environment [20].

Biofuels - environment friendly approach

As it has already been mentioned biofuel is renewable fuel that can be prepared from vegetable oils, animal fats, or recycled restaurant greases. Biodiesel is safe, biodegradable, and reduces serious air pollutants such as particulates, carbon monoxide, hydrocarbons, and air toxics. In spite of these facts progress in biofuel use is nowadays still discussed.

First-generation biofuels rely on food plant species (crops) as their feedstock. Corn, soy, rapeseed and sugarcane all have readily accessible sugars, starches and oils. Thus to change them into biofuels simply involves either fermenting the sugars or transform the fatty oils through transesterfication. **Second-generation biofuels** use lignocellulosic biomass as feedstock (mainly wood, ie trees), non-food plants like switchgrass (*Panicum virgatum*) and agricultural residue (as well as other organic wastes) such as corn stalks. Rather than improving the fuel-making process, **third-generation biofuels** seek to improve the feedstock. Designing oilier crops, for example, could greatly boost yield. Scientists (geneticists) have designed poplar trees (ie GM poplars) with properties to make them easier to process. Researchers have already mapped the genomes of sorghum and corn, which may allow genetic agronomists to change the genes controlling oil production. Thus, third generation biofuels are carbon neutral when consumed meaning that the crops consume the same amount of carbon from the atmosphere as they will release when combusted. This is done through GM and nowadays it is not yet commercially available. **Fourth-generation technology** combines genetically optimized feedstocks, which are designed to capture large amounts of carbon, with genomically synthesized microbes, which are made to efficiently make fuels. Key to the process is the capture and sequestration of CO_2, a process that renders fourth-generation biofuels a “carbon negative” source of fuel (in detail see [23]).

The major benefit of biofuels is the potential to reduce net CO_2 emissions to the atmosphere. Enhanced C management may make it possible to take CO_2 released from the fossil C cycle and transfer it to the biological C cycle to enhance food, fiber, and biofuel production as well as sequester C for enhancing environmental quality [10].

Phytoremediation - cost-effective green biotechnology

Environmental pollution with xenobiotics including toxic metals is still serious global problem. Development of phytoremediation technologies for the plant-based clean-up of contaminated substrates is therefore of significant interest. Phytoremediation is environment-friendly and cost-effective green technology for the removing of toxic metals and organic pollutants from the environment using the some species of the plants. There are several types of phytoremediation technologies currently available for clean-up of both contaminated soils and water. The most important of them are **phytoextraction**, **rhizofiltration**, **phytostabilization**, **rhizodegradation**, **hydraulic control and phytorestauration** [24, 25].

The most effective but also technically the most difficult phytoremediation technology is phytoextraction involving the cultivation of metal-tolerant plants that concentrate soil contaminants in their aboveground tissues. At the end of the growth period, plant biomass is harvested, dried or incinerated, and the contaminant-enriched material is deposited in a special dump or added into a smelter. The energy gained from burning of the biomass could support the profitability of this technology, if the resultant fumes can be cleaned appropriately. For phytoextraction to be effective, the dry biomass or the ash derived from above ground tissues of a phytoremediator crop should contain substantially higher concentrations of the contaminant than the polluted soil [26].

It should be stressed that from above-mentioned phytoremediation technologies the most frequent practical application has phytoextraction which has been growing rapidly in popularity world-wide for the last twenty years. In generally, this process has been tried more often for extraction of toxic metals than for organic substances. A living plant may continue to absorb contaminants until it is harvested. After harvest a lower level of the contaminant will remain in the soil, so the growth/harvest cycle must usually be repeated through several crops to achieve a significant cleanup. After the process, the cleaned soil can support other vegetation.

Energetic plants vs bioethics aspects

In connection with the increasing trend of biofuel use an important ethical problem occurred - perplexity whether crops (eg maize, cereals, potatoes, rapeseed, and sunflower) could be used exclusively for alimentary purposes or also as an alternative energy source. Astyk [27] published twelve ethical principles which describe all actual aspects (both positive and negative) of biofuels. Serious factor also is the increase of the soil portion designated for cultivation of technical crops at the expense of forests and natural vegetation, what could be reflected in the biodiversity decline. These assumptions evoked negative reflection in the world, too. Therefore, acceptance of fundamental principles of bioethics is needed.

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KOSZTY I KORZYŚCI WYKORZYSTANIA ROŚLIN ENERGETYCZNYCH - WYZWANIA DLA PRZYJAZNEGO ZARZĄDZANIA ŚRODOWISKIEM

Abstrakt: Energia biomasy jest uznana za jedno z najbardziej obiecujących i najważniejszych odnawialnych źródeł energii. Podkreślono, że oprócz gatunków roślin drzewiastych, jako rośliny energetyczne mogą być również wykorzystywane uprawy (głównie kukurydzy, rzepaku, słonecznika, soi, sorgo, trzciny cukrowej) i inne rośliny niespożywcze (np. proso, jatrofa, glony). Uprawa i zbiór **roślin energetycznych** wymaga niewielkich kosztów, a wykorzystuje się je do produkcji biopaliw lub bezpośredniego uzyskania energii (ogrzewanie lub produkcja energii elektrycznej). Ponadto, produkty uboczne upraw (odpady zielone) i inne rośliny niespożywcze mogą być także wykorzystywane do produkcji biopaliw. Podkreślono, że europejska produkcja biodiesla z roślin energetycznych stale rośnie w ostatnim dziesięcioleciu, koncentrując się głównie na oleju rzepakowym stosowanym w produkcji FAME (estry metylowe kwasów tłuszczowych). Podobne tendencje zaobserwowano w przypadku bioetanolu (jako biokomponentu benzyny), otrzymywanego przede wszystkim z kukurydzy i zbóż. Obecnie bioetanol i biodiesel, wytwarzane głównie z kukurydzy i rzepaku, są stosowane w transporcie. Natomiast w przeszłości rośliny te były używane tylko jako żywność. W konsekwencji pojawiły się nowe problemy etyczne wynikające z rozbieżność między wykorzystaniem kukurydzy i rzepaku jako żywności lub jako alternatywnego źródła energii. Nowe podejście biotechnologiczne pokazuje, że rośliny energetyczne mają również duże znaczenie dla przyjaznego zarządzania środowiskiem, szczególnie w fitoremediacji. Oczyszczanie za pomocą fitoremediacji jest uważane za technologię oszczędną i przyjazną dla środowiska. W skrócie zaprezentowano niektóre z obecnie wykorzystywanych rodzajów fitoremediacji.

Słowa kluczowe: alternatywne źródła energii, bioetyka, biopaliwa, rośliny energetyczne, środowisko, fitoremediacja

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CHEMICAL COMPOSITION AND ASSESSMENT OF DRINKING WATER QUALITY: LATVIA CASE STUDY

SKŁAD CHEMICZNY I OCENA JAKOŚĆ WODY PITNEJ. STUDIUM PRZYPADKU: ŁOTWA

Abstract: Assessment of drinking water quality in seven largest Latvia drinking water supply systems (Riga, Daugavpils, Liepaja, Ventspils, Jelgava, Jurmala, and Rezekne) in 2008 using mathematical statistical processing of chemical composition data is carried out. In all analyzed drinking water the concentrations of Hg, Cd, Pb, Cu, Ni, Cr (total), BrO_3^- and trihalomethanes (total) were observed in the level of their quantification or less than it or concentration changes were observed only in some cases that are significantly less than their maximum permissible values (MPV). The processed data show that higher concentrations of sulphate in Jelgava and Jurmala drinking water were observed. In Jelgava drinking water sulphate concentration exceed the accepted MPV for 97 mg/dm^3 and in Jurmala - for 26 mg/dm^3. Besides, high values of total iron (1.15±0.54 mg/dm^3) and turbidity (14.2±7.2 nephelometric turbidity units) were obtained also in Jelgava drinking water. Relative high concentration of aluminium in Liepaja drinking water (0.2 mg/dm^3) takes place that achieves the MPV. Confidence intervals of mean values were calculated using Chebyshev's inequality. The processed data testify well even very well quality of the analyzed largest Latvia drinking water supply systems.

Keywords: drinking water quality, chemical composition, mathematical statistics, Latvia

Introduction

Provision of a qualitative drinking water is an important precondition for improvement of the life quality. Drinking water quality directly affects human health. The impacts reflect the level of contamination of whole drinking water supply system (raw water, treatment facilities and distribution network to consumers). The primary goals of environmental especially drinking water management are to provide safe drinking water supply in international and national scale. The international organizations, eg World Health Organization (WHO) have major functions to propose regulations, guidelines, and recommendations in order to realize human right to have access to an adequate of safe drinking water independently of their stage of development and their social and economic conditions.

Latvia has rich water resources, especially freshwater, which well exceeds current and planned consumption. In general chemical structure of raw water resources ensure to meet adequacy requirements of drinking water quality determined by Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption and Republic of Latvia Cabinet Regulation No. 235 “Mandatory harmlessness and quality requirements for drinking water, and the procedures for monitoring and control thereof” (adopted 29 April 2003).

Management of drinking water quality is a matter of great importance in Latvia. Implementation of the State Investment Program 800+, drinking water regular and audit monitoring as well as other environmental projects are integral part of public health and environmental protection.

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The present study is devoted to assessment of drinking water quality in seven largest Latvia drinking water supply systems in 2008 using mathematical statistical processing of chemical composition data.

Materials and methods

Quality assessment of Latvia drinking water is carried out using chemical composition data of drinking water obtained from the Public Health Agency of the Ministry of Health. Drinking water was analyzed in 2008 in seven largest Latvia drinking water systems - Riga, Daugavpils, Liepaja, Ventspils, Jelgava, Jurmala, and Rezekne (Fig. 1).

Fig. 1. Latvia administrative map. The largest drinking water systems: Riga, Daugavpils, Liepaja, Ventspils, Jelgava, Jurmala, and Rezekne

Drinking water was sampled from the site of consumers and analyses were carried out considering the requirements (testing methods, sampling frequency, the necessary precision and accuracy, maximum permissible values (MPV) of the variables) in Republic of Latvia Cabinet Regulations No. 235 “Mandatory harmlessness and quality requirements for drinking water, and the procedures for monitoring and control thereof” (adopted 29 April 2003) and in Cabinet Regulations No. 118 adopted on March 12, 2002 “Regulations regarding the Quality of Surface Waters and Groundwaters” (with amendments). Drinking water quality was evaluated by the following variables: color, turbidity, pH, conductivity, aluminium, iron (total), fluorides, sulphates, ammonium, nitrates(V), nitrites(III), mercury, cadmium, lead, copper, nickel, chromium, bromates, trihalomethanes (total).

Data processing of drinking water chemical composition includes mathematical statistical calculations. The Q-test was applied for suitability estimation of drinking water data set. The mean and the confidence interval of chemical composition variables of drinking water was expressed using Chebyshev’s inequality (confidence level $\alpha = 0.06$): $\bar{x} - 4s/\sqrt{n} \leq \mu \leq \bar{x} + 4s/\sqrt{n}$, where μ - mathematical expectation, $\bar{x}$ - mean, and s - standard deviation, and $4s/\sqrt{n}$ - standard error of mean [1]. Rezekne drinking water supply system was characterized only by two measurements of the variables. Availability of the data for further their processing was evaluated using also Chebyshev's inequality: $|x_1 - x_2| < 4s$ (where x_1 and x_2 - results of measurements). It was used for estimation of Al, Fe, F^-, pH, turbidity, and conductivity values. Assessment of differences between sample means was carried out using Bartlett's test criterion.

Characteristic of Latvia drinking water supply

Latvia has rich water resources, especially freshwater, which well exceeds current and planned consumption. Water resources allow providing high quality drinking water for all population - 70% is composed from artesian and 30% from surface water sources (rivers and lakes). Total amount of surface waters comprises 13,300 m^3 per capita but in European Union (EU) it comprises at an average 7,250 m^3 per capita [2]. In most water supply systems hydrogen-carbonate calcium water with mineralization 0.3÷0.4 g/dm^3 is used. Chemical structure of rock and infiltration water is caused by hydrogen-carbonate calcium water.

Mostly artesian waters are used for the centralized water supply in Latvia towns. They are better protected than groundwater table. Drinking water sources for the capital of Latvia Riga comprise a mixture of surface, natural groundwater, and artificially recharged groundwater from Lake Mazais Baltezers that is the main source for artificial recharge plant supplying up to 25% of Riga drinking water [3]. Reservoir of Riga hydro-power plant on the Daugava River is used as a surface water source. The Daugava Waterworks is the largest surface water treatment plant in Latvia that purifies more than 100 000 m^3 per day using alum as a coagulant [4]. However, quality of water taken from the reservoir of Riga hydro-power plant depends on transboundary pollution that enters into the Daugava River from Russia and Belarus. In the period from 1990 to 2007 three large accidents happened in the river Daugava basin. In November 1990 during filling a railroad tank in a chemical plant "Polimir", Novopolock (Belarus) spill of acetone cyanohydrin (ACH operates on respiratory centers) occurred. Significant amount of ACH leaked into the Daugava River. Due to the pollution mass fish deaths were observed in the river. Therefore during one week water supply from the Daugava River was interrupted in Riga. The second accident involved sanitation leakage from Belarus in the middle of 1990s. The last accident, disruption of oil pipe line Unecha - Ventspils (enterprise „Zapad-Transnefteprodukt", Russia), caused the Daugava River ecosystem contamination with diesel fuel that happened 23 March 2007. Diesel fuel of 4,171 Mg entered into the territory of Latvia, but ~ 90% was collected from the Daugava River waters. The noted accidents can originate and affect Riga drinking water quality [5].

Statistical description of drinking water chemical composition

The analyzed drinking water data of seven largest Latvia drinking water supply systems are conditionally divided into two groups. The first group involves the variables whose values do not change. They are the concentrations of Hg, Cd, Pb, Cu, Ni, Cr (total), BrO_3^- and trihalomethanes (total). These variables were observed in the level of their determination or less than it or concentration changes were observed only in some cases. The lowest observed concentrations are the following (in $\mu g/dm^3$): Hg - 0.1, Cd - 0.5, Pb -

1.0, Cu - 0.2, Ni - 2.0, Cr (total) - 1.0, BrO$_3^-$ - 1.0, and trihalomethanes (total) - 10.0. Besides, the exceptions comprised total Cr concentration in Daugavpils drinking water - 20.0 µg/dm^3 and Ni concentration in Jelgava drinking water - 5.4 µg/dm^3 (1 measurement). Total concentrations of trihalomethanes of Riga drinking water varied in the wide range of 0.1÷50.1 µg/dm^3 (mean and standard error of mean 23.8±0.35 µg/dm^3). The same statistics for total concentrations of trihalomethanes of Liepaja drinking water are the following: range of 0.10÷1.14 µg/dm^3, mean and standard error of mean - 0.54±0.21 µg/dm^3. All noted concentrations are less than their MPV. Drinking water color modified in the range of 5÷10 units of Pt/Co scale with the exception of 20 units of Pt/Co scale in Daugavpils and Jurmala drinking water (1 measurement).

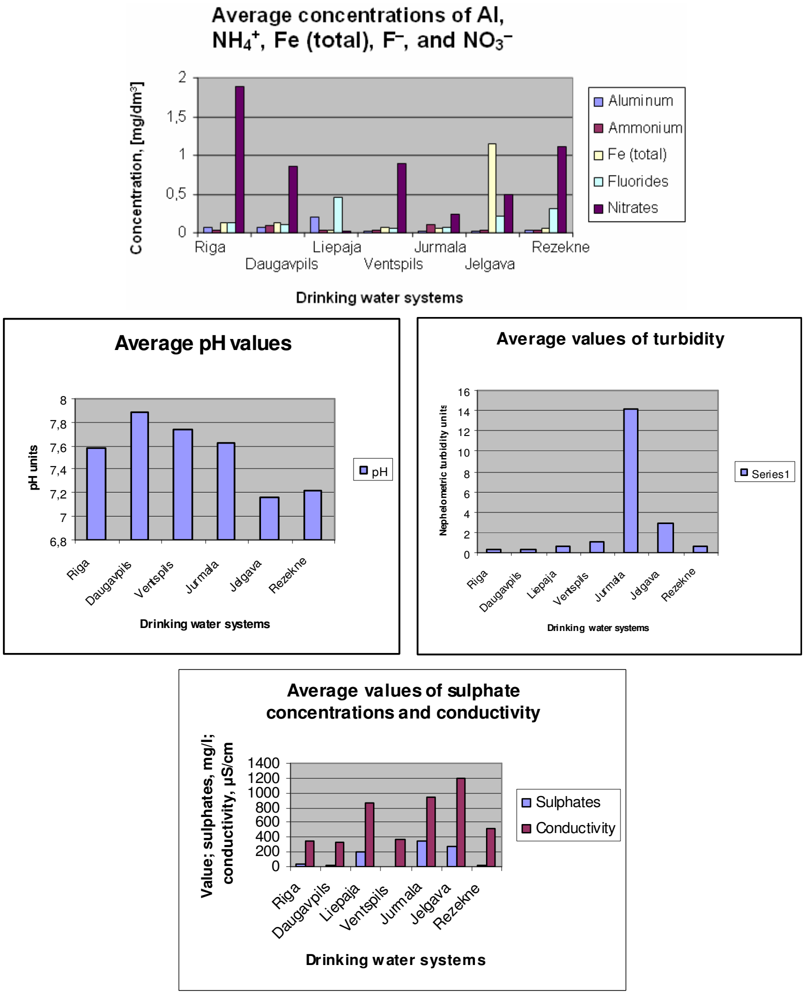

Fig. 2. Average values of Al, NH$_4^+$, Fe (total), F$^-$, NO$_3^-$, SO$_4^{2-}$, conductivity, turbidity, and pH in the largest Latvia drinking water systems

The second group includes the variables whose value changes were observed - turbidity, pH, and conductivity, concentrations of Al, Fe (total), F^-, SO_4^{2-}, NH_4^+, NO_3^- and NO_2^-. The obtained data of processing are summarized in Figure 2. Data set distribution character was estimated only for Riga drinking water variables (sample size n = 18) and its inadequacy to normal distribution was obtained. Therefore Chebyshev's inequality was applied to calculate confidence intervals of variable means because Chebyshev's theorem could be used to random variables of any distribution.

Comparison of variable mean and median shows that these statistics are not equal for all variables. Median is a statistic that is sensitive to data set symmetric or asymmetric distribution. Data symmetric distribution is observed if the mean and median are equal but in the opposite case - asymmetric distribution. Considering the diversity of sample sizes from n = 2 to n = 18 evaluation of data distribution character was not carried out. Comparison of differences between sample means at confidence level $\alpha = 0.05$ using Bartlett's test criterion testifies on the following assurance.

In all analyzed drinking water systems nitrate(III) and fluoride concentrations do not significantly differ. Mean concentration of aluminium in Liepaja drinking water system (0.2 mg/dm^3) significantly differs from its concentration in other drinking water systems that have statistically equal value 0.02 mg/dm^3. Concentration of aluminium in Liepaja drinking water is equal with MPV.

Total iron concentration (1.15±0.54 mg/dm^3) in Jelgava drinking water system significantly differs from total iron concentration of other systems but it exceed the MPV. High iron concentration is an important problem of drinking water quality in Latvia that is caused by high content of iron in groundwater tables. Therefore drinking water de-ironing is included in Latvia drinking water processing.

In Riga drinking water nitrate concentration has a wide dispersion that is specified by high standard deviation (±1.6 mg/dm^3). Mean concentration of nitrate (1.9 mg/dm^3) is significantly higher than in other drinking water systems that are in the range from 0.013 to 1.1 mg/dm^3.

Sulphate concentrations in Jelgava (347±41 mg/dm^3) and Jurmala (276±32 mg/dm^3) drinking water systems are significantly higher than in drinking water of Riga, Daugavpils, Liepaja, Ventspils, and Rezekne. Leakage from gypsum formations causes high sulphate concentrations in the noted drinking water systems. Comparison of sulphate concentrations with the MPV shows that in Jelgava drinking water average linear deviation is 97 mg/dm^3 and in Jurmala - 26 mg/dm^3.

In all drinking water systems conductivity mean values have a great dispersion with significantly high values of 1189±315 and 944±172 μS/cm in drinking water of Jelgava and Jurmala. It could be explained by high concentrations of sulphates.

Signinficantly high value of turbidity (14.2±7.4 nephelometric turbidity units, NTV) was observed in Jelgava drinking water. The Regulations No. 235 testifies turbidity values as acceptable to consumers and no substantial changes. In the case of surface water treatment, it should be striven to reach that turbidity caused by treatment plants does not exceed 1.0 (NTV).

Mean of drinking water pH falls in the range from 7.16 (Jurmala) to 7.88 (Daugavpils). pH of Riga and Jurmala drinking water significantly differs from pH of Daugavpils, Ventspils, Rezekne, and Jelgava drinking water owing their data great dispersion. Mean values pH stands in the pH range 6.5÷9.5 that satisfy the requirements of the Regulations No 235.

Conclusions

Assessment of chemical composition of the analyzed seven largest drinking water systems shows that drinking water quality satisfies to the harmlessness and quality requirements testified in the Regulations No. 235 with exception of higher concentrations of sulphate than the MPV in Jelgava and Jurmala drinking water as well as high values of turbidity and total iron in Jelgava drinking water. In all analyzed drinking water systems content of Hg, Cd, Pb, Cu, Ni, Cr (total), BrO_3^- and trihalomethanes (total) are in the level of their determination or less than it or some concentration changes were observed only in some cases that are significantly less than their MPV.

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SKŁAD CHEMICZNY I OCENA JAKOŚĆ WODY PITNEJ. STUDIUM PRZYPADKU: ŁOTWA

Abstrakt: W 2008 r. wykonano statystyczną ocenę jakości wody pitnej pobranej z siedmiu największych systemów wodociągowych Łotwy (Ryga, Daugavpils, Liepaja, Ventspils, Jelgava, Jurmala i Rezekne) na podstawie ich składu chemicznego. We wszystkich analizowanych wodach pitnych stężenia Hg, Cd, Pb, Cu, Ni, Cr (stężenie całkowite), BrO_3^- i trihalogenometanów (stężenie całkowite) były na granicy oznaczalności lub poniżej. Tylko w niektórych przypadkach obserwowano zmiany stężeń, ale były one znacznie mniejsze od dopuszczalnej wartości maksymalnej (MPV). Na podstawie analizy danych stwierdzono zwiększone stężenie siarczanów w wodzie pitnej z Jelgavy i Jurmaly. W wodzie pitnej Jelgavy stężenie siarczanów przekraczało maksymalne wartości dopuszczalne (MPV) o 97 mg/dm^3, a w Jurmale - 26 mg/dm^3. W wodzie pitnej z Jelgavy stwierdzono też duże całkowite stężenie żelaza (1,15 ± 0,54 mg/dm^3) i znaczne zmętnienie (14,2 ± 7,2 NTU). Stwierdzono stosunkowo duże stężenie glinu w wodzie pitnej z rzeki Liepaja (0,2 mg/dm^3), sięgające MPV. Przedziały ufności wartości średniej zostały obliczone z wykorzystaniem nierówności Czebyszewa. Analizowane dane świadczą o bardzo dobrej jakości wody pitnej z badanych sieci wodociągowych Łotwy.

Słowa kluczowe: jakości wody pitnej, skład chemiczny, statystyka matematyczna, Łotwa

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INSTRUMENTAL TECHNIQUES USED FOR ASSESSMENT OF FOOD QUALITY

INSTRUMENTALNE TECHNIKI OCENY JAKOŚCI ŻYWNOŚCI

Abstract: Two instrumental techniques applied for food quality assessment such as gas chromatography--olfactometry (GC-O) and electronic nose (e-nose) are characterized. The principle of each technique and typical analytical approaches in odour determination are briefly described. Furthermore, the examples of applications, performed also in the author's laboratory, are given.

Keywords: gas chromatography-olfactometry, electronic nose, volatile compounds, aroma, quality

Introduction

Growing interest and care about safe food products fulfilling high quality criteria is becoming one of the major priorities in food technology sector. One of the signs of this care is development and optimization of monitoring and control methods of both, food materials and their processing. Valuable source of information about quality of the particular product is the analysis of volatile compounds based on either classic sensor analysis or application of instrumental methods.

Food products flavour is mostly caused by many volatile compounds which appear in such product. Volatile compounds are mainly responsible for shaping the organoleptic quality of many kinds of food, even though they are found in relatively small quantities in such products [1]. For consumers, an organoleptic quality is equally important and often decisive in the purchase. From chemical point of view, the aroma of most food products is a complicated mixture, sometimes consisting of several hundred compounds. The presence of volatile compounds, their concentration and composition in food products can give valuable information about health quality of food.

In many companies a classical approach in evaluation of organoleptic quality of food products is quite often the only method used on this purpose. Classical methods are mainly based on the sensory analysis, carried out by a group of trained panelists. This analysis is a perfect tool in carrying out marketing tests of consumers but because of many limitations is not sufficient enough for quality control in industrial laboratories. Because of many disadvantages a good complement in the evaluation of organoleptic food properties is instrumental analysis. Detailed and complex qualitative and quantitative analysis of volatile components with the use of appropriate instrumental methods allow for identification of volatile compounds which influence on the flavour composition of food products [2]. The methods employed most often, allowing the creation and recognition of aromagrams are chromatographic techniques, in particular gas chromatography and so called electronic

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nose. Instrumental analysis used for characterization of the food aroma is dominated by two approaches.

First applies gas chromatography-olfactometry (GC-O) to examine single compounds responsible for the aroma of the product. In recent years, intensive studies have been carried out regarding sensory activity of the individual volatile components of various food products and the dependence between the odour and the chemical composition of the volatile fraction of these products, using gas chromatography with olfactometric detection (GC-O) [3, 4].

Second approach is the analysis of the aroma as a whole, without dividing it into individual aromatic compounds. This approach involves analytical methods applying sensors known as ‘electronic noses’ or ‘artificial noses’, which because of their simple construction and short time of analysis have become very popular lately.

Gas chromatography-olfactometry (GC-O)

GC-O is a technique based on sensory evaluation of the eluate from the chromatographic column by trained assessor or group of assessors. Quantitative and qualitative odour evaluation is possible thanks to the presence of a specially constructed attachment, called olfactometric port (Fig. 1).

Olfactogram appearance is highly dependent on the analyte isolation procedure and the quantitative method used. Identification of aroma active compounds is possible on the basis of simultaneous use of second detector. Mostly, second detector function performs mass spectrometer (MS) or flame-ionization detector (FID).

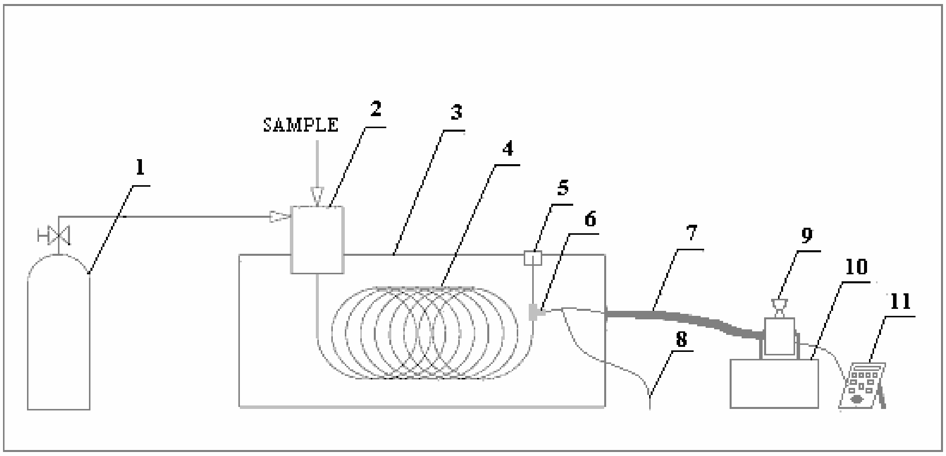

Fig. 1. Scheme of gas chromatograph coupled with olfactometric detector: 1 - carrier gas, 2 - injector, 3 - gas chromatograph, 4 - capillary column, 5 - detector, 6 - splitter of column flow, 7- heated transfer line, 8 - humified air, 9 - olfactometric port, 10 - controller of temperature and gas pressure, 11 - signal generator

Gas chromatography-olfactometry is mostly utilize to intensity evaluation, duration and description of odorous stimulus as well as determination which compounds are responsible for shaping unique aroma or taints of many food products [5]. Development in

GC-O technique results in devising few quantity methods. Each of those methods is based on different principles of the analysis. Three principles can be distinguished: aroma intensity perception, proportion of aroma concentration to detection threshold and the amount of the assessors who detected an odour [6]. Figure 2 presents three categories of GC-O quantitative methods.

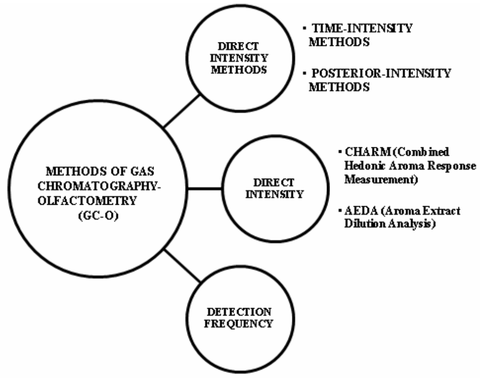

Fig. 2. Quantitative methods in gas chromatography-olfactometry

Although gas chromatography-olfactometry exists over 40 years, the new applications still emerge (Tab. 1). Even samples with complicated matrix can be analyzed by this technique.

Table 1

The examples of GC-O application to food products analysis

Food product	Purpose of the analysis	References
raw spirits	discrimination of different organoleptic quality raw spirits	[7]
honey	identification of distinctive aroma compound in different types of honey	[8]
cognac	quality control of the product on the basic of discrepancies in its volatile fraction	[9]
tarhana	influence of drying methods on active aroma compounds	[10]
olive oil	effect of extraction conditions on virgin oil sensory quality	[11]
champagne	effect of antioxidants on champagne flavour characteristics	[12]
banana	changes in aromatic compounds of banana during ripening	[13]
ham	characterizing of most aroma active compounds	[14]
beer	identification the hop aromatic compounds	[15]
chocolate	identification of key odorant compounds	[16]
Liquor Yanghe Daqu	identification of aroma compounds in young and aged liquors	[17]

Identification of volatile compounds in GC-O coupled with GC-MS analysis can be conducted as follows. As an example can be raw spirits and honeys analysis. In those cases identification of spirits and honey volatiles was made by comparison mass spectra with data in NIST Mass Spectral Database. Additionally, flavour compounds detected by sensory-panel were registered in the form of olfactograms by fingerspan method. Volatile flavour compounds were identified after comparison of their retention times from olfactogram with mass spectra. The predicted aroma of compound was additionally confirmed by literature data and standard substances [18].

Electronic nose (e-nose)

Electronic nose is a device consisting of a set (matrix) of electrochemical sensors, selective for certain volatile compounds. The instrument is equipped with appropriate system of pattern recognition capable of identification of simple and complex odours (Fig. 3) [19].

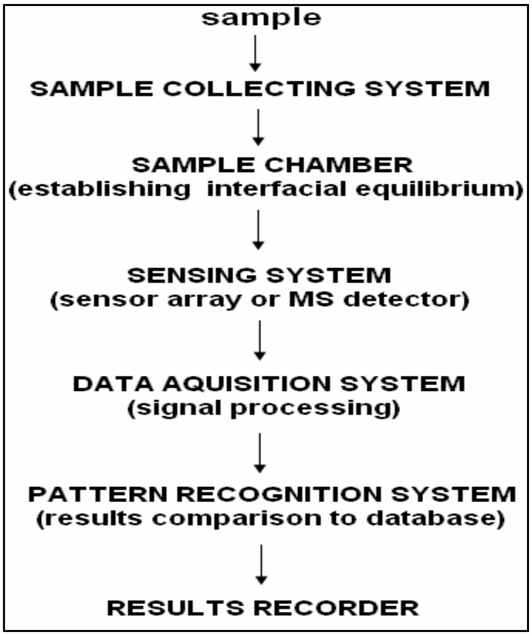

Fig. 3. Electronic nose construction scheme

Since ‘artificial noses’ [19, 20] are cheap and the time of analysis is short, they have become an alternative for relatively expensive methods of food quality assessment [20] such as gas chromatography-olfactometry, infrared spectroscopy or classic sensor analysis. The latter is still the mostly used technique in food analytics and the decisive factor in quality assessment of food products and semi products. However, sensor analysis is characterized by many limitations caused by presence of human factor (eg. olfactory sensitivity of the assessor, their self-well-being, physical and mental state, tiredness or certain anosmia), low repeatability and reproducibility of results, lack of possibilities to identify compounds which influence the aroma and taste or to perform quantitative analysis of them.

Quantitative analysis of odour may be an additional source of valuable information about quality of examined sample. Electronic nose, which creates such possibilities, can be treated as a new type sensor converting chemical signal (odour) into electrical signal. Odour, as well as colour is an analytical signal. However, the quantitative analysis of odour for many years could not have been conducted because of a lack of suitable equipment - sensors.

For the odour of a tested product responsible are not only single aromatic compounds (eg benzaldehyde is responsible for the aroma of bitter almonds), but also their complex mixtures. They give a resultant aromatic effect which is present eg in perfumes. On that account, it is necessary to employ a matrix of gas sensors (the examples of the most frequently utilized are presented in Table 1) [21]. Sensor matrix is built of at least few sensors, each of which is selective towards one different substance and generates appropriate signal, which does not overlap with other signals. The sensors are analogues of protein receptors in nasal epithelium. The electronic system which transfers signal from a sensor works like sensory neurons and Artificial Neural Networks (ANN) constitute its main part [22]. Microprocessor or computer play a role of a brain. There is a resultant signal from all the sensors collected in the computer and an electronic pattern of the odour is created. Similarly to human brain, the pattern is compared with computer database of odours, or more precisely, their chemical structure [19, 22]. Thanks to the pattern recognition system, the analysis can be conducted [23].

To sum up, electronic and human noses function similarly in spite of the differences in the way that signals about odorant are received. Functioning of biological olfactory cells is based on the phenomenon of depolarization of their epithelium (principle of the sodium-potassium pump). Sensors employed in e-nose mainly use the changes in resistance in the active material layer and other physical and chemo-physical phenomena [19, 21].

Electronic nose systems were designed to be used in many branches of industry, where aroma play significant role. Especially, food industry is the biggest and most promising market for these systems [19, 20, 24]. Applications of e-nose in the food analysis include mainly: quality control of raw and manufactured products, monitoring of process, freshness and maturity, shelf-life investigations, authenticity assessment of products and checking packaging for poisonous compounds containment (Fig. 4) [19, 24-26].

Table 2

Characterization of e-nose sensors [27]

Type of sensor	Principle of operation	Sensitivity	Advantages	Disadvantages
MO	Conductivity	5÷500 ppm	Inexpensive, microfabricated, fast response and recovery times	Operates at high temperature, sulphur poisoning, not many coatings available
CP	Conductivity	0.1÷100 ppm	Operates at room temp., microfabricated	Very sensitive to humidity
QCM	Piezoelectricity	1.0 ng mass change	Good reproducibility	MEMS fabricated, complex circuitry, poor signal-to-noise ratio
SAW	Piezoelectricity	1.0 ng mass change	Diverse range of coatings, high sensitivity	Interface electronics, difficult to reproduce
MOSFET	Capacitive charge coupling	ppm	Small, low cost sensors, integratable	Baseline drift
Optical	Fluorescence, Chemiluminescence	Low ppb	High electrical noise immunity	Restricted availability of light sources
MS	Atomic mass spectrum	Low ppb	Potential analytical accuracy	Sample concentration required
MO - Metal Oxide, CP - Conductive Polymer, QCM - Quartz Crystal Microbalance, SAW - Surface Acoustic Wave, MOSFET - Metal-Oxide Semiconductor Field-Effect Transistor, MS - Mass Spectrometry				

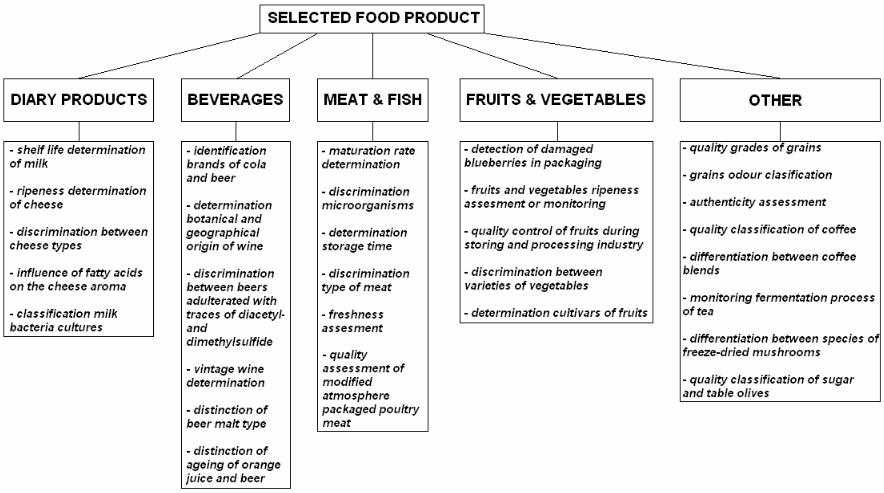

Fig. 4. Practical applications of electronic nose in food industry

Conclusions

Both presented techniques are better than classical organoleptic analyses which are widely applied in the industry. However, more promising seems to be e-nose. It is due to the following features: short time of analysis, high sensitivity and reproducibility, objective identification of odours, possible in situ analysis (portable devise). E-nose system has also a few disadvantages, like ageing material of sensors, possibility of sensors poisoning, in some cases humidity influence to sensor responses.

Nowadays, GC-O and e-nose are additionally applied or even replace methods so far used in the industrial laboratories. Essential feature of those techniques is the fact that such analysis give more detailed information about food products quality (human nose is even more sensitive than conventional detectors). The modern approaches of such type of analysis can point out key aroma compounds shaping flavour of the product and may help in technology improvement. The use of these methods also allow for discrimination raw material used in the production.

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INSTRUMENTALNE TECHNIKI OCENY JAKOŚCI ŻYWNOŚCI

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Abstrakt: Scharakteryzowano dwie techniki instrumentalne stosowane w ocenie jakości produktów spożywczych, tj. chromatografię gazową w połączeniu z detekcją olfaktometryczną oraz nos elektroniczny. Opisano zasady działania obu technik oraz typowe procedury analityczne stosowane w ocenie zapachu. Ponadto podano przykłady praktycznego zastosowania obu technik, włącznie z tymi, które wykorzystano w pracowni autorów.

Słowa kluczowe: chromatografia gazowa-olfaktometria, nos elektroniczny, związki lotne, zapach, jakość

Papers

Artykuły

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Małgorzata DESKA[1]

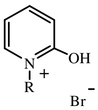
QUATERNARY AZAAROMATICS AS BIOLOGICALLY ACTIVE COMPOUNDS AND AS SPECIES BINDING NUCLEIC ACIDS

CZWARTORZĘDOWE ZWIĄZKI AZAAROMATYCZNE O WŁAŚCIWOŚCIACH BIOLOGICZNYCH ORAZ ZDOLNOŚCI WIĄZANIA KWASÓW NUKLEINOWYCH

Abstract: Quaternary azaaromatics are biologically active species showing antibacterial, antidiabetic and antitumor properties; some of them are used in treatment of osteoporosis and dermatological disorders. Due to binding of nucleic acids, the quaternary azaaromatics are promising in the investigation of biological processes. It should be mentioned that quaternary azaaromatics are also building blocks for sensors and optoelectronic devices; a growing attention is paid today to their application as components of supramolecular systems serving as molecular switches and machines. The work, besides the presentation of biologically active quaternary azaaromatics and corresponding sensors concerns the thermal analysis of quaternary salts of diazaphenanthrenes (daps): three isomeric quaternary salts of 1,5-, 1,6- and 4,6-daps with 2-bromoethanol (**14–16**) and one quaternary salt of 1,6-dap with 1,6-dibromohexane (**17**). The simultaneous thermal analysis (TG/DTG/DSC) of **14–17** was performed in the 20÷500°C temperature range. It was established that the thermal stability of **14–17** increases in the order **17** < **15** < **14** < **16**.

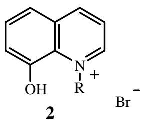
Keywords: biological activity, diazaphenanthrenes, quaternary azaaromatics, thermal analysis

In the first part of the article selected examples of quaternary azaaromatics are presented in view of their biological activities and ability for binding nucleic acids, along with corresponding sensors; in the second part the results of thermal analysis of quaternary salts of diazaphenanthrenes are described.



1

R=$C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$



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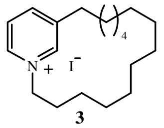

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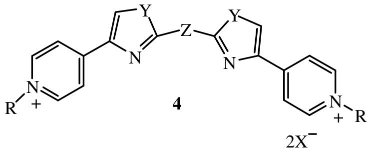
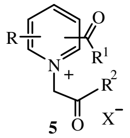


4

R = alkyl; Y = O, S

Z = bond, -CH=CH- , $-C_6H_4-$

X^- = Cl^-, NO_3^- , AcO^-, $EtCOO^-$



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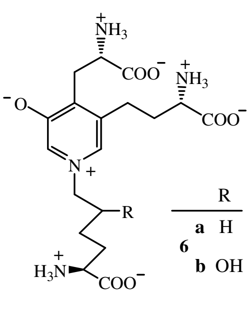
R = F, Cl, Br, SH

R^1= alkyl, aryl,

R^2= H, alkyl, aryl

X^-= Cl^- , Br^-, AcO^-



6	R
a	H
b	OH

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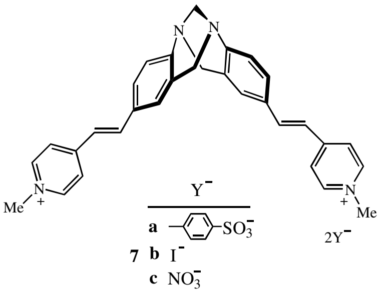
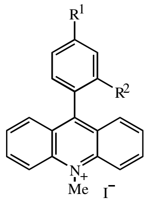
Quaternary azaaromatics show various biological activities and are used in investigation of physiological processes. As examples of species with antibacterial and antifungal activities may serve amphiphilic hydroxypyridinium and hydroxyquinolinium bromides **1** and **2**, respectively [1]; moreover the antibacterial and cytotoxic properties have been found in pyridinium alkaloid **3** [2]. It was established that compounds **4** show antitumor activity [3], pyridinium salts **5** are useful in diabetic and dermatological disorders [4] and compounds **6** in the treatment of osteoporosis [5].

Among applications of quaternary azaaromatics one should mention the use of lambda (Λ) shaped pyridinium salts **7** for quantitative protein assay; they show the aggregation-induced emission (AIE) behavior, which is nonemissive in solution, but strongly luminescent in the aggregate state [6]. The utilization of AIE effect for protein detection and quantification was investigated using bovine serum albumin (BSA) as a model protein. These bioprobes enable the determination of BSA by fluorimetric method. It should be pointed out that the use of **7** allows the detection of trace amounts of proteins.

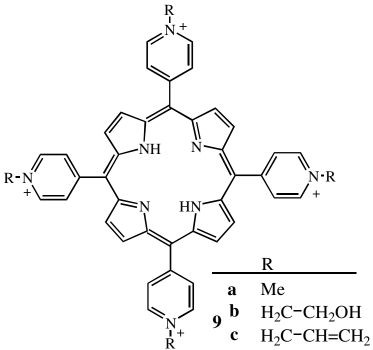
It was established that acridinium iodides **8** interact with single strand (ss) DNA, and not with double strand (ds) DNA. Due to this ability they may be used as fluorescent probes in biological processes [7].



7



8		R¹	R²
	a	Me	H
	b	Me	Me



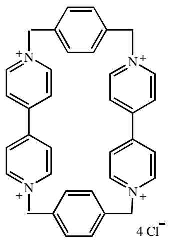
9		R
	a	Me
	b	H_2C-CH_2OH
	c	$H_2C-CH=CH_2$
	d	$H_2C-C(Me)=CH_2$

A special class of quaternary azaaromatics are porphyrins substituted in *meso* positions by pyridinium units; their interaction with DNA is important for medical applications, including photodynamic therapy and virus inhibition [8]. It was found that the characterization of ss-DNA may be performed with the use of **9a** which is the G-quadruplex binding agent [9]. The study of interaction of **9b–d** with RNA duplexes has shown that **9b** and **9c** act as intercalators, whereas **9d** forms outside-bound aggregates [10].

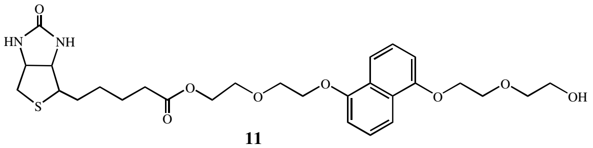
Supramolecular systems containing quaternary azaaromatics units are now a topic of an intense research, due to their promising properties as building blocks for molecular switches and machines. As an example of such supramolecular systems used in investigations of biological processes may serve the pseudorotaxane formed between the biotin-avidin bioconjugate and tetracationic cyclophane **10** [11, 12]. This behavior is promising for a number of biological applications including drug delivery and clinical diagnostics.

It was established that the biotinylated naphthalene derivative **11** strongly binds to avidin. Compound **11** contains the electron-donor naphthalene moiety, able to bind as a guest with the electron-acceptor host **10** to give a pseudorotaxane. The mixing of **10** and **11** in D_2O/ EtOH-d_6 solvent results in the appearance of the purple color.

The complex of **10** with **11** may be disrupted by tetrathiafulvalene **12** as a competing guest for **10**. This changing of guests is accompanied with purple to green color change, allowing a convenient monitoring of the process by UV-Vis spectroscopy. The above study allows preparation of the tuneable biotin-avidin bioconjugates useful for diagnostic applications and is promising for development of other tuneable protein-pseudorotaxane systems.



10



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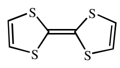

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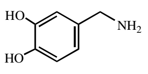
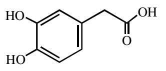
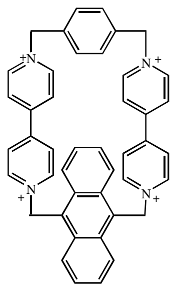


DA



DOPAC



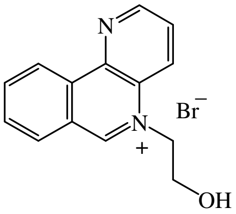
13

One should mention also the development of the indirect method for dopamine (DA) determination [13]. The DA determination attracts now a growing attention because of the great importance of this compound in biological processes. The electrochemical measurement of DA is difficult due to its low concentration in the central nervous system.

It was found that the DA determination may be performed indirectly by measurement of 3,4-dihydroxyphenylacetic acid (DOPAC), which is the main product of the enzymatic degradation of DA. In this method the electrochemical sensor for determination of DOPAC may be used. It consists of tetracationic cyclophane **13** adsorbed on single-walled carbon nanotubes (SWNTs), with **13** as the recognition element binding DOPAC by electrostatic interaction, and SWNTs serving as the electronic transducer. This sensor has high selectivity for DOPAC and is promising for applications in physiological investigations.

In the study of quaternary salts of diazaphenanthrenes (dap) of an antibacterial and antifungal activity, which are research topic of our Laboratory [14-19], the thermal analysis of four examples of these compounds has been performed. Three isomeric quaternary salts of 1,5-, 1,6- and 4,6-daps with 2-bromoethanol (**14–16**) and one quaternary salt of 1,6-dap with 1,6-dibromohexane (**17**) have been investigated by simultaneous thermal analysis (TG/DTG/DSC). The experiments were carried out on the NETSCH Simultaneous Thermal Analyzer STA 409C with the use of corundum nonhermetic crucibles in the 20÷500°C temperature range.

Compounds **14–17** have been obtained by quaternization reactions of the isomeric 1,5-, 1,6- and 4,6-daps with 2-bromoethanol in acetonitrile affording **14–16** and of 1,6-dap with 1,6-dibromohexane in benzene yielding **17** [20].



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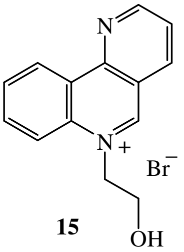

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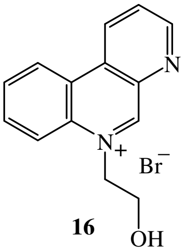
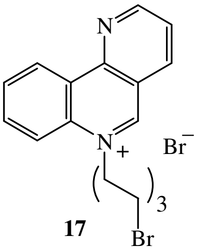


16



17

The above analysis allows to determine the thermal stability of considered compounds, which to some extent is connected with their melting points. The results are summarized in Table 1. For **15–17**, the first weight loss, observed on the TG curve is due to the moisture loss. The thermal stability of **14–17** increases in the order **17**< **15** < **14** < **16**, which is connected with the second weight loss, observed on the TG curve. The used apparatus was not coupled with gas analyzer, therefore the estimation of volatile products, formed during decomposition of investigated compounds was difficult, however on the basis of the weight balance it can be assumed that they are mainly nitrogen oxides and carbon oxides.

Table 1

Results of thermal analysis of 14–17

Compound	**TG**			**DTG**	**DSC**		
	T_i [°C]	**T_c [°C]**	**Δm [%]**	**T_p [°C]**	**T_o [°C]**	**T_p [°C]**	**ΔH [J/g]**
14	186.3	238.5	10.70	224.5	185.2	200.0 (endo)	59.04
	238.5	367.4	64.52	283.8	208.8	223.2 (exo)	–159.3
					258.2	292.1 (endo)	181.1
15	35.0	102.3	3.01	65.6	39.1	68.3 (endo)	
	161.8	252.1	9.15	226.3	175.6	189.9 (endo)	110.0
					204.0	225.6 (exo)	–116.4
	252.1	326.7	8.43	293.6			
	326.7	448.4	17.64	396.5			
16	40.1	89.9	1.21	64.2			
	191.7	264.2	9.55	226.6	202.7	228.8 (exo)	
	264.2	291.5	2.34	278.7			
	291.5	352.6	5.74	315.6			
	352.6	438.0	10.77	408.6			
17	51.0	78.2	1.36	65,2			
	96.6	161.0	5.40	131.5	123.7	142.0 (endo)	73.8
	161.0	234.6	15.82	206.7	156.2	171.1 (exo)	–103.8
	234.6	356.5	9.01				
	356.5	442.6	7.87	397.6			

T_i - temperature of the start of weight loss
T_c - temperature of the end of weight loss
T_p - peak temperature
T_o - extrapolated temperature of the peak start
Δm - weight loss
ΔH - enthalpy change
endo - endothermic transformation
exo - exothermic transformation

Compound 14

On the DSC curve of **14** at temperature T_o = 185.2°C the endothermic peak, connected with the weight loss Δm = 10.7% is observed. This peak is assigned to melting process of **14**. At temperature T_o = 208.8°C the peak, connected with the highest weight loss among those of **14–17** appears. Presumably during this transformation the intermediate products are formed, at the considerable participation of the air oxygen; subsequently they are decomposed during the endothermic process, at temperature T_o = 258.2°C. This transformation is accompanied by a large enthalpy change (ΔH = 181.1 J/g). During heating of **14** to 500°C, the total weight loss equal to 87% was observed.

Compound 15

The DSC curve of **15** at temperature T_o = 175.6°C shows the start of the endothermic transformation, with the enthalpy change ΔH = 110.0 J/g, accompanied by the weight loss Δm equal to 9.15%; in the 204.0÷259.2°C temperature range the exothermic transformation connected with the enthalpy change ΔH = –116.4 J/g occurs. The heating of **15** is accompanied by the total weight loss equal to 57%.

Compound 16

On the DSC curve of **16**, which is the most thermally stable among considered compounds, the transformation of the second range was observed; it is the so called "vitrification effect" not connected with heat emission or absorption. Starting from temperature T_o = 191.7°C, on the TG curve the gradual weight loss achieving 38%, is found.

Compound 17

On the DSC curve of **17**, which is the least thermally stable among **14–17**, at temperature T_o = 123.7°C the endothermic peak, connected with the enthalpy change ΔH = 73.8 J/g appears. At the temperature T_o = 156.2°C the exothermic transformation begins; its ΔH is equal to –103.8 J/g. The shape of the DSC curve (plateau) in the temperature range 207.1÷443.3°C indicates that the processes occur without the participation of the air oxygen. The heating of **17** is accompanied by the total weight loss equal to ca 44%.

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CZWARTORZĘDOWE ZWIĄZKI AZAAROMATYCZNE O WŁAŚCIWOŚCIACH BIOLOGICZNYCH ORAZ ZDOLNOŚCI WIĄZANIA KWASÓW NUKLEINOWYCH

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Abstrakt: Czwartorzędowe związki azaaromatyczne są układami biologicznie aktywnymi, wykazującymi właściwości antybakteryjne, antydiabetyczne i przeciwnowotworowe, niektóre z nich są stosowane w leczeniu osteoporozy i schorzeń dermatologicznych. Czwartorzędowe związki azaaromatyczne wiążą kwasy nukleinowe, co umożliwia badanie procesów biologicznych. Ponadto należy zwrócić uwagę na fakt, że czwartorzędowe związki azaaromatyczne są obiecujące w budowie czujników i urządzeń optoelektronicznych; obecnie ukazuje się wiele prac dotyczących ich zastosowania w układach supramolekularnych jako przełączników i maszyn molekularnych. Prezentowana praca, oprócz omówienia biologicznie czynnych czwartorzędowych związków azaaromatycznych i odpowiednich czujników, dotyczy analizy termicznej czwartorzędowych soli diazafenantrenów (daf): trzech izomerycznych soli 1,5-, 1,6- i 4,6-daf z 2-bromoetanolem **14–16** oraz jednej czwartorzędowej soli 1,6-daf z 1,6-dibromoheksanem **17**. Równoczesna analiza termiczna (TG/DTG/DSC) została przeprowadzona w zakresie temperatur 20÷500°C. Ustalono, że termiczna trwałość rozważanych związków wzrasta w porządku **17 <15 < 14 < 16**.

Słowa kluczowe: aktywność biologiczna, analiza termiczna, czwartorzędowe związki azaaromatyczne, diazafenantreny

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Iwona DESKA[1] and Anna SZEWCZYK[1]

INFLUENCE OF THE HYDRAULIC CONDUCTIVITY OF SOIL ON THE DIFFERENCE BETWEEN APPARENT AND ACTUAL THICKNESS OF LNAPL ON THE GROUNDWATER TABLE

WPŁYW WSPÓŁCZYNNIKA FILTRACJI GRUNTU NA RÓŻNICĘ MIĘDZY MIĄŻSZOŚCIĄ POZORNĄ I RZECZYWISTĄ LNAPL NA ZWIERCIADLE WODY PODZIEMNEJ

Abstract: The actual thickness of lighter-than-water non-aqueous phase liquid (LNAPL) on the groundwater table is always different from the apparent thickness (measured in the monitoring well). There are several methods developed for estimating the actual LNAPL thickness on the base of the apparent thickness, but the results obtained with different formulas are inconsistent and (in many cases) very imprecise. The obtained results of laboratory investigations indicate that the appropriate model for estimating the actual thickness of light non-aqueous phase liquid should include the properties of soil and LNAPL. The investigations confirmed that the hydraulic conductivity is very important parameter in the case of homogeneous soils. On the base of the results the empirical model was developed. This model includes the hydraulic conductivity of soil and the density and dynamical viscosity of LNAPL. The results of the verification of developed model indicate that the calculated values corresponded in many cases with the values obtained during laboratory investigations.

Keywords: LNAPL, actual thickness, apparent thickness, hydraulic conductivity, empirical model

The principal sources of soil and groundwater contamination with lighter-than-water non-aqueous phase liquids (LNAPL) are surface spills from cisterns and leakages from underground storage tanks and pipelines. If the layer of LNAPL floats on the groundwater table the initial remediation step should be its recovery [1-3]. A proper design of recovery requires an assessment of the contamination plume volume on the base of the LNAPL thickness [1]. Unfortunately, the thickness of LNAPL on the groundwater table (the actual thickness) is different from the thickness observed in the well (the apparent thickness) [4-6] and this difference depends on the properties of soil, and the properties and amounts of the free product floating on the groundwater table [7-9]. The results obtained with different formulas for estimating the actual thickness are inconsistent, and in many cases inaccurate [4, 5, 10]. The results of laboratory investigations indicate that the appropriate model for estimating the actual LNAPL thickness should include the properties of both: soil and LNAPL [9, 11]. The paper presents the developed empirical model for estimating the actual LNAPL thickness on the groundwater table. The model is based on the properties of soil (hydraulic conductivity) and properties of LNAPL (dynamical viscosity).

Materials and methods

Laboratory experiments were conducted in Plexiglas columns with filter-tubes as monitoring wells [7-9] with use of six model soils and six types of LNAPL. The properties of soils and LNAPLs are given in Tables 1 and 2.

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The experimental columns were packed with the soil samples (soils 1-6) and filled with tap water until the water table reached the assumed elevation. After 3-4 days 50 cm^3 of diverse LNAPLs, coloured with the pigment - Sudan III, were injected above the capillary fringe zone. After subsequent 3-4 days, the apparent and actual LNAPL thicknesses were measured in the well and in soil. This procedure was repeated at least 10 times. The actual thickness was the distance between LNAPL-water interface in the soil and air-LNAPL interface in the well (without the capillary fringe of LNAPL in the soil). The columns were hydraulically connected with equalizing columns which aim was to keep the water table constant during experiments. The styrofoam covers protected the top of columns.

Table 1

Properties of soils used in the experiments

Soil	Type of soil	Soil grain size [mm]	Medium soil grain size [mm]	Hydraulic conductivity at 10°C k [m/d]
1	fine sand	0.1÷0.25	0.175	11.33 ± 0.07
2	medium sand	0.25÷0.315	0.2825	23.255 ± 0.426
3	medium sand	0.315÷0.5	0.4075	71.334 ± 1.640
4	coarse sand	0.5÷0.63	0.565	107.285
5	coarse sand	0.63÷0.8	0.715	154.627
6	coarse sand	0.8÷1.0	0.9	231.884

Table 2

Properties of LNAPLs used in the experiments (at temperature of 20°C)

LNAPL	Notation	Type of LNAPL	Density ρ_o [kg/m^3]	Dynamic viscosity η_o [kg/m·s]
LNAPL 1	L1	Petroleum	820.5	$1.804\cdot10^{-3} \pm 1.42\cdot10^{-6}$
LNAPL 2	L2	Rape oil	918	$0.0718 \pm 5.94\cdot10^{-5}$
LNAPL 3	L3	Mineral oil “Lotos”	880	$0.3 \pm 2.54\cdot10^{-4}$
LNAPL 4	L4	Semi-synthetic oil “Orlen”	872	$0.219 \pm 2.52\cdot10^{-4}$
LNAPL 5	L5	Synthetic oil “Lotos”	855	0.181
LNAPL 6	L6	Synthetic oil “Orlen”	871	$0.194 \pm 2.51\cdot10^{-4}$

Results and discussion

On the base of the results the graphs were plotted which show the influence of the hydraulic conductivity of soil (k) on the difference between apparent and actual thicknesses ($H_0 - H_f$) for three selected actual thicknesses: 5, 10 and 15 cm. Figure 1 shows only the graphs plotted for two actual thicknesses: 5 and 15 cm. The results indicate that relationship between hydraulic conductivity and the difference between thicknesses has the logarithmic character. It can be confirmed by the high values of the determination coefficients.

On the base of the results of laboratory investigations the empirical model was developed that describes the relationship between apparent and actual LNAPL thicknesses (Equation 1). Model is based on the hydraulic conductivity and the dynamic viscosity of LNAPL. The actual LNAPL thickness can be derived from equation:

$$H_f = H_0 - (\omega\eta_o + \varphi)\ln k - \chi\eta_o - \xi \qquad (1)$$

where: H_f - actual LNAPL thickness [cm], H_0 - apparent LNAPL thickness [cm], k - hydraulic conductivity in 10°C [m/d], η_o - dynamic viscosity of LNAPL [$kg\cdot m^{-1}\cdot s^{-1}$], ω, φ, χ and ξ - factors depending on actual LNAPL thickness [-].

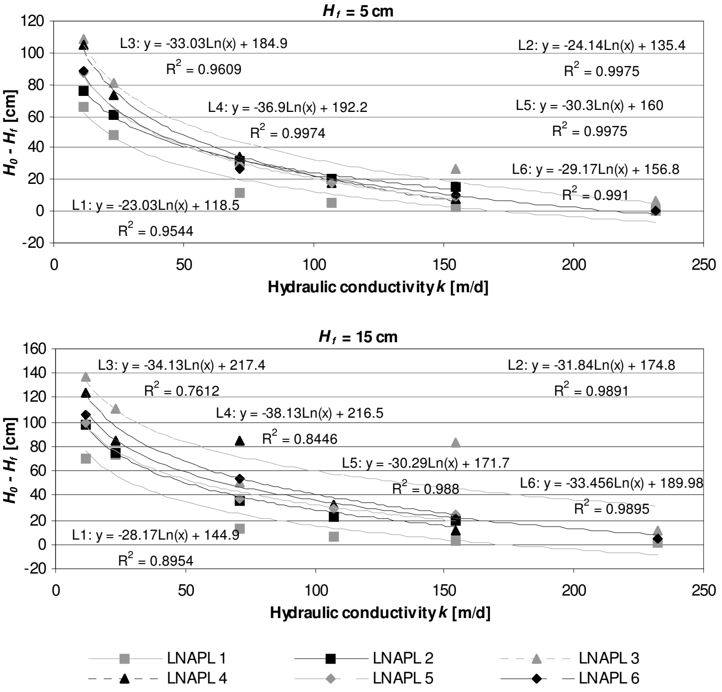

Fig. 1. The influence of the hydraulic conductivity on the difference between apparent and actual thicknesses

Factors ω, φ, χ and ξ can be calculated from the equations:

$$\omega = 1.989H_{fe} - 52.006 \tag{2}$$

$$\varphi = -0.6445H_{fe} - 19.457 \tag{3}$$

$$\chi = -0.878H_{fe} + 247.29 \tag{4}$$

$$\xi = 2.932H_{fe} + 104.29 \tag{5}$$

where H_{fe} - initial estimated actual LNAPL thickness [cm].

Use of proposed model requires initial estimation of actual thickness (H_{fe}) to determine the proper values of factors ω, φ, χ and ξ. The calculation should be repeated until the calculated actual thickness is equal to the initial estimated value.

Figure 2 presents the verification of the model. The relationships between apparent and actual thicknesses obtained from the model (the curves) were compared with the experimental data (point graphs). The results indicate that the values calculated from the developed model corresponded in many cases to the experimental data. In the case of soil 1 the best results were obtained for LNAPLs 1, 2 and 6. In the case of soil 2 the model curves are similar to the experimental graphs for all LNAPLs. The best fits are also reached for the compositions: soil 3 - LNAPL 4; soil 4 - LNAPLs 2, 4, 5; soil 5 - LNAPLs 1, 5, 6 and for soil 6 - LNAPL 6. Only for a few compositions of soils and LNAPLs the results derived from the developed model differ from the experimental data. The most unfavorable results were obtained for the compositions: LNAPL 1 - soils 3, 4, 6 and LNAPL 4 - soil 5. The relationships between apparent and actual thicknesses drawn on the base of the proposed model have the linear character. Further studies aimed at the improvement of the model should include the check of the importance of the hydraulic conductivity in the case of the heterogeneous soils. The experiments should also include the examination of the influence of other parameters eg the equivalent diameter and the coefficient of uniformity.

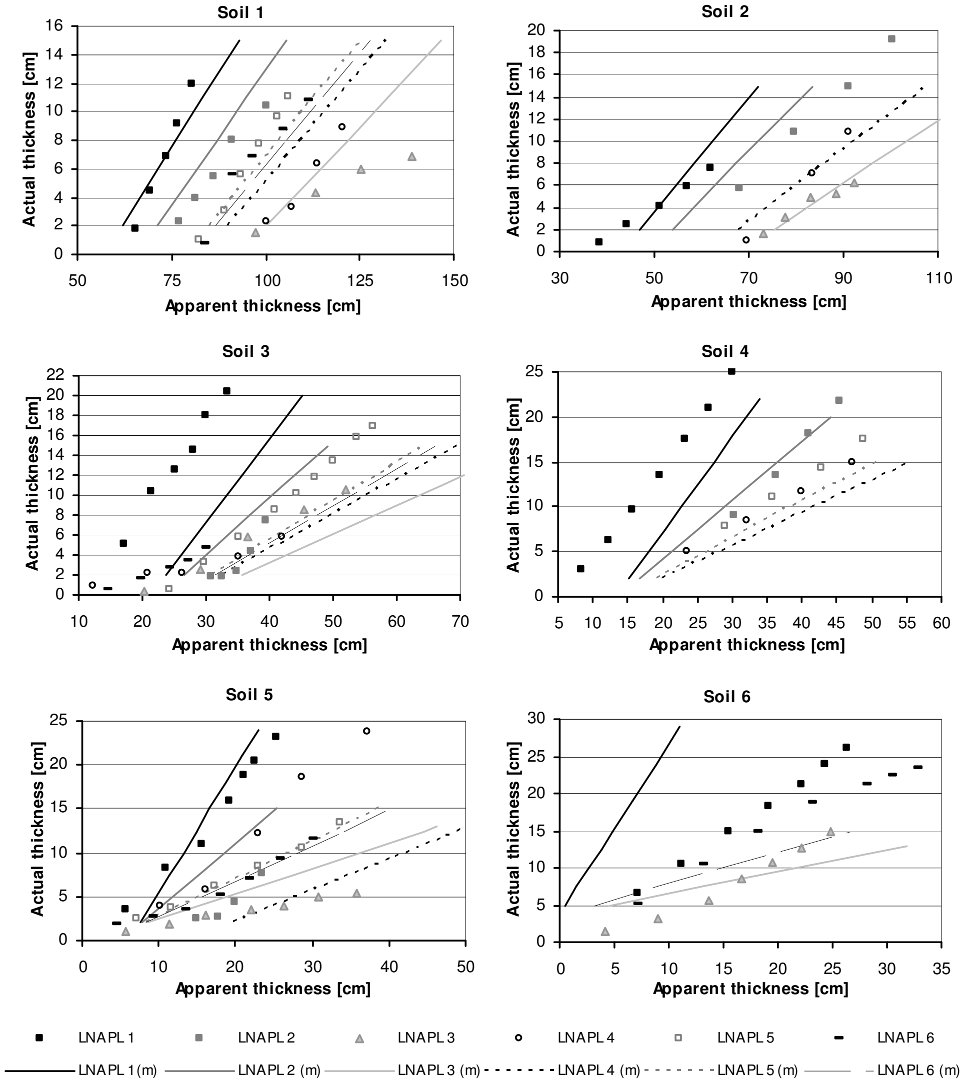

Fig. 2. The verification of the developed model on the base of laboratory investigations

Conclusions

1. The difference between apparent and actual thicknesses increases with the decrease of the hydraulic conductivity of soil. This relationship has a logarithmic character.
2. The appropriate model for estimating the actual LNAPL thickness on the groundwater table should include the properties of both: soil and LNAPL.
3. The values of actual LNAPL thickness calculated from the developed model are in many cases consistent with the results of the laboratory investigations. The best results were obtained in the case of the fine-grained soils.
4. Further studies aimed at the improvement of the proposed empirical model should include the laboratory experiments with the use of heterogeneous soils to check the importance of the hydraulic conductivity in this case and to study the influence of other parameters of soils eg the equivalent diameter and the coefficient of uniformity.

Acknowledgements

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WPŁYW WSPÓŁCZYNNIKA FILTRACJI GRUNTU NA RÓŻNICĘ MIĘDZY MIĄŻSZOŚCIĄ POZORNĄ I RZECZYWISTĄ LNAPL NA ZWIERCIADLE WODY PODZIEMNEJ

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Abstrakt: Rzeczywista miąższość lekkiej cieczy organicznej (LNAPL) na zwierciadle wody podziemnej zawsze różni się od miąższości zmierzonej w studni (tzw. miąższości pozornej), a różnica między nimi jest zależna od właściwości gruntu i LNAPL oraz od ilości cieczy organicznej na zwierciadle wody. Metody stosowane obecnie do ustalania rzeczywistej miąższości LNAPL na podstawie zmierzonej miąższości pozornej pozwalają na uzyskiwanie wyników bardzo rozbieżnych i w większości przypadków nieprecyzyjnych. Metody te są bardzo uproszczone, uwzględniają zbyt małą liczbę parametrów (jedynie właściwości gruntów lub jedynie właściwości LNAPL). Poza tym poprawne ustalenie wartości niektórych parametrów uwzględnionych w metodach jest bardzo trudne zarówno w warunkach laboratoryjnych, jak i terenowych. Na podstawie uzyskanych wyników badań laboratoryjnych ustalono, że poprawnie opracowany model obliczania rzeczywistej miąższości LNAPL powinien uwzględniać zarówno właściwości gruntu, jak i LNAPL. Ustalono, że w przypadku gruntów jednorodnych bardzo równomiernie uziarnionych jednym z ważniejszych parametrów jest współczynnik filtracji. Na podstawie analizy kluczowych parametrów wpływających na zależność między miąższością pozorną i rzeczywistą opracowano model empiryczny uwzględniający współczynnik filtracji gruntu oraz współczynnik lepkości dynamicznej LNAPL. Weryfikacja modelu potwierdziła, że w większości przypadków jego zastosowanie pozwoliło na uzyskanie wyników zbliżonych do ustalonych w warunkach laboratoryjnych.

Słowa kluczowe: LNAPL, miąższość rzeczywista, miąższość pozorna, współczynnik filtracji, model empiryczny

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KINETICS STUDIES OF THE BIODEGRADATION OF VOLATILE ORGANIC COMPOUNDS IN A BATCH REACTOR

BADANIA KINETYKI REAKCJI BIODEGRADACJI LOTNYCH ZWIĄZKÓW ORGANICZNYCH W REAKTORZE OKRESOWYM

Abstract: The aim of this work is to present Volatile Organic Compounds degradation by microorganisms. Vinyl acetate was utilized by laboratory strain *Pseudomonas fluorescens* PCM 2123 or environmental strain EC3_2001, identified as *Pseudomonas putida*. Styrene was decomposed by bacteria from genus *Pseudomonas*, described as E-93486. The experiments were conducted for different initial concentration of vital substrate in a batch reactor. During research influence of organic compound concentration on microorganisms' growth was studied.

Keywords: biodegradation, batch culture, growth kinetic

The consequences of civilization progress are changes in our environment, for example the composition of air. Our atmosphere contains gases, solid particles and liquids which can be harmful both to people and living organisms. Natural air pollutants come from forest fires and eruption of volcanoes. Pollutants whose source is human activity are more dangerous. A large group of air pollutants are volatile organic compounds (VOCs). They are any organic chemical compounds having vapor pressures (under normal conditions) more than 0.07 kPa and an initial boiling point less than or equal to 260°C. Harmfulness of emission of these compounds to the atmosphere derives not only from their toxicity for living organisms but also from the fact, that they participate in photochemical reactions. Products of these reactions (ozone, hydrogen peroxide, peroxyacetylnitrate (PAN)) cause photochemical smog to form and they have influence on human health, plants and climate.

There are several different ways to reduce the emission of harmful organic compounds. In recent years many common materials and products used indoors (paints, coatings, cleaning solvents, wood preservatives) have been improved and they have low VOCs content. Also some new methods of removal pollutants from exhaust gases have been developed. Some of these base on the possibility of using microorganisms to degrade VOCs. Biological methods are effective, efficient and they do not generate secondary pollutants. Biodegradation is especially positive when gas stream is large, unstable in time and the concentration of volatile organic compounds is not high.

Vinyl acetate and styrene belong to the group of VOCs and they are on the list of hazardous air pollutants. Vinyl acetate is commonly used in industry to produce polyvinyl acetate and other polymers or resins. It is intermediately used in paints, coatings, textiles and acrylic fibers. Breathing its vapor causes symptoms of intoxication.

Styrene is an important monomer which is used for the production of polystyrene or rubber (SBR, SAN). Irritation of the upper respiratory system, reduced time response, weakness, headache, nervousness are observed in persons exposed to styrene.

Both styrene and vinyl acetate can be the source of energy and carbon for the microorganisms and they can be utilized for carbon dioxide and water [1, 2].

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Experiments

A bioreactor Biostat B was used for the batch processes. The reactor was filled with a mineral salts medium, which contained (per 1 dm^3) EDTA-Na (Titriplex) 0.2 g; $MgSO_4 \cdot 7H_2O$ 0.58 g; $CaCl_2 \cdot 2H_2O$ 0.067 g; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.0002 g; $FeSO_4 \cdot 7H_2O$ 0.002 g; $(NH_4)_2SO4$ 1 g; KH_2PO_4 3.4 g; $Na_2HPO_4 \cdot 12H_2O$ 4.5 g. Then, the suspension of biomass was added in the amount ensuring a constants value of cell mass concentration. After inoculation a certain volume of vinyl acetate or styrene was added to the medium directly. The equipment of the bioreactor enables to control and to keep fixed process parameters (pH, oxygen saturation, stirring, temperature). Experiments were conducted at the temperature 30°C, agitation 300 rpm, oxidation 5 $mg \cdot dm^{-3}$ and pH = 7. The pH was maintained by adding either KOH or KH_2PO_4. All experiments were carried out without aeration so, to overcome the oxygen limitation problem, H_2O_2 was used as an additional oxygen source in water.

Liquid samples were periodically withdrawn from the reactor. The experiments were conducted for different initial concentrations of vital substrate in the batch reactor.

Analytical methods

Cell concentrations in samples were estimated by measuring the absorbance A at $\lambda = 550$ nm. The A values were converted to dry cell mass using a calibration curve.

The changes in the substrate and by-products concentration were determined by gas chromatography. Samples were taken from the cultures and directly analyzed by injection of 0.15 mm^3 samples on a Varian 3800 gas chromatograph, equipped with a 30 m length, CP-wax 52 CB column and a flame ionization detector FID. Helium was used as the carrier gas.

Microorganisms

In the research on the vinyl acetate biodegradation, laboratory strain *Pseudomonas fluorescens* PCM 2123 coming from the Polish Collection of Microorganisms (IITD Wroclaw) or bacteria isolated from samples of soil, described as EC3_2001 and identified as *Pseudomonas putida* were used. To isolate bacterial strain able to degrade vinyl acetate the classical enrichment techniques were used [3]. Strains were kept on agar slopes at 4°C. Procedure of activation and adaptation to use organic compound present in medium, was the same for both strains. It is described in detail in [4].

For the microbiological decomposition of styrene bacteria from genus *Pseudomonas*, coming from VTT Culture Collection (Finland) and described as E-93486, were chosen.

Results and discussion

The first part of the present work was focused on vinyl acetate biodegradation. We have attempted to compare the efficiency of microbiological breakdown of vinyl acetate in aerobic conditions by laboratory strain and by environmental strain. Multiplication of adapted bacteria was carried out on 500 cm^3 Erlenmeyer's flasks in standard conditions (temp. 30ºC, shaking 130 rpm, vinyl acetate concentration 400 ppm).

During experiments the influence of organic compound concentration on the growth of microorganisms was studied. Cultures in the batch reactor included the same number of cells in medium but the initial substrate concentration was changed.

In the research on vinyl acetate biodegradation the initial concentration of this compound was changed from 30 to 185 ppm. The smallest dose of substrate was used on maintenance and no biomass growth was observed. Increasing doses of substrate allowed to observe differences in biomass growth. Regular growth was obtained by introducing vinyl acetate to *Pseudomonas fluorescens* culture.

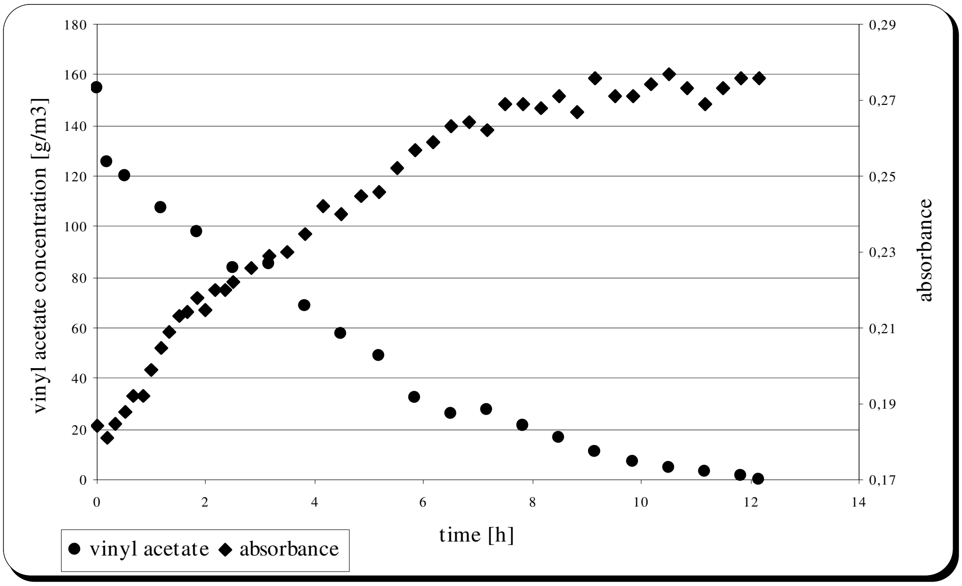

Fig. 1. Changes of the vinyl acetate concentration and absorbance during the aerobic biodegradation by *Pseudomonas fluorescens* in a batch bioreactor for initial substrate concentration 155 ppm

Rys. 1. Zmiany stężenia octanu winylu i absorbancji w czasie tlenowej biodegradacji przez *Pseudomonas fluorescens* w reaktorze okresowym dla początkowego stężenia substratu 155 ppm

In EC3_2001 cultures initial intensive growth of bacteria was stopped after a short time. It is an effect of metabolites appearing in cultures with initial substrate concentration higher than 50 ppm. Hydrolyses of ester bond caused vinyl acetate to decompose into acetic acid and vinyl alcohol. Vinyl alcohol is unstable and, under normal conditions, tautomerizes to acetaldehyde. This compound can be oxidized to acetic acid or reduced to ethanol. In samples from *Pseudomonas fluorescens* cultures acetaldehyde and ethanol were not present. Acetic acid appeared in cultures with initial substrate concentration equal or higher than 124 ppm. The formed acetaldehyde is immediately oxidized to acetic acid.

In the first stage EC3_2001 bacteria only hydrolyzes vinyl acetate and acetaldehyde concentration increases. Now, all energy is used to transform toxin to acetic acid and excess is transformed to ethanol. When concentration of acetaldehyde decreases, alcohol is oxidized to acetaldehyde and farther to acetic acid. These stages of vinyl acetate degradation reflect biomass growth. Initial, intensive increase of the number of cells is inhibited by the appearance toxicological acetaldehyde. When toxin concentration decreases, growth is more intensive but not as fast as earlier.

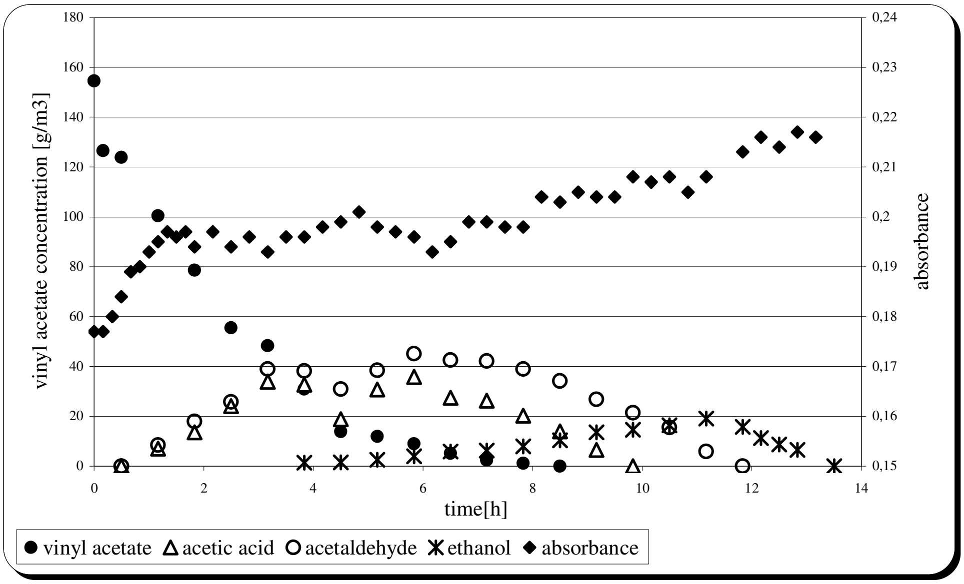

Fig. 2. Changes of the vinyl acetate concentration and absorbance during the aerobic biodegradation by EC3_2001 strain in a batch bioreactor for initial substrate concentration 155 ppm

Rys. 2. Zmiany stężenia octanu winylu i absorbancji w czasie tlenowej biodegradacji przez szczep EC3_2001 w reaktorze okresowym dla początkowego stężenia substratu 155 ppm

Taking into consideration the above *Pseudomonas fluorescens* strain was chosen to farther research on air purification in trickle bed reactor.

Next, styrene biodegradation was studied in the batch reactor. Trials of strain *Pseudomonas fluorescens* adaptation to use this pollutant as energy and carbon source resulted in failure. The adaptation of environmental strain EC3_2001 was not successful, either although this strain was isolated from soil samples collected in the area of chemical company.

In connection with above results, we decided to buy E-93486 strain, coming from VTT Culture Collection (Finland). Activation and adaptation of this strain was conducted according to the instructions from VTT Culture Collection.

Biodegradation of styrene by E-93486 strain was studied in the batch reactor. Initial substrate concentration was changed from 5 to 90 ppm. Introducing styrene to E-93486 strain culture obtained regular growth. A clear lag phase was observed in culture with initial substrate concentration higher than 15 ppm. The metabolism of microorganisms from genus *Pseudomonas,* which are capable of styrene biodegradation, proceeds through styrene oxide. Other metabolites are phenylacetaldehyde and phenylacetate. Only styrene appeared in liquid samples collected from reactor whereas metabolites were not present. Inhibitory influence wasn't noted in the given range of initial styrene concentration.

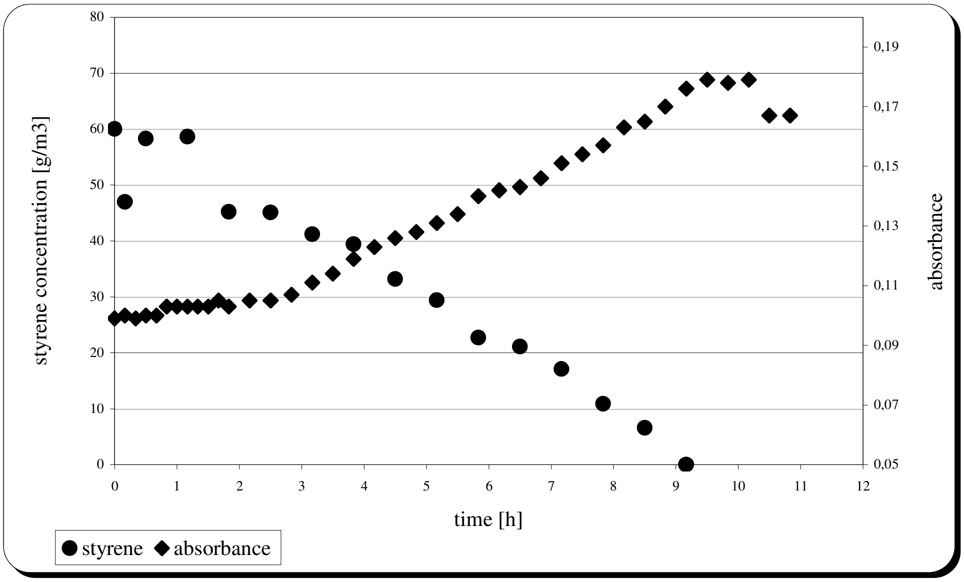

Fig. 3. Changes of the styrene concentration and absorbance during the aerobic biodegradation by E-93486 strain in a batch bioreactor for initial substrate concentration 60 ppm

Rys. 3. Zmiany stężenia styrenu i absorbancji w czasie tlenowej biodegradacji przez szczep E-93486 w reaktorze okresowym dla początkowego stężenia substratu 60 ppm

Conclusions

Vinyl acetate and styrene could be degrade by selected microorganisms.

Growth in the presence of vinyl acetate is not only limited by the initial concentration of substrate, but also by presence of intermediates. Especially acetaldehyde has toxic influence on living organisms.

The adapted bacteria, able to grow in the presence of vinyl acetate, were not able to grow in the presence of styrene as vital substrate. Strain E-93486 degrades styrene well in concentration below 100 ppm.

Acknowlegement

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BADANIA KINETYKI REAKCJI BIODEGRADACJI LOTNYCH ZWIĄZKÓW ORGANICZNYCH W REAKTORZE OKRESOWYM

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Abstrakt: Przedstawiono wyniki badań procesu degradacji lotnych związków organicznych przez mikroorganizmy. Badania dotyczyły rozkładu octanu winylu przez dwa różne szczepy: laboratoryjny *Pseudomonas fluorescens* oraz EC3_2001, zidentyfikowany jako *Pseudomonas putida*. Druga część badań dotyczyła degradacji styrenu przez szczep E-93486. Omówiono wyniki pomiarów prowadzonych w reaktorze okresowym, w którym utrzymywano stałą temperaturę 30°C, pH = 7, prędkość mieszania 300 rpm oraz natlenienie podłoża na poziomie 5 $mg \cdot dm^{-3}$. W czasie trwania hodowli okresowo pobierano próbki płynu hodowlanego w celu określenia aktualnego stężenia substratu i ewentualnie produktów pośrednich (za pomocą chromatografu gazowego Varian 3800) oraz stężenia biomasy (poprzez pomiar absorbancji). Badania przeprowadzono w reaktorze okresowym dla szerokiego zakresu zmian początkowego stężenia substratu życiowego. Zgromadzona baza danych eksperymentalnych umożliwi wyestymowanie stałych równania opisującego szybkość wzrostu mikroorganizmów oraz współczynników wydajności biomasy dla obu analizowanych substratów wzrostowych.

Słowa kluczowe: biodegradacja, VOCs, hodowla okresowa

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EFFECT OF SOIL CONTAMINATION WITH HEAVY METALS IN A MIXTURE WITH ZINC AND NICKEL ON THEIR CONTENT IN BROAD BEAN (*Vicia faba* L. ssp. *maior*) PODS AND SEEDS

WPŁYW SKAŻENIA GLEBY METALAMI CIĘŻKIMI W MIESZANINIE Z CYNKIEM I NIKLEM NA ICH ZAWARTOŚĆ W STRĄKACH I NASIONACH BOBU (*Vicia faba* L. ssp. *maior*)

Abstract: The investigations aimed at an assessment of soil contamination with mixtures of heavy metals (Pb, Cu and Cd) with zinc and nickel on two levels of pollution on heavy metal concentrations in broad bean pods and seeds. Soil contamination with zinc used separately and in a mixture with Cd, Pb or Cu on III level acc. to IUNG classification prevented formation of pods and seeds by broad bean. Heavy metal concentrations in broad bean pods and seeds assumed the following order: Zn > Ni > Cu > Pb > Cd. The soil contamination with nickel in mixture with Cd, Pb or Cu on II level of pollution acc. to IUNG Classification does not cause an increase in the levels of the above-mentioned metals in broad bean seeds, but nickel concentrations increase by ca 2÷4 times. The soil pollution with nickel in a mixture with Cd, Pb or Cu contributed to an increase in the above metals concentrations in broad bean pods relatively to the applied dose, except for copper, whose level did not significantly differ from this element content in the unpolluted pods.

Keywords: mixtures of heavy metals, soil pollution, *Vicia faba* L. ssp. *maior*

Heavy metals present in soil jointly often reveal a different effect on growth and development of plants than when occurring singly [1].

The work aimed at an assessment of soil contamination with mixtures of heavy metals (Pb, Cu i Cd) with zinc and nickel on two levels of pollution on heavy metal concentrations in broad bean pods and seeds.

Material and methods

Broad bean, White Windsor c.v. was cultivated in a control soil with natural heavy metal concentrations and in the soil contaminated with the mixtures of heavy metals or with single metals:

- Cd - 2.25 mg $\cdot$ kg^{-1} d.m. +Zn - 350 mg $\cdot$ kg^{-1} d.m. (ZnII+CdII)
- Cd - 4 mg $\cdot$ kg^{-1} d.m. + Zn - 1000 mg $\cdot$ kg^{-1} d.m. (ZnIII+CdIII)
- Cu - 65 mg $\cdot$ kg^{-1} d.m. + Zn - 350 mg $\cdot$ kg^{-1} d.m. (ZnII+CuII)
- Cu - 85 mg $\cdot$ kg^{-1} d.m. + Zn - 1000 mg $\cdot$ kg^{-1} d.m. (Zn III+CuIII)
- Pb - 175 mg $\cdot$ kg^{-1} d.m. +Zn - 350 mg $\cdot$ kg^{-1} d.m. (ZnII+PbII)
- Pb - 530 mg $\cdot$ kg^{-1} d.m. + Zn - 1000 mg $\cdot$ kg^{-1} d.m. (ZnIII+PbIII)
- Ni - 62.5 mg $\cdot$ kg^{-1} d.m. + Zn - 350 mg $\cdot$ kg^{-1} d.m. (NiII+ZnII)
- Ni - 110 mg $\cdot$ kg^{-1} d.m. + Zn - 1000 mg $\cdot$ kg^{-1} d.m. (NiIII+ZnIII)
- Cd - 2.25 mg $\cdot$ kg^{-1} d.m. + Ni - 62.5 mg $\cdot$ kg^{-1} d.m. (NiII+CdII)
- Cd - 4 mg $\cdot$ kg^{-1} d.m. + Ni - 110 mg $\cdot$ kg^{-1} d.m. (NiIII + CdIII)

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- Cu - 65 mg · kg^{-1} d.m. + Ni - 62.5 mg · kg^{-1} d.m. (NiII+CuII)
- Cu - 85 mg · kg^{-1} d.m. + Ni - 110 mg · kg^{-1} d.m. (NiIII+CuIII)
- Pb - 175 mg · kg^{-1} d.m. + Ni - 62.5 mg · kg^{-1} d.m. (NiII+PbII)
- Pb - 530 mg · kg^{-1} d.m. + Ni - 110 mg · kg^{-1} d.m. (NiIII+PbIII)
- Cd - 4 mg · kg^{-1} d.m. (CdIII)
- Cu - 85 mg · kg^{-1} d.m. (CuIII)
- Pb - mg · kg^{-1} d.m. (PbIII)
- Ni - 110 mg · kg^{-1} d.m. (NiIII)
- Zn - 1000 mg · kg^{-1} d.m. (ZnIII)

The level of soil contamination corresponded to II and III class of pollution acc. to the classification suggested by IUNG in Puławy [2]. The plants were cultivated in plastic pots with 9.8 kg d.m. of soil under field conditions. Detailed description of the methods of heavy metal supply into the soil was presented in another publication [3]. The experiment was conducted in 2008 on degraded chernozem developed from loess with acid reaction (pH in 1 mol·dm^{-3} KCl solution was 5.5 and in water 6.3) and organic carbon content 1.13%. On objects where the soil was contaminated with single heavy metals, the soil polluted in the previous year, in which also broad bean was cultivated in 2007, was used.

Samples for chemical analyses were collected at milk ripeness of seeds. Plant material was washed in tap and in distilled water, dried at 105°C to a constant weight and ground to fine powder, then mineralized and dissolved in 10% HNO_3. After filtration: Zn, Pb, Ni, Cu and Cd content was measured using Flame Atomic Absorption Spectrometry (AAS) [4, 5]. The quality of the analytical procedure was controlled by using samples of the reference material in each series of analysis (Certified Reference material CTA-OTL-1 Oriental Tobacco Leaves). The data were processed using software Statistica to compute significant statistical differences between samples ($p < 0.05$) according to Tukey's multiple range test.

Results and discussion

The applied heavy metal doses (particularly higher ones) led to a considerable weakening of broad bean plant growth and prevented seed formation on some experimental objects. Soil contamination with zinc and other analyzed metals mixture on a lower level led to ca 2-3-fold increase in this metal content in broad bean pods. The highest Zn contents were registered in broad bean pods growing in the soil contaminated with a mixture of zinc and lead (108 mg · kg^{-1} d.m.). On the other polluted soil objects Zn content in broad bean pods was on the same level (84÷88 mg · kg^{-1} d.m.) (Tab.1). Among the objects where the soil was contaminated with Zn broad bean seeds were produced only on the soil contaminated with a mixture with cadmium in a lower dose. Zinc content in seeds on this object was higher by ca 30% in comparison with the control (Tab. 1).

Broad bean pods cultivated in the soil contaminated with a mixture of Ni and copper in a higher dose revealed the highest content of nickel (ca 15 times greater than noted in the control plants) (Tab. 2). Nickel applied together with Cd or Pb in a higher dose caused about 6-8-fold increase in Ni content in pods, whereas at lower contamination level this element concentrations were between ca 4 times higher for the object where the soil was contaminated with Ni and Zn, and ca 7 times higher on the soil contaminated with Cu and Ni (in comparison with the control). Seeds of plants cultivated in the soil contaminated with Ni in a mixture with Cd or Pb in a lower dose contained ca 4 times greater amounts of this metal than the control seeds. On the other hand, seeds from the plants exposed to Ni and Cu mixture had c.a. twice more of nickel than the control ones (Tab. 2). In the Author's previous investigations, examining the effect of soil contamination by a joint dose of heavy metals (Pb, Zn, Ni, Cu and Cd) on I level of contamination acc. to IUNG (except Cd used on II level of pollution) only a significant increase in nickel concentrations was found in broad bean seeds, whereas concentrations of the other metals remained on the same level as in the control plants. In pods, a marked increase in metal concentrations was registered for nickel, cadmium and zinc [6].

Table 1

The mean content of Zn [mg · kg^{-1} d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 1

Średnia zawartość Zn [mg · kg^{-1} s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	Content of Zn in pods	Content of Zn in seeds
Control(1)	35.00 a	44.94 a
Control	32.54 a	43.26 a
C+NPK(1)	35.25 a	42.65 a
C+NPK	32.56 a	42.85 a
NiII+ZnII	87.48 b	-
ZnII+CdII	88.56 b	62.88 b
ZnII+PbII	108.49 c	-
ZnII+CuII	84.20 b	-

Table 2

The mean content of Ni [mg · kg^{-1} d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 2

Średnia zawartość Ni [mg · kg^{-1} s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	Content of Ni in pods	Content of Ni in seeds
Control(1)	4.19 a	6.04 c
Control	2.70 a	4.17 a
C+NPK(1)	5.71 a	5.90 bc
C+NPK	4.30 a	4.54 ab
NiII+ZnII	18.63 b	-
NiII+CdII	24.52 c	23.13 e
NiII+PbII	21.41 bc	22.81 e
NiII+CuII	31.17 d	13.04 d
NiIII+CdIII	23.46 c	-
NiIII+CuIII	62.94 e	-
NiIII+PbIII	33.02 d	-

Cu content in broad bean pods cultivated in the soil contaminated with this metal or with its mixtures with lower doses of Zn or Ni was similar to the control plants. On the other hand, a higher dose of Cu in a mixture with Ni caused a slight increase in copper level in broad bean pods (Tab. 3). Among the objects with Cu polluted soil, seeds were obtained only from plants exposed to copper applied separately and in a mixture with Ni on a lower level. In the first case seeds accumulated slightly bigger quantities of Cu than the unpolluted plant seeds, in the second even less than the control seeds. A similar effect was noted in previous research [6].

Table 3

The mean content of Cu [mg · kg^{-1} d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 3

Średnia zawartość Cu [mg · kg^{-1} s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	**Content of Cu in pods**	**Content of Cu in seeds**
Control(1)	8.08 ab	10.17 c
Control	8.14 ab	9.29 bc
C+NPK(1)	7.57 a	9.33 bc
C+NPK	6.64 a	8.77 b
CuIII(1)	7.20 a	11.62 d
NiII+CuII	6.25 a	2.21 a
ZnII+CuII	8.13 ab	-
NiIII+CuIII	9.82 b	-

Cd content in broad bean pods reached the highest values when it was grown in the soil contaminated with a single metal. However, Ni admixture led to about 1/3 decrease in Cd level (Tab. 4). Cd content in broad bean seeds growing in the soil contaminated with this metal separately was ca 3-fold higher than in the unpolluted seeds. On the other hand, a lower dose of metal mixtures did not affect significantly Cd concentrations in the seeds.

Table 4

The mean content of Cd [mg · kg^{-1} d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 4

Średnia zawartość Cd [mg · kg^{-1} s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	**Content of Cd in pods**	**Content of Cd in seeds**
Control(1)	0.33 c	0.14 a
Control	0.07 a	0.20 ab
C+NPK(1)	0.14 a	0.24 b
C+NPK	0.23 b	0.22 b
CdIII(1)	0.82 f	0.67 c
NiII+CdII	0.47 d	0.17 ab
ZnII+CdII	0.37 c	0.20 ab
NiIII+CdIII	0.58 e	-

Lead in a mixture with a higher dose of Ni caused almost twice higher concentration of this metal in broad bean pods than noted when the metal was added separately (Tab. 5). Pb content in this object was ca 5-fold higher than in the control objects. At the lower pollution level, lead supplement in mixtures with Zn or Ni only slightly elevated this metal concentrations in pods. Seeds originating from the plants cultivated in the soil contaminated with Pb and Ni mixture in lower dose were characterized by a similar lead content as the unpolluted seeds.

Table 5

The mean content of Pb [mg · kg^{-1} d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 5

Średnia zawartość Pb [mg · kg^{-1} s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	Content of Pb in pods	Content of Pb in seeds
Control(1)	0.22 a	0.36 ab
Control	0.60 b	0.26 a
C+NPK(1)	0.24 a	0.32 ab
C+NPK	0.27 a	0.44 b
PbIII(1)	1.50 d	0.63 c
NiII+PbII	0.93 c	0.27 a
ZnII+PbII	0.74 bc	-
NiIII+PbIII	2.78 e	-

Conclusions

1. Soil contamination with zinc used separately and in a mixture with Cd, Pb or Cu on III level acc. to IUNG classification prevented formation of pods and seeds by broad bean.
2. Heavy metal concentrations in broad bean pods and seeds assumed the following order: Zn>Ni>Cu>Pb>Cd.
3. The soil contamination with nickel in mixture with Cd, Pb or Cu on II level of pollution acc. to IUNG Classification does not cause an increase in the levels of the above mentioned metals in broad bean seeds, but nickel concentrations increase by c.a. 2÷4 times.
4. The soil pollution with nickel in a mixture with Cd, Pb or Cu contributed to an increase in the above metals concentrations in broad bean pods relatively to the applied dose, except for copper, whose level did not significantly differ from this element content in the unpolluted pods.

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WPŁYW SKAŻENIA GLEBY METALAMI CIĘŻKIMI W MIESZANINIE Z CYNKIEM I NIKLEM NA ICH ZAWARTOŚĆ W STRĄKACH I NASIONACH BOBU (*Vicia faba* L. ssp. *maior*)

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Abstrakt: Celem pracy było określenie wpływu skażenia gleby mieszaninami metali ciężkich (Pb, Cu i Cd) z cynkiem i niklem na dwóch poziomach zanieczyszczenia na ich zawartość w strąkach i nasionach bobu. Skażenie gleby cynkiem zastosowanym oddzielnie oraz w mieszaninie z Cd, Pb lub Cu na poziomie III stopnia wg klasyfikacji IUNG prowadzi do niewykształcania strąków i nasion przez bób. Zawartość metali ciężkich w strąkach i nasionach bobu kształtowała się w następującym porządku: Zn > Ni > Cu > Pb > Cd. Skażenie gleby niklem w mieszaninie z Cd, Pb lub Cu na poziomie II stopnia zanieczyszczenia wg klasyfikacji IUNG nie powoduje podwyższenia poziomu wymienionych metali towarzyszących w nasionach bobu, natomiast zawartość niklu wzrasta ok. 2-, 4-krotnie. Skażenie gleby niklem w mieszaninie z Cd, Pb lub Cu przyczyniało się do wzrostu zawartości wymienionych metali w strąkach bobu odpowiednio do zastosowanej dawki, z wyjątkiem miedzi, której poziom nie odbiegał statystycznie istotnie od zawartości tego pierwiastka w strąkach niezanieczyszczonych.

Słowa kluczowe: mieszaniny metali ciężkich, skażenie gleby, *Vicia faba* L. ssp. *maior*

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DOSE-DEPENDENT ABSORPTION AND RETENTION OF ZINC IN RATS

ABSORPCJA I RETENCJA CYNKU U SZCZURÓW W ZALEŻNOŚCI OD ILOŚCI STOSOWANEJ DAWKI

Abstract: The aim of this study was to evaluate the influence of doses of zinc on its metabolism (absorption and distribution) in the carcass of animals. The examinations involved male Wistar rats divided into two groups. All the rats were given intragastrically for 28 days traces of zinc chloride labeled with radioactive zinc-65. Group I (the controls) fed a standard laboratory diet LSM and tap water containing traces of zinc (0.03 mg/dm^3). Rats in group II were fed as those in group I except for the drinking water that was supplemented with 23.3 mg Zn/dm^3 increasing about twice the dietary zinc intake as compared with that in group I. Radiozinc was measured in the carcass 3 h, 6 h, 1 d, 2 d, 4 d, 7d, 14 d, and 28 d after dosing with the use of scintillation counter equipped with well-type crystal that permitted the detection of radioactivity in the body of tested rats. Results included concentrations of radiozinc in the carcass at the time points which were used to calculate the AUC values. The obtained AUC values permitted the evaluation of carcass retention of zinc within 28 days postdosing. Data indicated that the percentage content of the isotope in group II was lower than that in the controls and differences were statistically significant at 6 h and day 1, 2, 4, and 28. The AUC values showed that the dietary zinc uptake in group II decreased by about 23% as compared with that in group I. The results supported evidence that increased dietary zinc intake results in decreased zinc retention in the body.

Keywords: radiozinc, carcass, distribution, rat

Zinc is generally considered a relatively non-toxic metal [1] although zinc salts in higher concentrations can injure epithelial tissue. In addition, intake of excess zinc has been reported to affect activities of pancreatic enzymes [2] and lipoproteins in serum [3], alter the metabolism of copper [4] and iron [4, 5], and disturb immunological function [6].

Bioavailability of zinc may vary and is influenced by dietary zinc supplements and numerous food constituents [7].

The aim of the present studies was to compare zinc absorption and retention in rats received a non-supplemented diet and a diet comprising a twofold higher zinc concentration.

Material and methods

Ninety male Wistar rats weighing 202 g ± 11 g were used. The animals were randomly assigned into two dietary groups of 45 rats each after an acclimatisation period of one week. Rats in group 1 were offered a standard rodent chow LSM (Fodder Manufacture Motycz, Poland) and tap water containing 0.02 mg zinc/dm^3 *ad libitum.* The total zinc content of the LSM diet was 23.3 mg/kg according to the manufacturer. Rats in group 2 fed the same chow but drank tap water supplemented with 23.3 mg Zn/dm^3. The animals were on these diets for the whole experimental period. Body weight gains and feed and water consumption were recorded weekly during the feeding period.

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Traces of labelled zinc chloride (zinc-65, Polatom, Poland) in a 0.5 mdm^3 water solution comprising about 20 kBq per rat was given daily by an intragastric tube to all rats for 28 days except weekends. Rats were killed by immersion in gaseous carbon dioxide at 3 hr, 6 hr, and day 1, 2, 4, 7, 14, 28 d after dosing. Radiozinc in the carcass (whole body without the stomach and intestines) was measured using a whole-body counter ZM 701 (Polon, Poland). Reference standards for quantification of carcass radiozinc were prepared by intraperitoneal injection of the appropriate solution to rats which were killed 45 min thereafter.

The area under the curves (AUC) of radiozinc content versus time points was calculated by the trapezoidal rule. Data were analysed statistically using Student's *t*-test at $P<0.05$.

Results

Rats in all groups demonstrated similar feed and water intake and the body weigh gain (Tab. 1). Further, the organ to body ratio for the liver, spleen, heart, testes, and kidneys was similar in the two groups examined. The blood values including erythrocytes, haematocrit, haemoglobin, and leukocyte for the two groups of rats were also similar (not shown).

Table 1

Body weight gains and organ to body ratio

Group	**Initial weight [g]**	**Final weight [g]**	**Liver to body ratio**	**Kidneys to body ratio**	**Testes to body ratio**	**Heart to body ratio**
Group 1	205 ± 15	384 ± 20 (87%)	3.89 ± 0.25	0.72 ± 0.07	0.89 ± 0.19	0.29 ± 0.02
Group 2	206 ± 12	374 ± 29% (83%)	4.06 ± 0.34	0.70 ± 0.006	0.88 ± .08	0.28 ± 0.02

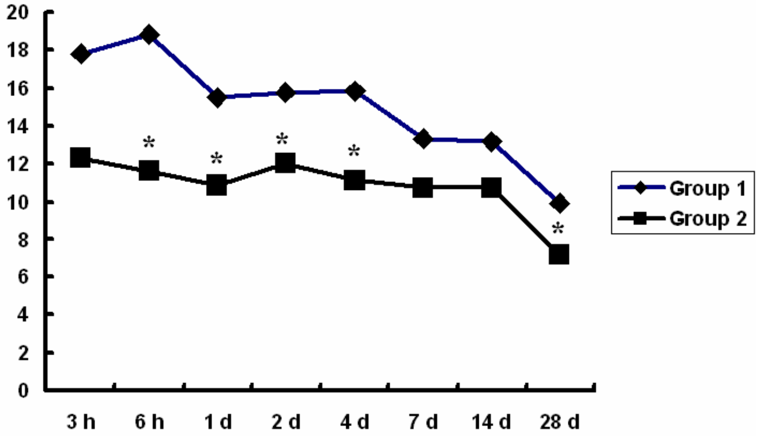

Fig. 1. Zinc content in the carcass (% of total dose), * - $P<0.05$

The time-course distribution of zinc-65 in the carcass within a 28-d period after the exposure is shown in Figure 1. Zinc-65 content in the carcass of rats in group 2 was visibly lower in comparison with that in group 1. Significant differences were found at 6 hr, and on days 1, 2, 4, and 28. The AUC values showing integrated exposure to zinc-65 in group 1 and group 2 were 8324 and 6404, respectively. These values indicated that the percentage absorption of zinc in group 2 was lower by about 23%.

Discussion

No significant alterations in organ to body ratios and body weight gains in the rats exposed to higher dose of zinc suggested that two-fold increase of dietary zinc did not affect unfavourable the health state of animals. It is in agreement with other reports [7, 9] that moderate supplements of zinc may be well tolerated by the organism. The toxic effects of zinc such as depressed rate of growth and feed intake in experimental animals may be produced by markedly higher dietary zinc intakes including doses of hundreds of milligrams per kilogram [10].

In the present studies the intestinal absorption following intragastrical administration for 28 days of traces of zinc-65 chloride to rats exposed to the consumption of adequate and supplemental zinc was compared by carcass radioactivity counting. The results indicate that zinc-65 in the two groups studied accumulated in a similar pattern irrespective of the dose administered. However, the percentage of the dose found in the carcass was significantly lower in the case of supplemental zinc indicating that the relative zinc absorption was reduced with increasing dose. When AUC values for the two groups tested were compared, it was seen that the rate of zinc-65 absorption was reduced by about 23% in rats fed supplemental zinc. On the other hand, the total amount of absorbed zinc was markedly higher in the rats fed supplemental zinc.

There are numerous factors including trace elements, forms of zinc, and several other food constituents that influence the gastrointestinal absorption of zinc [7, 11-14]. However, considering the presented data it seems reasonable that changes in zinc bioavailability are mainly associated with various tested doses of zinc applied.

A dose-dependant bioavailability of zinc was reported by other researches [14, 15] who found that low dietary zinc results in increased zinc retention and that at higher dietary zinc levels absorption of zinc is reduced within an 11-day post dosing period. The results presented here are in accordance with the above findings. Further, the data indicated that a low relative retention of zinc given intragastrically at higher than the recommended level may persist for a longer period in the body.

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ABSORPCJA I RETENCJA CYNKU U SZCZURÓW W ZALEŻNOŚCI OD ILOŚCI STOSOWANEJ DAWKI

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Abstrakt: Określono wpływ ilości podawanego cynku na jego metabolizm (przyswajanie i rozmieszczenie) w korpusie zwierząt, którym przed pomiarami usunięto przewód pokarmowy z treścią. W badaniach użyto szczurów samców szczepu Wistar, które podzielono na dwie grupy. W obu grupach podawano dożołądkowo przez 28 dni śladowe ilości chlorku cynku znakowanego radioaktywnym cynkiem-65. Grupę I stanowiły zwierzęta kontrolne karmione standardową paszą LSM i wodą pitną zawierającą śladowe ilości cynku (0,03 mg/dm^3 w Puławach), a grupę II zwierzęta karmione podobnie jak zwierzęta w grupie I z tym, że otrzymywały wodę pitną wzbogaconą w chlorek cynku w stężeniu (23,3 mg/dm^3), odpowiadającym stężeniu tego pierwiastka w podawanej zwierzętom paszy LSM. Radiocynk oznaczano w korpusie 3 h, 6 h, 1 d, 2 d, 4 d, 7 d, 14 d, 28 d po aplikacji za pomocą licznika scyntylacyjnego ze studzienkowym kryształem umożliwiającym pomiar radioaktywności całego ciała badanych zwierząt. Wyniki omówiono, biorąc pod uwagę wartości stężeń radiocynku w badanych przedziałach czasowych, które zastosowano do wyliczania metodą trapezowych pól wartości parametru AUC. Wartość tego parametru pozwoliła ocenić zawartości radiocynku w korpusie w okresie od 3 h do 28 dni po zakończeniu aplikacji. Dane opisujące rozmieszczenie radiocynku w korpusie wskazują, że procentowa zawartość tego izotopu była mniejsza aniżeli w grupie I, a różnice były statystycznie istotne po 6 h, 1 d, 2 d, 4 d i 28 d. Na podstawie obliczonego parametru AUC stwierdzono, że u zwierząt otrzymujących dwukrotnie większą od zalecanej dawki cynku jej procentowe przyswajanie zmniejszyło się o około 23% w porównaniu do odpowiednich wartości uzyskanych w grupie kontrolnej. Otrzymane wyniki potwierdzają wcześniejsze badania sugerujące, że podawanie cynku w ilościach większych od zalecanych powoduje zmniejszone jego przyswajanie.

Słowa kluczowe: radiocynk, korpus, rozmieszczenie, szczur

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FLUSHING WATER AND SEDIMENTS UTILIZATION FROM SELECTED WATER TREATMENT STATION

UZDATNIANIE WÓD POPŁUCZNYCH I UTYLIZACJA OSADÓW NA STACJACH UZDATNIANIA WODY

Abstract: The development of urbanized areas increases the intensity of problems connected to wastes utilization. The products formed during water treatment are usually omitted as irrelevant. These products cover flushing water and sediment. The researches reported in the literature show that pollutant concentration in these products may reach very high values. The effects of their utilization absence may be very significant for the environment. The existing methods of water treatment by-products utilization are still not satisfactory and usually cause significant ecological problems. The problem is not solved by sediments storage, even after its primary dewatering. Sediments combustion, after significant reduction of its volume, may cause increase of the water treatment station functioning costs and also is not commonly accepted by the public. The Authors, basing on the water treatment station in Simferopol, Ukraine, present the method of flushing water treatment and sediments utilization. The proposed technology, based on application of several reagents, enables limitation of water loss. Application of sediments as cement component, in road construction etc. additionally reduces the costs of system functioning. The results of rinsed sewage of Simferopol water treatment station researches are presented as an example of the described problems. The methods of drains treatment and sediments usage in order to break stone chips production are offered and substantiated. The studies are supported by the preliminary economical analysis.

Keywords: water treatment station, flushing water, sediments

The problems of waste products utilization, generated by human life activity get more and more actual every year. Considerable territories, which are allocated for domestic waste disposal, are seriously technologically endangered. This situation may lead to the vast catastrophes. The common understanding of this problem is denoted favourably for the reprocessing or utilization technology development [1, 2].

Among the waste products utilization technologies the fritting technology becomes widespread. The admixtures, which are located in waste products or formed during their burning, considerably increase at the end of burning process in many cases. Receiving the burning products of lower quantity, but ecologically dangerous, requires the development of their utilization technology [3, 4].

In a number of these problems the technological drains utilization on drinking water treatment stations acquires particular significance practically all over the territory of former Soviet Union countries every year. During the period of developing drinking water treatment stations acting standards (BDaR (*Building demands and rules in Ukraine*) 11-31-74, BDaR 2.04.02-84) these problems are not taken into consideration. This explains why there is usually not any solution of technological drains processing or such solutions are extremely simplified on most of drinking water treatment stations. Herewith, as a rule, the ecologically safe technologies of sediments utilization are not expected. There are high concentrated admixtures, which are typical for particular water supply source like a SiO_2,

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Fe_2O_3, CaO, MgO, BOD (*biological oxygen demand*), COD (*chemical oxygen demand*), nitrogen, water colouring etc., and water treatment technologies. The presence of different admixtures in sediments is caused by using different reagents in technology of water purification, its disinfections and conditioning (Al_2O_3, Fe_2O_3, chloroorganic compounds, aluminates, and products, which are created during the water pollution with an oxygen contact etc.) [5]. A considerable concentration of referred products on silt (technological) sediments is marked unfavourably on technological burden in region of their arrangement. Pollutions from these sediments transported by surface water infiltrate also to underground aquifer [6].

Simferopol hydroelectric power centre and water station

Our investigations concerning admixtures contents in the sediments on drinking water treatment stations of Simferopol hydroelectric power centre, affirm that the concentration of different admixtures visibly differs from typical limiting values for river Salgyr, where they can be discharged. The mentioned indices differ from limiting values demands, which are signalised in the technological rules on drinking water treatment station (Tab. 1). Besides that our research showed that the quality of rinsed water differs considerably according to many parameters from the filtering station demands (attached to returning rinsed water in a mixer on filtering station) and from river-water demands (attached to rinsed water draining to the river).

Table 1

The indices of water quality in Simferopol basin, river Salgyr, limited values of purified rinsed water before discharge into river Salgyr and before recycling

Denomination	**Rinsed water**[a]	**Simferopol basin**[b]	**Limiting values of discharging into Salgyr**	**Limiting values of returning into works mixer**
Unsedimentable suspension [mg/dm^3]	99.1	4.45	12	8
Dry residue [mg/dm^3]	529.2	340.1	530	440
Nitrogen of ammonia [mg/dm^3]	0.093	0.18	0.1	0.23
Nitrates(III) [mg/dm^3]	0.028	0.0124	0.027	0.016
Nitrates(V) [mg/dm^3]	7.69	2.79	7.9	8
BOD_5 [mg O_2/dm^3]	2.047	1.67	2.0	2.3
Permanganate oxidation[c] [mg O_2/dm^3]	7.62	4.67	4.9	6
Iron [mg/dm^3]	0.164	0.15	0.17	0.2
Aluminium [mg/dm^3]	4.76		0.07	0.3
Chloroform[d]	15.61	1.1		15
Carbon tetrachloride	0.57	0.02		0.03
Bromodichloromethane	45.28	5.11		6.6
Dibromochloromethane	5.53	0.94		1.22
Chlorides [mg/dm^3]	32.85	35.39		45
Sulphates [mg/dm^3]	53.8	67.64		87

[a] - average data for 2002-2003 years; [b] - average data for 1992-2003 years; [c] - average data for 2003 year; [d] - during the experiment data.

Simferopol hydroelectric power station contains the technical solutions focused on the improvement of rinsed waters quality based on two-hours desilting incapable to ensure not only necessary lowering the content of admixtures like dibromochloromethane and bromodichloromethane, carbon tetrachloride, but also unsedimentable suspension.

Moreover, the technological drains and sediments localization on the mentioned hydroelectric power centre requires a considerable territory; which is absolutely unprofitably from the economic point of view in Crimea conditions.

Different reagents application (eg “Polvak” or “Magnaflok” produced by Ciba - part of BASF company) for intensification of the desilting process as sulphuric acid aluminium, iron chloride, permits to increase the efficiency of this process. The necessary efficiency of mentioned process is achieved by the applied reagents dose regulation. The choice of reagent is realized on the technological and economic calculations base. Herewith it should be taken into consideration that increased dose of reagents results in their increased presence in sediments.

The essential reduction of admixtures in rinsed water by its preliminary desilting and filtration allows adding prepared in this way water into a mixer of drinking water treatment facility [7]. The research of rinsed water treated by the “Polidadmak” (Loser Chemie GmbH, Germany) reagent treatment process affirms that such admixtures as dibromochloromethane and bromodichloromethane, aluminium etc. cause volume decreases noticeably. The level of mentioned reduction reaches 40÷90%.

The proper choice of reagents and technologies of preliminary rinsed water treatment results in the further effective water treatment process and creates a possibility of reagents return into mixer on drinking water treatment station. This solution along with an ecological effectiveness has an economic expediency. Daily wastes in a money equivalent are about 600÷800 \$/24 hours with a quantity of twenty-four-hour fault of rinsed water 3000÷4000 m^3 on Simferopol hydroelectric power centre “Petrovs’ki skeli”, with a total cost of about 0.2 $\$/m^3$.

Proposed method of rinsed water reuse and sediment treatment or utilisation

The necessity of technical solutions providing effective rinsed water treatment application is obvious. The return of purified rinsed water into a mixer on drinking water treatment stations leads to utilization of formed sediments in order to receipt the product which is not dangerous for environment.

On drinking water treatment station “Petrovs’ki skeli” 500 tones of sediments are accumulated roughly (Fig. 1). Sediments lose more than 70% of their mass while the frying, which affirms that its burning is possible.

A presence of various harmful admixtures in sediment confirms such decision. It shows the technological reasons of its incineration fixation in the process of sediments utilization. Application of the other technologies of sediment utilization is considered (eg in road linen, using as concrete ingredients, application for cement production), but the technical and economical parameters of these methods are required [1-5, 8, 9]. In a number of cases such methods of utilization of sediments result in lower quality of eventual products and these methods are not appropriate ecologically.

The chart of technological drains treatment and utilization of sediment is proposed (Fig. 2) as a solution for the described problem. The basic feature of such a process is treatment of the technological drains on the filters with a floating load (filter with a fused charging), able to work with rinsed water containing the high concentration of mechanical admixtures. The arrangement of pumps and capacities keeping rinsed water for their washing are not required. Possibilities of simultaneous application of two processes (filtration and sedimentation) in one technological unit (device), allow to abandon the expensive constructions and results in reduction of sizes of the rinsed drains cleaning station.

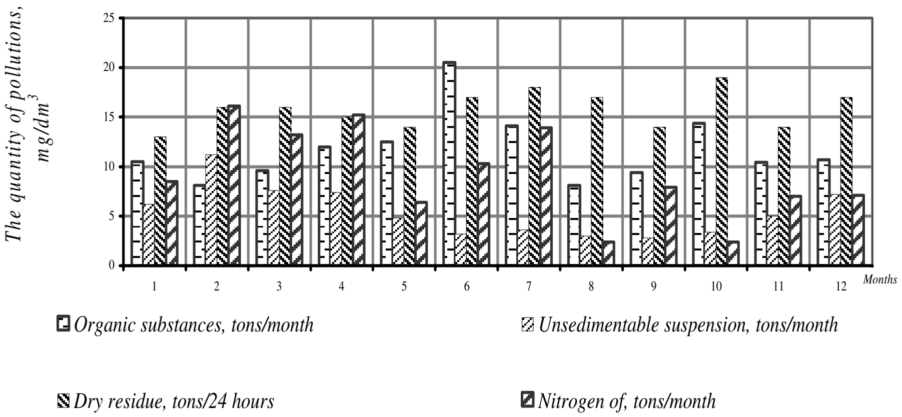

Fig. 1. Quantity of pollutions, which get into water treatment works "Petrovs'ki skeli" with water from Simferopol' basin (there are other mentioned admixtures in sediment)

The rinsed water is treated by reagent processes before it enters the fused charging filter. The dose of reagent depends on quality of rinsed water. The technological and structural parameters of filter depend on quality of the aggregates formed in the rinsed water after its treatment by the reagent. The partly cleared water fulfils the requirements of technological regulation on the drinking water treatment station and can be delivered to the mixer.

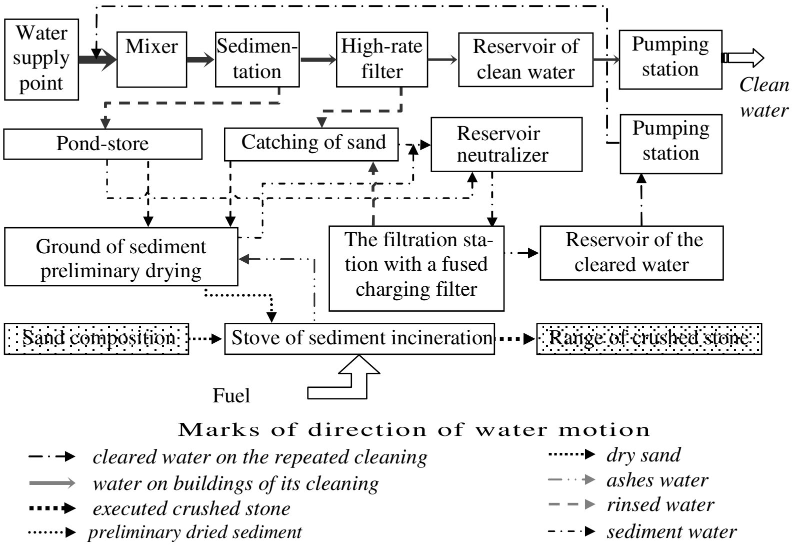

Fig. 2. Chart of technological flows cleaning and sediment utilization on the water drinking treatment station

The sediment localized in a pond-store and in grid chamber goes to the zone of its preliminary drying. Water polluted by ashes generated during sediments incineration is directed to the grounds of sediment preliminary drying. The surface of drying zone ground is covered by hydroisolation to prevent the infiltration through the moistened surface.

The process of sediments preliminary drying in the Crimea conditions, characterized by high average annual temperatures, can also be successfully conducted in the natural terms. Application of natural sediment preliminary drying technology effects positively on technical and economic indexes of the proposed process of technological water cleaning.

The next step of conducted process is sediments drying in the high temperature. According to the haydite gravel requirements described by State Standardizing 9759-71 the gravel standards obtained during our experiments after burning the sediment at the temperature of 1120 and 600°C match the “B” class.

The conducted researches show that: as a result of 500 tons of sediment containing about 80% combustible substances incineration, annually over 100 tons of mineral admixtures obtained from water flux will be utilized. That means that 100 tons of harmful for environment technological admixtures can be neutralized annually on water treatment works “Petrovs’ki skeli”.

Conclusion

The considerable sediment mass containing environmentally harmful admixtures accumulates on the drinking water stations. Sediments contain up to 80% of combustible admixtures.

The application of filters with a floating load into the technological scheme of sediment treatment is expedient. These filters are able to work with rinsed water containing the high concentration of mechanical admixtures.

Safe sediment utilization on the drinking water treatment stations can be attained by the application of the high temperature sediments treatment with a crush stone acquirement as a finished product.

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UZDATNIANIE WÓD POPŁUCZNYCH I UTYLIZACJA OSADÓW NA STACJACH UZDATNIANIA WODY

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Abstrakt: Postępująca urbanizacja prowadzi do nasilenia problemów z utylizacją powstających odpadów. Pośród nich często pomija się jako mało ważne (zdaniem niektórych) produkty powstające w procesie uzdatniania wody. Należą do nich wody i osady popłuczne. Badania dostępne w literaturze wskazują, że koncentracja zanieczyszczeń w tych produktach może być bardzo wysoka. Konsekwencje braku ich utylizacji mogą być bardzo znaczące dla środowiska. Istniejące metody utylizacji produktów ubocznych stacji uzdatniania wody wciąż nie są satysfakcjonujące i często prowadzą do znacznych ekologicznych problemów. Składowanie osadów, nawet po wstępnym ich odwodnieniu, nie rozwiązuje problemu. Spalanie osadów przy znacznym ograniczeniu ich objętości podnosi także znacząco koszty pracy stacji uzdatniania. Nie jest ono także powszechnie akceptowalne przez opinię publiczną. Na przykładzie stacji uzdatniania wody w Symferopolu autorzy prezentują metodę uzdatniania wód popłucznych i utylizacji osadów. Zaproponowana technologia, przy zastosowaniu szeregu reagentów, umożliwia ograniczenie strat wody. Zastosowanie osadów jako komponentów do cementu, budowy dróg itp. dodatkowo zmniejsza koszty funkcjonowania stacji uzdatniania. Autorzy prezentują wyniki badań zanieczyszczeń zawartych w wodach popłucznych wybranej stacji uzdatniania, omawiają zastosowany proces technologiczny oraz prezentują wyniki badań jego efektywności. Rozważania uzupełnione zostały o wstępną analizę ekonomiczną.

Słowa kluczowe: stacje uzdatniania wody, wody popłuczne, osady

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QUALITY CONTROL DURING ANALYSIS OF POLLUTANTS IN BEER USING STATISTICAL CONTROL OF SURROGATE STANDARD

STATYSTYCZNA ANALIZA JAKOŚCI KONTROLI ZANIECZYSZCZEŃ PIWA ZA POMOCĄ STANDARDÓW ZASTĘPCZYCH

Abstract: In the framework of good laboratory praxis the emphasis is placed on the quality of reproducible results. Methods of mathematic statistics are very useful for this reason. In the form of regulating diagrams they can help to quick identification of that results which are outside the required tolerances. There are a lot of various samples used for statistical regulation. One possibility how to control the efficiency of whole analytic process, from preparing to the final determination, is using of surrogate standard. 2,4,5,6-tetrachloro-*m*-xylene and decachlorobiphenyl were used as surrogate standards for the determination of PCB in various types of water. These compounds were examined during relatively complicate determination of PCB in beer. The concentration was determined by Shewhart's diagrams for individual values and sliding span according to standard ČSN ISO 8258. This example of PCB determination in beer indicates the possibility of using surrogate standards as a powerful tool for determination of quality results.

Keywords: pollutants, PCB, surrogate standard, Shewhart's diagrams, beer

Good laboratory practice places great emphasis on systems assuring quality control of produced results [1]. Not always analytical methods work in the way to provide acceptable measurement results. The objective of quality assurance is to keep the produced results within constant and defined limits of which we assume to be correct. Ideally, the analysis results of check samples move around to the average value. But the size of this oscillation is time-dependent. The accuracy of measurement results might then be out of required tolerance and can get worse as a consequence of uncontrolled variation sources. Analysts need to have a tool available which would warn them soon enough that the analytical system moves out of the required zone of stability. Such a time warning can be provided by statistical quality control [2].

Walter A. Shewhart is considered the father of modern statistical control, who published a comprehensive work in 1931, dealing with this subject. The first control charts used for process quality control were elaborated already in 1924, when he was employed at Bell's laboratories of Western Electric Company [3].

Statistical quality control gained its ground mainly in production processes as a significant tool for production control. Nevertheless, a measurement process as well, including chemical analysis, is a certain kind of production, too. In this case, it is data production, on which similar quality requirements like on the product are posed. Very useful tools in statistical quality control are the control charts, which allow a fast visual check of the status of the system being controlled.

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In general terms, control charts are used for the analysis of repeated processes, thus analytical measurements in our case. In principle, they are represented by continuous charts with statistically defined control elements.

The central line is the first control element, which is considered to be the mean value of measurement results that are uniformly distributed around it. It would be optimal if this line (its numerical representation) was identical with the real content of the check material. In reality, this never happens and therefore, the value of the central line is derived from the mean value of a series of measurement by a stabilised system, about which it can be stated that it is in the status of statistical control. To determine this value, at least 20 repeated measurements in a sufficiently long period of time are recommended to be carried out, which is necessary to involve the time dependence of the system.

Control limits are the second element and are defined as ± k-multiple of the mean-root-square error of determination. Since the deviations can be both positive and negative, also these limits are called upper and lower limits [2, 4]. Shewhart's diagrams are the most frequently used type of control diagrams in chemical analytical laboratories for the control of the accuracy of the analytical method. The controlled parameter is the value of the concentration of the corresponding analyte, which is plotted against the time axis representing individual measurements. The diagram produced from individual results is called the diagram for individual values. A second alternative is the measurement of the check sample repeatedly at each series, most frequently in a pair. In this case, average values of repetition are plotted in the diagram. The diagram produced from average values represents a more sophisticated but more exacting and more expensive alternative. Its advantage is that the influence of the random error is reduced by using the averages of repeated measurements of the same check sample and the probability of the detection of systematic errors, which fundamentally determine the accuracy of the analytical procedure, increases.

These diagrams are frequently used in connection with the diagrams for moving ranges, which are designated for the control of the presence of random errors and are not influenced by a possible systematic error. The moving range is the absolute value of the difference of a pair of measurement following each other in a measurement series, ie the difference between the first and second measurement, then between the second and third measurement, etc. [2, 4].

For statistical control, various check samples are used for the measurement, such as a blank test of the method used (checks, whether the laboratory environment, chemicals or equipment do not contain contaminated substances or other interference compounds), a fortified matrix (a real sample with known addition of the analyte being determined is analysed in the same way as the real sample, it is used to discover systematic errors), a laboratory duplicate (two parts of the same sample are analysed separately in the same way, it is used for monitoring of the accuracy of the laboratory measurement, it discloses possible random errors) [5, 6].

Even if these check samples are analysed with a pre-defined frequency, they can not involve a random error which could occur when preparing a certain sample. This problem can be resolved by using the surrogate standard.

The surrogate standard is an additional check analyte. It is an organic compound, the presence of which in the sample is in practical terms unlikely and which has similar physical and chemical properties as the analytes being determined. The known quantity of this substance is added to all blank tests, calibration and check standards, to each sample (including duplicates and reference samples), always before the analytical procedure is carried out. The samples are then analysed ordinarily. The purpose of this additional analyte is to monitor the effectiveness of the analytical method absolutely for each sample [7, 8].

The determination of PCB in beer is quite complicated and consists of several steps - separation, purification, concentration increase [9]. These operations are critical points of the entire method and can be a source of errors. The control of the whole analytical procedure by means of a suitable surrogate standard is thus an important part in the assurance of the quality of produced results. For PCB determination in various water types, the following two substances were proven to be the best to be used as the surrogate standard: 2,4,5,6-tetrachloro-*m*-xylene and decachlorobiphenyl [10, 11].

The aim of the work was to test the possibility of using these two substances for statistical control using Shewhart's control diagrams as the tools of quality control for the determination of PCB in beer.

Experimental

Chemicals and standards

n-hexane SupraSolv, diethyl ether p.a., Extrelut - Merck, Germany;
Florisil PR 60 - 100 mesh, anhydrous natrium sulphate - Fluka, Switzerland;
Ultra-pure water - Milli-RO 5 plus - Millipore, USA;
Nitrogen of grade 4,6 and nitrogen of grade ECD - MGO, Czech Republic;
PCB - Mix 3 containing PCB congeners No. 28, 52, 101, 118, 138, 153, 180. The mixture was in isooctane and acetonitrile solutions, each congener at the concentration of 10 ng/mm^3 - Dr. Ehrenstorfer, Germany;
8080 Surrogate Spike Mix containing 2,4,5,6-tetrachloro-*m*-xylene and decachlorobiphenyl, each substance in acetone solution at the concentration of 1 ng/mm^3 - Supelco, USA.

GC conditions

Gas chromatograph: Chrompack CP 9001;
Column: DB-5, length 30 m, diameter 0.32 mm, film thickness 0.25 μm;
Carrier gas flow rate (nitrogen): 1.8 cm^3/min;
Detector: ECD;
Detector temperature: 310°C;
Injector temperature: 260°C;
Injection: splitless for 36 s, then split ratio 1:20;
Temperature program: 70°C (2 min), 25°C/min to 200°C (0 min), 2°C/min to 250°C (0 min), 50°C/min to 290°C (10 min)
Injection: 1 mm^3

Sample preparation

20 mm^3 of the surrogate standards (2,4,5,6-tetrachloro-*m*-xylene and decachlorobiphenyl, each at the concentration of 1 ng/mm^3 in acetone) are added into an exactly measured quantity of about 1 dm^3 of a thoroughly defoamed beer sample. Then the sample is extracted by 20 cm^3 of hexane for 15 minutes on a laboratory shaker with the

swing amplitude of 40 mm and a frequency of 70 to 80 swings per a minute. For separating the extract emulsion from the analyse beer, Hrivňák's adapter is used. The destruction of the emulsion is made by its sucking through the column filled with 6 g of Extrelut sorbent being previously activated by application of 9 cm^3 of ultra-pure water. The flow rate of the extract through the column with Extrelut is accelerated by connecting a water pump.

In the following step, it is necessary to purify the received hexane eluate in the column with approximately 3 g of Florisil sorbent with a layer of about 0.5 g of anhydrous natrium sulphate above it. Just before the use, the column is flushed with about 5 cm^3 of hexane. The hexane eluate received after having gone through the column with Extrelut sorbent is thickened at laboratory temperature by blowing-off under nitrogen atmosphere to a volume of about 1 cm^3 and then it is applied in the column prepared together with Florisil sorbent. Elution is carried out by 25 cm^3 of a 6% solution of diethyl ether in hexane. The concentration of the purified extract is first increased in Kuderna-Danish apparatus and finally under a gentle stream of nitrogen to a final volume of 200 mm^3. 1 mm^3 of such purified extract is then used for the analysis by the gas chromatography.

Results and discussion

The concentration of both surrogate standards was measured in 30 different beer samples (8 pale draft beer samples, 13 pale lager samples, 3 dark draft beer samples, 4 dark lagers and 2 non-alcoholic beer samples). From these results, the values for the central line and the control limits for Shewhart's control diagrams for individual values and the moving range were calculated according to the following relations:

Diagram for individual values X:

Central line = X

Upper control limit = X + 3/1.128 * R

Lower control limit = X - 3/1.128 * R

Diagram for moving range R:

Central line = R

Upper control limit = 3.267 * R, lower control limit = 0,

where X is the average concentration of the surrogate standard, R is the average moving range [3].

The concentrations of the surrogate standards from the samples analysed for the purpose of routine checking were gradually plotted in such prepared diagrams. The control diagrams for individual values and the moving range of both surrogate standards are shown in Figures 1 and 2. From the course of these diagrams, an important fact can be observed - the system is stable and it is in the state of statistical control. The positions of the measured points at their time course meet the criteria used for the interpretation of this type of control diagrams: none of the points lies out of the control limits and, eight consecutive points do not lie on the same side of the central line [2].

Conclusion

2,4,5,6-tetrachloro-*m*-xylene and decachlorobiphenyl were successfully used as surrogate standards for the determination of PCB in beer. By means of the statistical control of the concentration value of these substances in the form of Shewhart's control diagrams, it is possible to control the maintenance of the measurement process in a statistically managed status and detect as well as eliminate systematic errors and, as a result of this, the quality of produced results can be systematically assured.

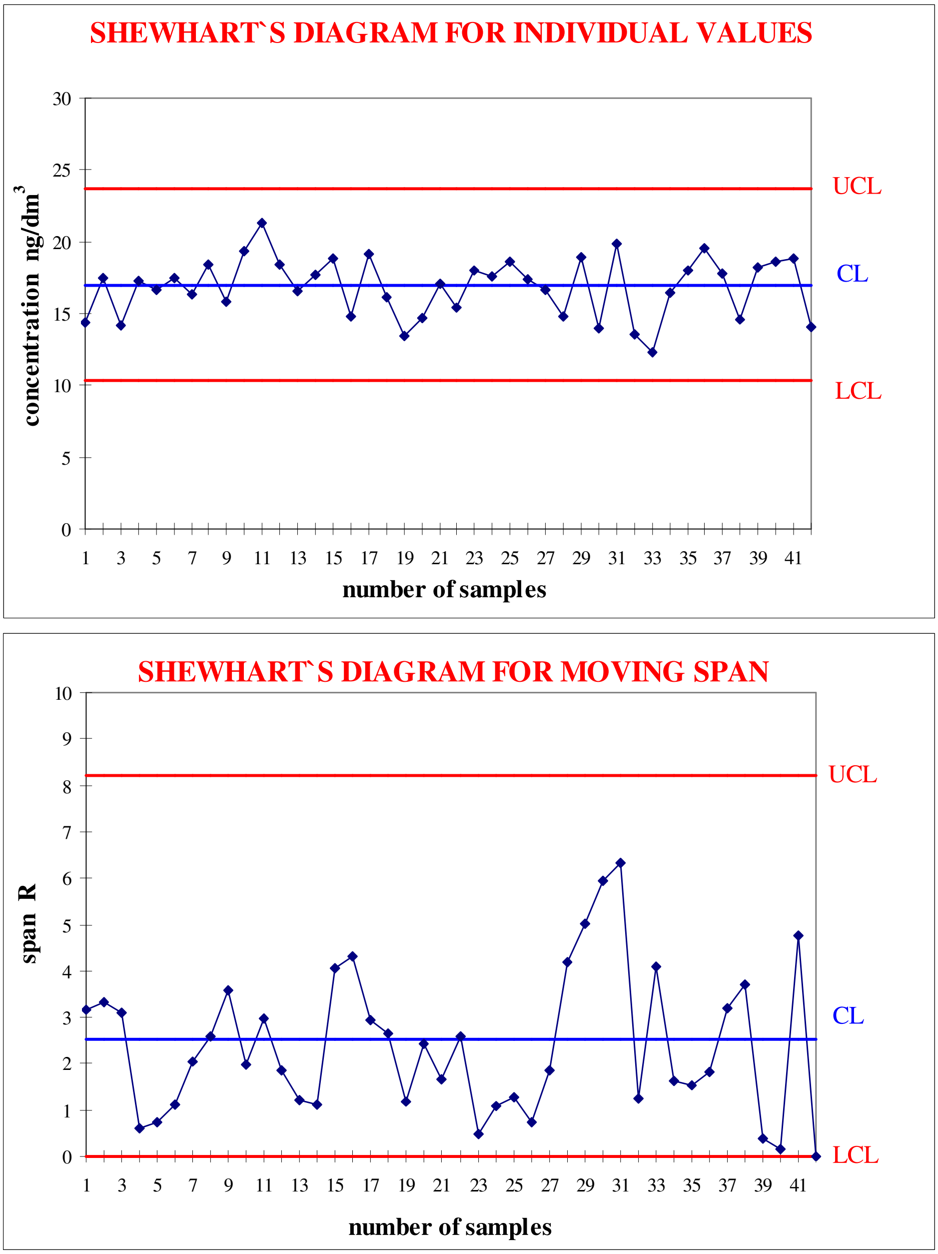
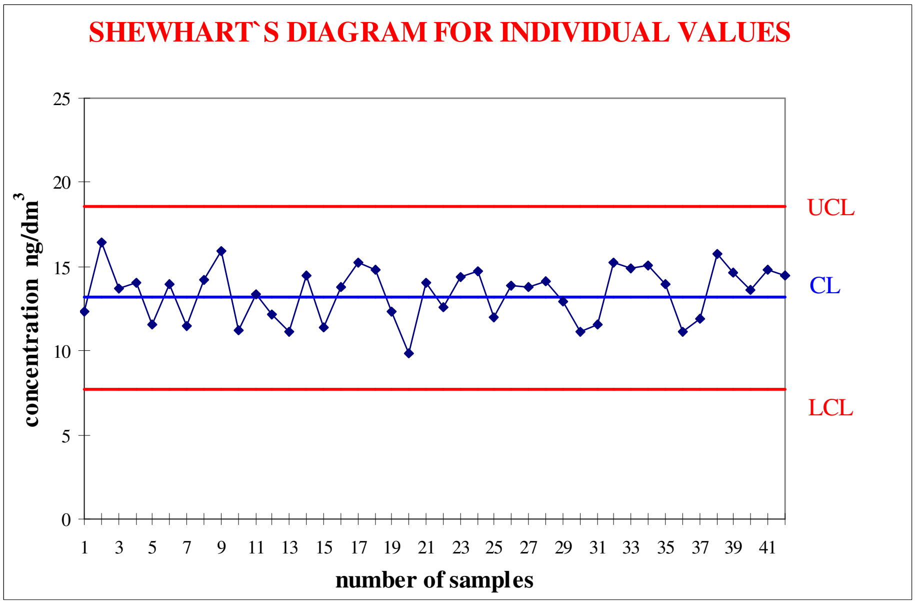


Fig. 1. Shewhart’s control diagrams for individual values of concentration and moving span of 2,4,5,6-tetrachloro-*m*-xylene



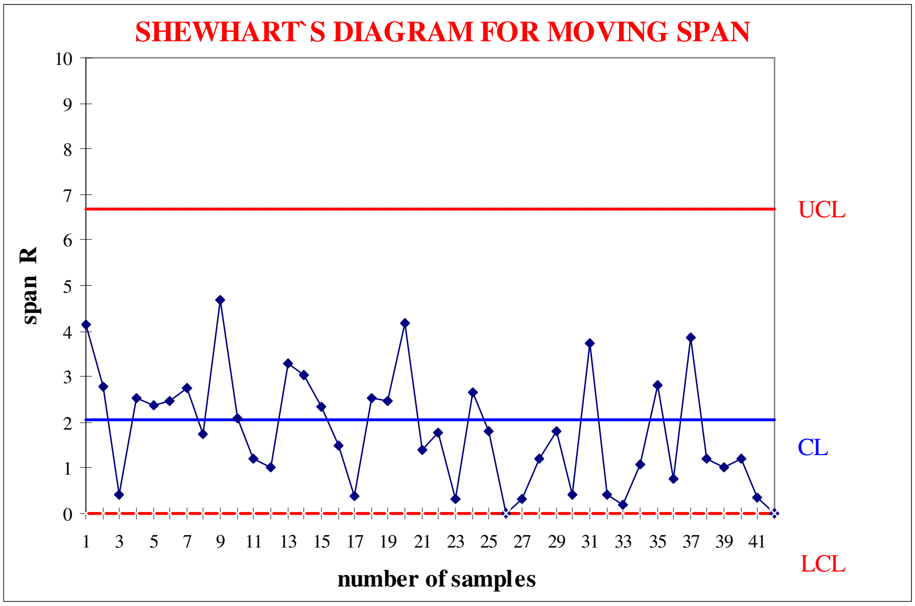


Fig. 2. Shewhart's control diagrams for individual values of concentration and moving range of decachlorobiphenyl

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STATYSTYCZNA ANALIZA JAKOŚCI KONTROLI ZANIECZYSZCZEŃ PIWA ZA POMOCĄ STANDARDÓW ZASTĘPCZYCH

Abstrakt: W ramach dobrej praktyki laboratoryjnej kładzie się nacisk na jakość uzyskanych wyników. Do ich oceny wykorzystuje się matematyczne metody statystyczne. Za pomocą schematycznych diagramów można szybko zidentyfikować wyniki pozostające poza wymaganymi granicami tolerancji. Istnieje wiele różnych metod statystycznych używanych do tych celów. Jedną z możliwości kontroli efektywności całego procesu analitycznego, od przygotowania próbki do końcowego oznaczenia, jest użycie standardów zastępczych. Do oznaczania PCB w różnych rodzajach wody użyto 2,4,5,6-tetrachloro-*m*-ksylenu i dekachlorobifenylu jako wzorców zastępczych. Związki te zostały zbadane podczas stosunkowo skomplikowanego oznaczania PCB w piwie. Stężenie zostało określone za pomocą schematów Shewharta dla poszczególnych wartości, zgodnie z normą ČSN ISO 8258. Ten przykład oznaczania PCB w piwie wskazuje na możliwość korzystania ze standardów zastępczych jako istotnego narzędzia do określenia jakości wyników.

Słowa kluczowe: zanieczyszczenia, PCB, standard zastępczy, diagramy Shewharta, piwo

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STUDIES OF ALCOHOLS CONTENT IN WINTER WINDSCREEN WASHING LIQUIDS

BADANIA ZAWARTOŚCI ALKOHOLI W ZIMOWYCH PŁYNACH DO SPRYSKIWACZY

Abstract: Analyses of 12 winter windscreen washing liquids available on the home market were performed. Methanol, ethanol and 2-propanol were determined. Results of the investigations indicated that in the all samples ethanol was present. In four samples methanol was also determined. One product contained 2-propanol. The total alcohols content in all samples was calculated. Alcohols content in winter windscreen washing liquids was between 30÷50%.

Keywords: windscreen washing liquids, cabin air quality, methanol, ethanol, 2-propanol

Indoor air quality is more and more important due to the time people spend indoors (more than 80% of their time) [1-3]. As well as indoor air quality in various buildings, air quality in mobile cabins (CAQ - Cabin Air Quality) including cars, trains, buses, aircrafts and subway is also important [3, 4].

In big city agglomerations people have spent more time in their cars. It is a result of the increase in numbers of vehicles as well as a bad road organization. Hence, the main roads in cities in the rush hours are full of cars stuck in a traffic jam.

According to accessible literature, in car cabin the volatile organic compounds such as acetone, ethanol, limonene, 2-propanol, 2-butanone are present very often [3, 5]. Ethanol and limonene are those, whose content in cabin air is the biggest. They are often ingredients of car cosmetics such as waxes, air-freshners, cleaning and conditioning agents. Windscreen washing liquids rank among this group of products. They may contain ethanol, 2-propanol and methanol. The above compounds have harmful impact on a human body by causing the symptoms such as sickness, irritation of an eye, nose or throat mucosa. Ethyl alcohol decreases the motor and muscle coordination and reaction time becomes slower, what may endanger the road safety. Both ethyl and methyl alcohols badly affect the eyesight and methyl alcohol may lead to irreversible damage of the eyesight [6].

Materials and methods

The studies of alcohols contents in the windscreen washing liquids were performed by using gas chromatography method. The subject of examinations was 12 of winter windscreen washing liquids available on the home market.

The samples of analyzed product (5 mm^3) were injected into 1114 cm^3 containers, tightly closed with the screw caps containing the silicon membranes. The samples were evaporated in a thermostat chamber at the temperature of 50°C. Next, they were stabilized at the room temperature for 30 minutes. After equilibration, the gas samples of 1 cm^3 volume were drawn with a Hamilton gas-tight syringe and analyzed on a gas

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chromatograph equipped with flame-ionization detectors (FID). The qualitative and quantitative analyses of windscreen washing liquids components were performed using a CHROM 5 gas chromatograph with a FID detector. The chromatograph was provided with a steel chromatographic column of 1 meter long of 3 mm inside diameter packed with Chromosorb 102, (80÷100 mesh). The following temperatures were applied: column 100°C, injector 120°C, detector 150°C. Nitrogen (40 $cm^3 \cdot min^{-1}$) was used as the carrier gas.

Identification was performed with the aid of methanol, ethanol and 2-propanol standards (standards for GC purity ≥99.9%). The identification of the alcohols was confirmed using an Agilent 6890N gas chromatograph with a 5973N mass selective detector.

Quantitative analyses of the windscreen washing liquids components were performed on the CHROM 5 gas chromatograph with a FID detector. The apparatus was calibrated using the standard gas mixtures prepared in the same containers and conditions in which the tested samples were prepared. Correlation coefficients of calibration curves for determined compounds were in the range of 0.9953÷0.9967. Alcohols content in the examined products was calculated.

Results and discussion

Results of the quantitative analyses of alcohols content in the examined samples are given in Table 1.

Table 1

Results of the determination of alcohols content in products

Product	Substance	Content [% vol.]	Standard deviation SD	Coefficient of variation RSD [%]
WL1	ethanol	34.90	0.97	2.79
WL2	methanol	4.57	0.14	3.15
	ethanol	34.41	0.21	0.62
WL3	methanol	38.66	1.30	3.35
	ethanol	6.00	0.21	3.53
WL4	ethanol	37.09	1.39	3.75
WL5	methanol	3.92	0.16	4.06
	ethanol	31.53	1.08	3.42
WL6	ethanol	48.61	1.74	3.57
WL7	methanol	8.87	0.12	1.35
	ethanol	12.37	0.21	1.71
	2-propanol	11.34	0.43	3.77
WL8	ethanol	35.02	0.92	2.64
WL9	ethanol	35.14	0.56	1.60
WL10	ethanol	40.41	0.37	0.91
WL11	ethanol	42.61	0.37	0.86
WL12	ethanol	41.75	0.42	1.02

Results of the investigations indicated that in the all samples ethanol was present. Its content in the most winter windscreen washing liquids was about 30÷50%. Only in two samples ethanol content was lower and averaged 6.00% for the sample WL3 and 12.37% for the sample WL7.

In four samples methanol was determined. Methanol content in sample WL3 was very high and achieved nearly 40%. In our opinion using of this product is dangerous. The large amount of methanol has harmful effect on human health, especially on sight. In the three other samples (WL2, WL5 and WL7) methanol content was considerably lower. Additionally in the WL7 sample, 2-propanol was identified. Its content in this product was about 11%.

The summary alcohols content in the samples was calculated. Results are presented in Figure 1. Alcohols content in winter windscreen washing liquids was between 30÷50%. In the majority of products this value varied from 30 to 40%. The maximum alcohols content was found in samples WL3 (44.66%) and WL6 (48.61%).

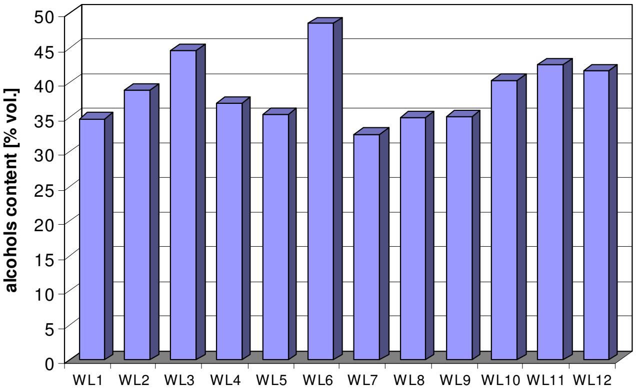

Fig. 1. Summary alcohols (methanol, ethanol and 2-propanol) content in winter windscreen washing liquids

The accuracy of the described method was determined on the basis of calculation of alcohols recovery from winter windscreen washing liquids of strictly determined composition. For this purpose composed four types of model washing liquids with different content of methanol, ethanol and 2-propanol. Alcohols content and composition of the model windscreen washing liquids were selected in the way to respond to the commercial product composition. Their characteristics and alcohols recoveries are given in Table 2. The mean alcohols recovery for the analysed products was 101.49%.

Table 2

Data of accuracy and precision of determination method of alcohols content in windscreen washing liquids

Model windscreen liquid	Substance	Content [% vol.]	Recovery [%]	Standard deviation SD	Coefficient of variation RSD [%]
TWL1	methanol	10	101.86	1.91	1.87
	ethanol	10	94.13	3.10	3.30
	2-propanol	10	94.76	2.38	2.55
TWL2	methanol	40	111.00	0.62	0.56
	ethanol	5	96.85	2.50	2.59
TWL3	methanol	5	106.43	2.88	2.71
	ethanol	40	103.36	0.63	0.61
TWL4	ethanol	50	103.50	0.50	0.48

Precision of the method was determined by evaluation of repeatability of the results calculating the standard deviation and a coefficient of variation. The coefficients of variation for the obtained results were below 5%.

Conclusions

The analyses of 12 winter windscreen washing liquids available on the home market were performed.

Summary alcohols content in winter windscreen washing liquids was between 30÷50%. In the tested products three alcohols (methanol, ethanol and 2-propanol) were identified. Ethanol was present in the all samples. In four samples methanol was determined. Its content was very high (39%) in one product. One sample contained about 11% of 2-propanol.

The described methodology may be used to control the quality of windscreen washing liquids and may be used to evaluation of cabin air quality. The method is repeatable and accurate. The mean alcohols recovery was 101.49%.

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BADANIA ZAWARTOŚCI ALKOHOLI W ZIMOWYCH PŁYNACH DO SPRYSKIWACZY

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Abstrakt: Przedmiotem badań było 12 rodzajów zimowych płynów do spryskiwaczy dostępnych na rynku krajowym. W badanych produktach zidentyfikowano i oznaczono ilościowo etanol, metanol i 2-propanol. Wyniki badań wskazują, że we wszystkich badanych produktach obecny jest etanol. Metanol zidentyfikowano w czterech próbkach. Jeden z produktów zawierał 2-propanol. Obliczono sumaryczną zawartość alkoholi w próbkach. Mieściła się ona w granicach 30÷50%.

Słowa kluczowe: płyny do spryskiwaczy, jakość powietrza w kabinach, metanol, etanol, 2-propanol

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FORMATION OF THE COMPREHENSION OF ENVIRONMENTAL BASIC CONCEPTS IN CHEMISTRY AND BIOLOGY

WYJAŚNIANIE PODSTAWOWYCH POJĘĆ W CHEMII I BIOLOGII ŚRODOWISKA

Abstract: In the background of successful acquiring of every learning subject there is comprehension of the basic concepts and problems of the corresponding field of science. Precise explanation of the basic concepts from the teacher's side facilitates the corresponding development of student's knowledge and comprehension. There are several substantial problems every teacher facing during learning process in chemistry and biology lessons. Students are defining the basic concepts/ideas not precisely because there is different explanation or even this explanation is lacking in different teaching aids. It is difficult for student to find definitions and explanations of concepts learned in preceding years without assistance. As for student as well for teacher there is lack of concise, clear source of information to be used both for self-education as well to prepare for examinations. And finally the actualities are changing in the long run. Obvious example is inclusion of actual environmental items in the chemistry course. Since the approval of new National Education standards in Latvia in 2006 environmental items were regarded mainly during biology lessons. Though there is the need to elaborate contemporary teaching aids assisting to solve the mentioned problems. The serious attention should be paid to the process of the formation of the basic concepts. Actual paper provides principles for the formation of the basic concept „acid rain".

Keywords: acquiring of chemistry and biology, comprehension of the basic concepts, basic concept, acid rain

The task of teacher when starting to teach every new learning subject is to raise interest of students regarding this new subject. Both in biology as well in chemistry the teacher shows demonstrations accompanied by exciting narration. Quite often the teacher to be carried away by demonstrations and experiments forget that he should set the background for knowledge starting from the very first lessons. Starting on that how good the student will acquire the basic concepts of biology and chemistry there will be dependent his following success during the acquiring of the whole biology or chemistry course as students are learning with deeper interest those issues understandable or at least partly understandable to them. To provide that during learning process student obtain qualitative knowledge teacher should ensure effective organization of student's thinking being systemic, logical and consequent.

In the studies of chemistry and biology the acquiring of the basic concepts is the background for successful acquiring of the themes related to surrounding environment, even more because according to the National standards in chemistry and biology students should acquire issues related to the environment [1]. Up to the approval of new National Education standards in Latvia in 2006 environmental items were regarded during biology lessons mainly where only the small part from the whole number of lessons were devoted to ecological items. To acquire the environmental items in the chemistry lessons there are not quite ready nor teachers nor students as the environmental items in the chemistry textbooks are viewed in general way or even the themes concerning environmental issues are missing.

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Though there is the need for teaching aid to facilitate the work of the teachers and students during acquiring environmental items.

Theoretical background of the methodology

The studying of chemistry and biology is cognitive process resulting in development of stable, practically applied knowledge founded on the acquiring of the basic concepts.

The basic concepts are knowledge in generalized way reflecting the crucial features of objects (substances) as well as the rules. Only gradually formed and developed basic concepts conceived complete and deeply by the students are suitable for characterization of already known facts by them as well as to allow them to use these basic concepts as effective cognitive tool [2]. Though when developing the basic concepts it is not possible to stay only with the description of definition of the basic concept or to stay with that the student knows concerning the object or phenomenon what it simply is. Teacher should develop knowledge (skills) allowing revealing the real substance of the basic concept, to allow view interconnections (contiguity between the basic concepts).

Formation of the basic concept is going on gradually. In the process of studying chemistry and biology there are two levels discerned - empirical and theoretical. The empirical basic concepts formed at empirical level allow arranging diversity of qualities of the studying object according to the unsubstantial features, though alleviating cognitive process. In the theoretical level the basic concepts are formed by extracting the substantial inner connections and connecting them together, though allowing the basic concepts became more concrete.

Some authors provide several models to form the basic concepts, though the differences between these models are minimal.

Table 1

Stages of the development of chemistry basic concepts [4]

Stage	Content of action	Result of action in the mind of student
I	Observation of several equal objects or phenomenon, revealing of equal external features. Primary synthesis as a result of comparison. Discovering the substance and way of cognition of the basic concept	The notion is forming The model of the basic concept is forming, the initial plan for development of basic concept
II	Study (analysis) and extraction (synthesis) of the substantial features of objects (phenomenon) or their structural parts	There is forming empirical basic concept
III	Inclusion of new facts in the content of the given basic notion. Detection of new connections and attitudes between given basic concept and other concepts (analysis and synthesis). Use of concept	Concretization and fastening of the empirical basic concept
IV	Detection of the site of given basic concept within the whole system of knowledge	Formation of the theoretical basic concept

For instance Russian scientists provide the following model to form the basic concepts [3]:

Stage 1 - there is gathered information obtained (received) by senses - hearing, sight and touch;
Stage 2 - formation of notions;
Stage 3 - generalization of notions results in formation of the basic concepts.

In the given scheme of classification first two stages corresponds to the empirical cognitive level but last one stage - to the theoretical cognitive level.

There are authors dividing 4 main stages in the development of the basic concepts [4].

In this case first three stages correspond to the empirical level, but fourth in its turn - to the theoretical level.

If comparing the models of formation of basic concepts it could be seen that in the formation of the basic concepts there is dominating principle - from the general towards concrete, substantial.

Methods and materials

In contemporary world even more popular become the educational aids allowing the interested persons to acquire basis of respective science field in concise way. These are handbooks, small glossaries of definitions, tables and assignments for calculations, not addressed to any particular class. They could be successfully used both in lessons as well as at home preparing for lessons. They could be used both by students and their parents as well as by teachers. Thus we regard that elaborated teaching aids in chemistry and biology could be important [5]. The basic concepts included in teaching aids are based on requirements of National Education standards of Latvia in chemistry and biology. Explanation of the basic concepts is based on principle - from the general towards concrete, more characteristic. Teaching aid is approbated and tested in practice in schools. Initial approbation is wide because the material is available for all of the students in Latvian schools; it is used by students, teachers, parents and applicants, as well as lecturers in universities for comparison aspect to be informed about what the students should know. One of the aspects of the teaching aid application is its use to form (develop) the basic concepts.

To form the basic concepts following scheme consisting of three stages could be used:
Stage 1 - gathering of experience, specification;
Stage 2 - formation of notions;
Stage 3 - division of most important features of the basic concept.

Results and discussion

Development of the basic concept "acid rain" in chemistry and biology

Development of the basic concept "acid rain" could be performed in three stages:

- Gathering of experience (What the students know already about the acid rain?)
- Formation of notions (What the acid rain really is?)
- Division of the most important features of the basic concept (How the acid rain forms? How it influence the surrounding environment?)

In the process of the development of the basic concept there could be used diverse studying methods. During the stage of the gathering of experience brainstorm will be useful. For the formation of notions there could be used experiments and demonstrations [6], for example, demonstration of sulphur reaction with oxygen and solving of obtained sulphuric(IV) oxide in water. Educational films [7] as well as pictures and presentations could be shown. When dividing the most important features, in biology the laboratory work, for example, "Observing the Influence of Acid Rain on Plant Growth" [8] could be performed, but in chemistry - “Forming of Acid Rains” [9] or Acid Effects on Metals [8]. Both in biology and in chemistry it is suggested to perform solving of problem situations by using real facts.

Development of the basic concepts in the elaborated chemistry and biology teaching aids

In the elaborated chemistry and biology teaching aids the basic concepts and regularities are reflected by the models and schemes comprehensive to students, basic concepts are explained in the language easy understandable to students, based on principle from the general towards concrete, coinciding with the stages of formation of the basic concepts. As an example there is given formation of the basic concept “acid rain” from the elaborated teaching aid (see Fig. 1).

Acid rain	Precipitations polluted by great amounts of sulphuric and nitric oxides.
Formation of acid rain	Sulphuric oxides reacting with water vapour in the air are making sulphuric acid drops. $2SO_2 + O_2 \rightarrow 2SO_3$ (*sulphuric(IV) oxide + oxygen → sulphuric(VI) oxide*) $SO_3 + H_2O \rightarrow H_2SO_4$ (*sulphuric(VI) oxide + water→ sulphuric(VI) acid*) Sulphuric oxides are forming by burning of fossil fuels, for instance, coal as well as are discharged during eruptions of volcanoes. The similar process occurs with nitric oxides, which also forming due to burning of fossil fuel. $2NO + O_2 \rightarrow 2NO_2$ (*nitric(II) oxide + oxygen → nitric(IV) oxide)* $3NO_2 + H_2O \rightarrow 2HNO_3 + NO$ *(nitric(IV) oxide + water → nitric(V) acid + nitric(II) oxide)*
Consequences of acid rain	• Metallic constructions are rusting; • Architectural monuments are damaged; • Lichens and coniferous trees are perishing; • By increasing soil acidity there are forming solvable toxic metallic compounds; • By decreasing of water pH value in lakes, there are disappearing many fish species.

Fig. 1. Fragment from the teaching aid in chemistry

Development of this basic concept in the teaching aid occurring as follows:

- At first there is given the definition what the “acid rain” is. By reading or learning this definition there is established just general perception what the “acid rain” is, its substance is still not clear.
- Then there are revealed substantial features of the “acid rain” - what are the reasons of forming it and how the “acid rain” influence surrounding environment. By reading this information becomes clear the reasons of “acid rain” and its consequences on surrounding environment.

By using these teaching aids student could fasten his present knowledge as well as to find out unclear items not discussed during lessons.

After the approbation of teaching aid there are received positive references and approving evaluations both from students and their parents as well as from teachers.

Conclusions

1. In the forming of the environmental basic concepts there could be regarded principle of logical sequence - from the general towards concrete.
2. In the forming of the environmental basic concepts it is suggested to use diverse methods both empirical and theoretical.
3. In the process of learning of the environmental basic concepts the teaching aids elaborated by the author and accepted by the target groups could be useful.

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WYJAŚNIANIE PODSTAWOWYCH POJĘĆ W CHEMII I BIOLOGII ŚRODOWISKA

Abstrakt: Podstawą zdobywania wiedzy z każdego przedmiot nauczania jest zrozumienie podstawowych pojęć i problemów związanych z odpowiednią dziedziną nauki. Dokładne wyjaśnienie podstawowych pojęć przez nauczyciela ułatwia odpowiednie przyswojenie wiedzy przez ucznia. Każdy nauczyciel w trakcie procesu nauczania chemii i biologii napotyka na kilka ważnych problemów. Studenci definiują podstawowe pojęcia/zwroty nieprecyzyjnie, dlatego że nie znają innego wyjaśnienia, a także brakuje jeszcze niektórych pomocy naukowych. Studentowi trudno jest bez pomocy podać definicje i wyjaśnienia pojęć poznanych w poprzednich latach. Zarówno w przypadku studentów, jak i nauczycieli brak jest zwięzłych, jasnych źródeł informacji, które mogą być wykorzystywane w procesie samokształcenia bądź przygotowywania się do egzaminów. I wreszcie aktualizacja informacji zachodzi w dłuższym czasie. Przykładem jest uwzględnienie rzeczywistej ochrony środowiska w trakcie nauczania przedmiotu chemia. Od czasu zatwierdzenia w 2006 r. nowych norm krajowych edukacji na Łotwie zagadnienia ochrony środowiska realizowane są głównie podczas lekcji biologii. Istnieje potrzeba opracowania współczesnych pomocy naukowych i szukania sposobów w celu rozwiązania wymienionych problemów. Należy zwrócić uwagę na proces kształtowania podstawowych pojęć. W pracy przedstawiono zasady tworzenia podstawowego pojęcia „kwaśny deszcz”.

Słowa kluczowe: rozumienie chemii i biologii, rozumienie podstawowych pojęć, pojęcia podstawowe, kwaśne deszcze

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DEVELOPMENT AND OPTIMIZATION OF EXTRACTION PROCEDURE OF MILK FAT FOR SIMULTANEOUS DETERMINATION OF DIOXINS AND DIOXIN-LIKE COMPOUNDS

OPRACOWANIE I OPTYMALIZACJA PROCESU EKSTRAKCJI TŁUSZCZU Z MLEKA W CELU RÓWNOCZESNEGO OZNACZANIA DIOKSYN I ZWIĄZKÓW DIOKSYNOPODOBNYCH

Abstract: Polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls are unintentionally produced, ubiquitous, persistent organic pollutants. Due to bioaccumulation in food chains, the main source of human exposure to the compounds is food of animal origin. European Community food safety strategy is provided through official monitoring of foodstuffs. This results in necessity of development of reliable and validated analytical method based on instrumental techniques. Milk and milk products are recognized to be good indicator of environmental exposure for persistent organic pollutants. Effective extraction of fat from complex matrix consisting of colloidally distributed sugars, vitamins, proteins and minerals is difficult. Conservative methods of fat extraction (liquid-liquid extraction) are particularly time and labour consuming; moreover require large amounts of organic solvents and laboratory space. In order to efficiently extract emulsified fat globules suspended in the aqueous phase of the milk, they have to be broken scrupulously. This condition is met with Accelerated Solvent Extraction method based on elevated pressure and temperature. Method of fat extraction based on ASE for further procedure of simultaneous determination of 35 analytes with high resolution gas chromatography-high resolution mass spectrometry from milk samples was developed. After optimisation, method was proven effective and reliable. Classical methods such as liquid-liquid extraction and Soxhlet extraction were used for comparison. Method was comprehensively validated according to EU requirements; Certified Reference Materials were used. Developed and optimised fat extraction method from milk based on Accelerated Solvent Extraction is currently routinely applied in the frame of the official monitoring of dioxins and dioxin-like compounds.

Keywords: milk, ASE, extraction, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, HRGC-HRMS

The surveillance of dioxins and dioxin-like compounds in milk has been conducted since early 90's in many European countries. Raw milk and its products are recognized to be a fine indicator of environmental exposure for persistent organic pollutants (POPs). Ruminants are contaminated through vegetable feedstuffs, once dioxins have been absorbed by aerial deposition on the vegetation. Contaminants due to their lipophilicity and their low biodegradability bioaccumulate in cows, next they are largely excreted from the body of lactating cows by transfer to milk. Milk and milk products are one of the major contributors to the human exposure to PCDD/Fs and PCBs. Moreover, during the last decade official monitoring programmes revealed numerous dioxins crisis situations, particularly often in milk or beef meat [1-3].

Conservative fat extraction from milk products such as liquid-liquid extraction (LLE) are particularly time and labour consuming multistep methods; moreover large amounts of organic solvents and plenty of laboratory space are required. Furthermore, extraction of fat from complex matrix consisting of colloidally distributed sugars, vitamins, proteins and

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minerals is difficult. Emulsified fat globules suspended in the aqueous phase of milk, have to be broken scrupulously in order to efficiently extract lipophilic contaminants from the matrix. Accelerated Solvent Extraction (ASE) is modern extraction technique that efficiently reduces extraction effort. Functionality of ASE is based upon automation; the quintessence is to apply the liquid solvent in conditions of elevated temperature (50÷200°C) and pressure (10÷20 MPa). The pressurized solvent and elevated temperature increase the solubility of the analytes and the kinetic rate of desorption of the analytes from the matrix.

Method of fat extraction based on ASE for further procedure of simultaneous determination of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and polychlorinated biphenyls with gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) in milk samples was developed, optimized and extensively validated.

Materials and methods

Two fat extraction techniques were comprehensively compared: classical liquid-liquid extraction and Accelerated Solvent Extraction (Tab. 1). Extraction in Soxhlet apparatus was additionally used as a reference technique. Prior to all extractions the mixtures of ^{13}C labelled solutions comprised of 35 analogues of contaminants analysed were added.

Liquid-liquid extraction. Raw milk (30 g) was transferred to 1000 ml separatory funnel; 4 cm^3 of saturated solution of KOH was added in order to digest the fat content; 200 cm^3 of anhydrous ethanol and 100 cm^3 of diethyl ether were added in order to denature the proteins; fat was extracted twice vigorously for 5 min with 140 cm^3 of *n*-pentane. Inorganic phase was discarded, while organic phase dehydrated with saturated solution of Na_2SO_4, twice. Finally organic phase was left with 50 g of Na_2SO_4 for final dehydration.

Soxhlet extraction. Extraction was performed with toluene and dichloromethane/*n*-hexane (1/1, v/v), both in triplicates. Freeze-dried milk (5 g) was extracted for 6 h in 200 ml Soxhlet extractors (Bibby Sterlin, Great Britain) at the speed of 6 siphons per h.

Accelerated Solvent Extraction. 200 g of milk sample was frozen (~20°C, 6 h). The water was eliminated in laboratory freeze-drier (1 mbar, –40°C) (Alpha 1-4 LSC, Martin Christ, Germany). Sample (5 g) was mixed with diatomaceous earth (4/1, w/w) (Hydromatrix, Varian, USA) for better dispersion. ASE 300 (Dionex, USA) with 34 cm^3 cells was used, filled up to 85÷90%. Pressure for all experiments was set to 10 MPa.

Consecutive parameters of ASE were optimized: solvents (or its mixtures), temperature (50÷150ºC), number of static cycles (1-4), time (1÷5 min) and flush volume (60÷120%). Dichlorometane, *n*-hexane, methanol and their mixtures were used. Extraction time and extract volume were measured. Final validation experiments were performed with *n*-hexane/dichloromethane/methanol (5/2/1, v/v/v) [4], pressure of 10 MPa, temperature of 100°C, within 3 extraction cycles of 2 min each and with a flush volume of 80%.

Further analysis was performed according to the method based on isotope dilution mass spectrometry (IDMS) with gas chromatography coupled to high resolution mass spectrometry. Samples were defatted on acidic silica columns and further purified and fractionated on Florisil® and Carbopack C. PCDD/Fs, non-*orto*-PCBs and mono-*orto*-PCBs fractions were analysed with HRGC-HRMS (MAT 95XP, Thermo Scientific, Germany) on a DB-5MS (60m, J&W Scientific, USA). Expanded uncertainty was estimated at the level of interest and was established below 20% for WHO-TEQs (Toxic Equivalency Factors of World Health Organization).

Validation study. ASE and LLE with subsequent PCDD/Fs and PCBs quantification were comprehensively validated according to EU standards [5]. Certified Reference Materials (IRMM, Geel, Belgium) were used to determine trueness [6, 7].

Table 1

Comparison of method validation results of Accelerated Solvent Extraction and liquid-liquid extraction

Extraction method	**ASE**	**Liquid-liquid**
Levels of contaminants	[pg/g fat]	
WHO-PCDD/F-TEQ	1.7 / 3.4 / 8.3	1.8 / 3.6 / 9.1
WHO-PCDD/F-PCB-TEQ	2.9 / 6.4 / 12.3	2.9 / 6.6 / 13.1
LOQ	[pg/g fat]	
WHO-PCDD/F-TEQ	0.14	0.29
WHO-PCDD/F-PCB-TEQ	0.19	0.33
Within-laboratory reproducibility, C.V.	[%]	
WHO-PCDD/F-TEQ	3.1	5.1
WHO-PCB-TEQ	3.6	6.5
Repeatability, C.V.	[%]	
WHO-PCDD/F-TEQ	1.8÷6.9	4.0÷6.2
WHO-PCDD/F-PCB-TEQ	2.8÷3.7	2.9÷6.5
Recovery	[%]	
TeCDD/Fs, PeCDD/Fs, HxCDD/Fs	60.1÷15.2	51.7÷106.7
HpCDD/Fs, OCDD/Fs	55.9÷107.4	59.7÷118.9
dl-PCBs	50.8÷118.3	42.7÷124.8
Within-laboratory reproducibility of fat extraction, C.V.	[%]	
	4.5	6.5
Repeatability of fat extraction, C.V.	[%]	
	1.4÷4.7	3.6÷9.0

Results

The fat extraction efficiency obtained with Soxhlet apparatus for BCR-607 was 26.9% for toluene (n = 3) and 26.5% for dichloromethane/*n*-hexane (1/1, v/v) (n = 3), whilst 26.8% for liquid-liquid extraction (n = 20).

ASE extractions with dichlorometan, *n*-hexane and methanol and their mixtures (1/1, v/v) led to lower extraction yields in comparison with reference techniques. Denaturized proteins instead of fat were obtained when methanol and its mixtures (1/1, v/v) were used; *n*-hexane, dichlorometane and their mixtures were insufficiently effective. When a mixture of *n*-hexane/dichloromethane/methanol (60/25/15, v/v/v) was used, the extraction efficiency was comparable to Soxhlet extraction and surpassed liquid-liquid extraction. Temperature of process was not significant parameter; efficiency increased of 5% with temperature increase from 50 to 100ºC. However during extraction in temperatures exceeding 100 to 150ºC, milk fat was accompanied by not-fatty fraction; share of non-fatty fraction within extract increased with temperature. Insufficient extraction was obtained within one extraction cycle, independently to its length (1÷5 min). There was no significant distinction in extraction yields between 2 to 5 extraction cycles. Extraction phase of one minute was not sufficient, however there was not difference between extraction cycles from

2 to 5 min. Flush volume of 60, 80, 100 and 120% was applied and 80% was found as most proficient.

After optimization of ASE method validation was performed for both techniques with naturally contaminated samples at the level of interest ranging from 50 to 200% of respective maximum levels for PCDD/Fs and the sum of PCDD/Fs and dl-PCBs (Tab. 2) [8]. Within-laboratory reproducibility of WHO-TEQs and fat extraction efficiency was estimated with 4 series of BCR-607 each in six replicates. Repeatability was calculated at each of three levels of analytes (n = 6). The fat extraction efficiency for BCR-607 (n = 24) was 28.9%. All values of within-laboratory reproducibility and repeatability were far below required value of 15%. Recovery of analytes was in the required range of 60÷120% for TeCDD/Fs to HxCDD/Fs. Method was proved sensitive with limits of quantifications for both extraction types far below required levels (0.6 and 1.2 pg of WHO-PCDD/F-TEQ and WHO-PCDD/F-PCB-TEQ, respectively) [5, 8], however LOQ values for LLE were much lower.

Trueness of PCDD/Fs estimated with BCR-532 and BCR-607 was usually below ±5% for both ASE and LLE, complying with EU requirement (< 20%) (Tab. 2) [5]. Trueness of some PCDD/Fs with high values of uncertainty (~40÷100%) ranged up to 36%.

Table 2

Trueness of Certified Reference Materials analysed (BCR-532, BCR-607)

CRM	**BCR-532 (n = 6)**			**BCR-607 (n = 24)**		
Extraction method	**[pg/g]***	**LLE [%]**	**ASE [%]**	**[pg/g]***	**LLE [%]**	**ASE [%]**
2.3.7.8-TCDD	0.10 ± 0.02	–10.45	1.69	0.25 ± 0.03	–0.87	4.32
1.2.3.7.8-PeCDD	0.29 ± 0.06	–9.07	–7.42	0.79 ± 0.04	–0.36	–2.93
1.2.3.4.7.8-HxCDD	0.17 ± 0.04	–6.00	–7.77	0.42 ± 0.07	–6.17	–5.69
1.2.3.6.7.8-HxCDD	0.43 ± 0.09	–1.75	–5.31	0.98 ± 0.11	–6.55	–4.19
1.2.3.7.8.9-HxCDD	0.16 ± 0.04	–8.32	–15.41	0.34 ± 0.05	–7.15	–2.86
2.3.7.8-TCDF	0.16 ± 0.08	–24.02	–31.87	0.05 ± 0.03	–22.00	–35.56
1.2.3.7.8-PeCDF	0.10 ± 0.10	–15.22	–16.93	0.05 ± 0.01	–0.84	10.10
2.3.4.7.8-PeCDF	0.67 ± 0.13	2.14	–4.06	1.81 ± 0.13	–1.98	–5.52
1.2.3.4.7.8-HxCDF	0.34 ± 0.07	–3.78	–7.73	0.94 ± 0.04	–2.74	–6.87
1.2.3.6.7.8-HxCDF	0.34 ± 0.07	–3.12	–7.06	1.01 ± 0.09	–1.04	3.87
1.2.3.7.8.9-HxCDF	0.39 ± 0.08	1.50	8.87	1.07 ± 0.05	–0.59	0.01
WHO-PCDD/F-TEQ	3.22	–4.83	6.48	9.02	–2.88	0.00

* - certified value ± uncertainty of measurement

Discussion

During routine work, liquid-liquid extraction of milk fat was found labour and time consuming. Vast amounts of organic solvents used and excessive laboratory space requirements led to search of other solutions. Fat from various foodstuffs is routinely extracted with ASE after desiccating the matrix with diatomaceous earth, sodium or magnesium sulphate. One of the pitfalls of chemical desiccation with diatomaceous earth is limited volume of sample, resulting in inadequate limits of detection. The other group of chemical desiccants is even more hazardous, when used in elevated temperatures with polar solvents might be harmful for ASE systems. More sophisticated dehydration solution applies physical desiccation of sample by means of freeze-drying was applied. Dehydration of the sample was an important factor, allowing for solvent penetration through dispersion with diatomaceous earth.

Extraction mixture was the major operational parameter of ASE. Mixture of solvents *n*-hexane/dichloromethane/methanol (5/2/1, v/v/v) was found selective and efficient for extraction of saturated and unsaturated milk fats as well as not-hydrolysed phospholipids. Other tested solvents or mixtures led to non-selective extraction with proteins and sugars next to fat. Temperature was another significant parameter. Effective extraction was obtained with low temperature of 100ºC, what can be explained with low melting point of milk fat, consisting in 98% of fatty acids and triglycerides. Whilst ASE extraction of POPs are usually performed in high temperatures (150÷200°C), there is potential of obtaining coextractives of fat when milk would be ASE extracted in such temperatures; in such conditions sugars were extracted. Efficient extraction of fat is critical step of POPs analysis, because concentrations of PCDD/Fs and PCBs in foodstuffs are expressed per gram of fat. Presence of coextractives might be confusing and severe misjudgement of analytical results might happen. Time and number of extraction cycles were of minor importance for extraction efficiency; however optimization allowed to finish the process in 16 min (3 cycles of 2 min each).

ASE and LLE methods were comprehensively validated according to EU requirements [5] and proven fully reliable. Generally, validation parameters were satisfactory for both methodologies. In terms of analytes recoveries ASE was found slightly more consistent. Within-laboratory reproducibility and repeatability of both respective WHO-TEQ values as well as fat extraction was significantly better for automatic technique. Considerable distinction between LOQs for ASE technique in comparison to LLE might be explained with increase of background noise due to high temperature and pressure for the former technique.

Only few automated methods of milk extraction for further analysis of POPs are described. This method was developed and optimised for simultaneous analysis of 7 PCDDs, 10 PCDFs, 12 dl-PCBs and 6 ndl-PCBs with GC-ID-HRMS. Further, the method may be applied to other emerging environmental contaminants eg hexachlorobenzene, mixed bromo-chlorinated dibenzo-*p*-dioxins and furans, organochlorine pesticides, polybrominated biphenyls, polybrominated diphenyl ethers, polybrominated dibenzo-*p*-dioxins and furans, polychlorinated naphtalenes, polycyclic aromatic hydrocarbons and toxaphens. The most time and labour consuming step of liquid-liquid extraction was successfully replaced with the automated process. Satisfactory results of validation make the method very attractive for the routine analysis of divers POPs.

Conclusions

1. Procedure of fat extraction from milk for subsequent multianalyte determination of PCDDs, PCDFs, dl-PCB and ndl-PCBs was developed and optimised. Method may be adapted to the other lipophilic environmental contaminants.
2. Automated method of fat extraction based on Accelerated Solvent Extraction with subsequent analysis of 35 dioxins, furans and polychlorinated biphenyls with high resolution mass spectrometry with isotope dilution was comprehensively validated according to European Community standards. Certified reference materials were used.

Measurement uncertainty was estimated. Finally, method was accredited according to ISO EN-PN 17025:2005.

3. The most profound parameters of effective milk extraction with Accelerated Solvent Extraction were solvent mixture and temperature.
4. The method based on Accelerated Solvent Extraction is currently routinely applied in the National Laboratory for Dioxins and PCBs in Feed and Food in the frame of official surveillance program.

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OPRACOWANIE I OPTYMALIZACJA PROCESU EKSTRAKCJI TŁUSZCZU Z MLEKA W CELU RÓWNOCZESNEGO OZNACZANIA DIOKSYN I ZWIĄZKÓW DIOKSYNOPODOBNYCH

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Abstrakt: Polichlorowane dibenzo-*p*-dioksyny, dibenzofurany i bifenyle są wszechobecnymi, trwałymi zanieczyszczeniami organicznymi. Z powodu bioakumulacji w łańcuchach troficznych głównym źródłem narażenia człowieka na dioksyny jest spożywanie żywności zwierzęcego pochodzenia. Bezpieczeństwo żywności zapewnianie jest we Wspólnocie Europejskiej (WE) w drodze kontroli urzędowej. W celu zapewnienia jej prawidłowego funkcjonowania WE zobowiązuje laboratoria do posługiwanie się wiarygodnymi i zwalidowanymi metodami analitycznymi wykorzystującymi techniki instrumentalne. Surowe mleko i jego produkty są uznawane za dobry wskaźnik zanieczyszczenia środowiska trwałymi związkami organicznymi. Metody ekstrakcji tłuszczu z mleka obarczone są licznymi wadami, ponieważ złożona matryca zawiera również koloidy cukrów, witamin, białek i minerałów. Tradycyjne metody ekstrakcji (ekstrakcja techniką ciecz-ciecz) są wyjątkowo czaso- i pracochłonne; dodatkowo zużywają duże ilości rozpuszczalników organicznych i wymagają znacznej przestrzeni laboratoryjnej. Podstawowym warunkiem wydajnej ekstrakcji tłuszczu z mleka jest efektywne rozbicie zemulgowanych drobin tłuszczu zawieszonych w fazie wodnej. Ten warunek w pełni spełnia technika przyśpieszonej ekstrakcji za pomocą rozpuszczalników dzięki zastosowaniu podwyższonych ciśnienia oraz temperatury. Opracowano metodę ekstrakcji tłuszczu z próbek mleka z wykorzystaniem techniki przyśpieszonej ekstrakcji za pomocą rozpuszczalników w celu dalszego równoczesnego oznaczania 35 toksycznych związków techniką chromatografii gazowej sprzężonej ze spektrometrią mas wysokiej rozdzielczości. Za metody odniesienia przyjęto ekstrakcję techniką ciecz-ciecz oraz ekstrakcję w aparacie Soxhleta. Przygotowaną metodę poddano walidacji z wykorzystaniem m.in. certyfikowanych materiałów odniesienia. Opracowana i zoptymalizowana metoda ekstrakcji tłuszczu z mleka z wykorzystaniem techniki przyśpieszonej ekstrakcji za pomocą rozpuszczalników jest obecnie rutynowo wykorzystywana w urzędowej kontroli próbek na obecność dioksyn, furanów i polichlorowanych bifenyli.

Słowa kluczowe: mleko, ASE, ekstrakcja, polichlorowane dibenzo-*p*-dioksyny, polichlorowane dibenzofurany, polichlorowane bifenyle, HRGC-HRMS

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INFLUENCE OF ENVIRONMENTAL CONDITIONS ON LIPOLYTIC ACTIVITY OF *Bacillus* sp. STRAINS

WPŁYW WARUNKÓW ŚRODOWISKA NA AKTYWNOŚĆ LIPOLITYCZNĄ SZCZEPÓW *Bacillus* sp.

Abstract: The aim of the research was the evaluation of lipolytic activity of *B. cereus* and *B. mycoides* strains, in reference to carbon source, pH and the temperature. In the research, two strains of *Bacillus cereus* and *Bacillus mycoides* each, isolated from the soil and water, were applied. The sources of carbon in culture media were fatty substrates: tributyrin, Tween 40, Tween 60, Tween 80 and glucose. The lipolytic activity was measured by means of titration in pH ranging from 5 to 8 and the temperature ranging from 30 to 60°C. The results were noted as the amount of liberated µmoles of fatty acids. In the conducted research, the amount of liberated µmoles of fatty acids depended on the type of fatty substrate in the medium, pH and the temperature. The strains under study showed the lowest activity at pH 5 and 6, and the highest activity at pH 7 and 8. In these conditions, most of the strains showed the lipolytic activity, even in case of the lack of fatty substrate in the medium. The highest amount of µmoles of fatty acids was liberated at pH 8 in the medium with Tween 40, and the highest results (52.5 µmol) were noted for the soil strain *B. cereus*. When analysing the influence of the temperature on the lipolytic activity, it was found that the highest amount of µmoles was noted at 30 and 40°C, and the lowest at 50 and 60°C. The best results were obtained for most of the strains at 30°C, in medium with Tween 40, and the most active was the soil strain of *B. mycoides* (52.5 µmol). The exception is *B. cereus*, as it liberated 82.5 µmol, in the medium with glucose. Taking into account all analysed sources of carbon and parameters, it seems that the most active were *B. mycoides* strains.

Keywords: *Bacillus* sp., lipases, tributyrin, Tween

Lipases, defined as hydrolases of glicerol esters EC 3.1.1.3, are the enzymes of high catalytical potential. They are produced by plants, animals and microorganisms, of which the last group remains in the centre of attention. Many kinds of bacteria possess the ability to produce them, among others bacteria of *Bacillus* kind [1].

A common interest in bacterial lipases is connected with their role as biocatalysts in many biochemical processes. They are used, among others to produce detergents, food, paper, pharmaceuticals and in the environmental protection. As shown by data in literature, they are varied in terms of their enzymatic activity, which depends on the species of microbes and the culturing conditions (eg pH of the growth medium, temperature, source of nitrogen and presence of lipids in the medium) [1-3].

The aim of undertaken research was the evaluation of lipolytic activity of *B. cereus* and *B. mycoides*, isolated form the natural environment, in reference to carbon source, pH and the temperature.

Materials and methods

The objects of the study were 4 *Bacillus* strains:

- 2 *Bacillus cereus* strains marked as: A96 and G10, isolated from soil and water, respectively;

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- 2 *Bacillus mycoides* strains marked as: A134 and G3, isolated from soil and water respectively.

The sources of carbon in culture media were the following fatty substrates: tributyrin, Tween 40, Tween 60, Tween 80 and glucose. The cultures were maintained in Erlenmeyer flasks of 250 cm^3 capacity containing 50 cm^3 of respective growth medium with an inoculum of density equal to E = 2, obtained from the 48-hour culture on a nutrient broth. Incubation was conducted on a rotary shaker for 2 days at 30°C.

Samples were collected after 2 days of culturing and centrifugated for 20 minutes at 4000 rpm. The extracellular lipolytic activity was determined in the obtained supernatant by means of titration towards the same substrates as the ones added to the growth media (the proper treatment). In the control treatment the supernatant was replaced with water. Lipolytic activity was estimated at pH ranging from 5 to 8, and at temperature ranging from 30 to 60°C. The amount of liberated fatty acids was determined by titration with 0.05 M NaOH solution against 2% phenolphthalein as an indicator, and calculated as a subtraction between the proper treatment and the control treatment results. The result was presented as the amount of liberated μmoles of fatty acids.

Results

In presented paper 4 bacterial strains of *Bacillus* kind were screened for their ability to synthesize lipolytic enzymes on the growth media containing different sources of carbon, at pH ranging from 5 to 8, and the temperature ranging from 30 to 60°C.

In conducted research, the amount of liberated μmoles of fatty acids depended on the carbon source in the growth medium, pH and the temperature.

The strains of *B. cereus* and *B. mycoides* under study were the least active at pH 5 and 6. However, the highest activity was noted at pH 7 and 8 (Figs. 1 and 2). Most of the strains showed lipolytic activity under these conditions, even in case of the lack of fatty substrates in the medium. The exception is *B. mycoides* A134, which did not show activity at pH 7, and in the presence of glucose (Fig. 1).

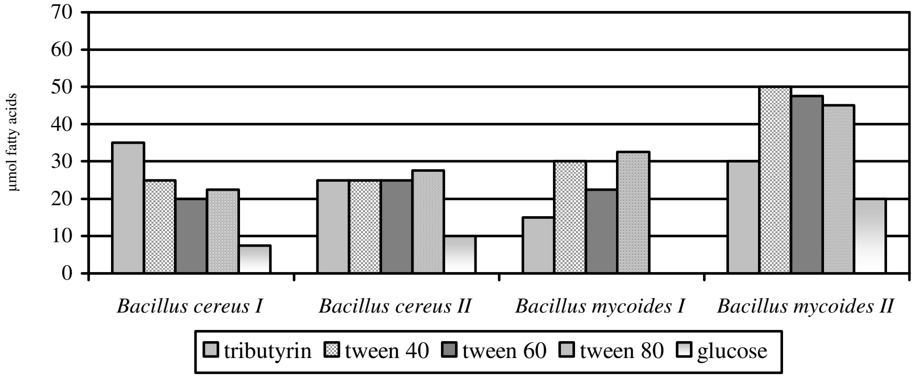

Fig. 1. Lipolytic activity of *B. cereus* and *B. mycoides* strains at pH 7

All strains under study showed the lowest activity at pH 7 on the medium with glucose as the source of carbon. Whereas the highest activity depended on the strain itself. *B. cereus* strain liberated the highest amount of fatty acids (35.0 µmol) in the presence of tributyrin, strains *B. cereus* G10 and *B. mycoides* A134 on the medium with the addition of Tween 80 (27.5 and 32.5 µmol respectively), and *B. mycoides* G3 strain in the presence of Tween 40 - 50.0 µmol (Fig. 1).

However, the highest amount of µmoles of fatty acids was liberated at pH 8. All strains under study showed favourable activity towards the medium with Tween 40, and the highest amount (52.5 µmol) was obtained for the soil strain *B. cereus* A96. The exception was *B. mycoides* G3, which as at pH equal to 7, preferred the medium with Tween 60, and liberated 60.0 µmol of fatty acids (Fig. 2).

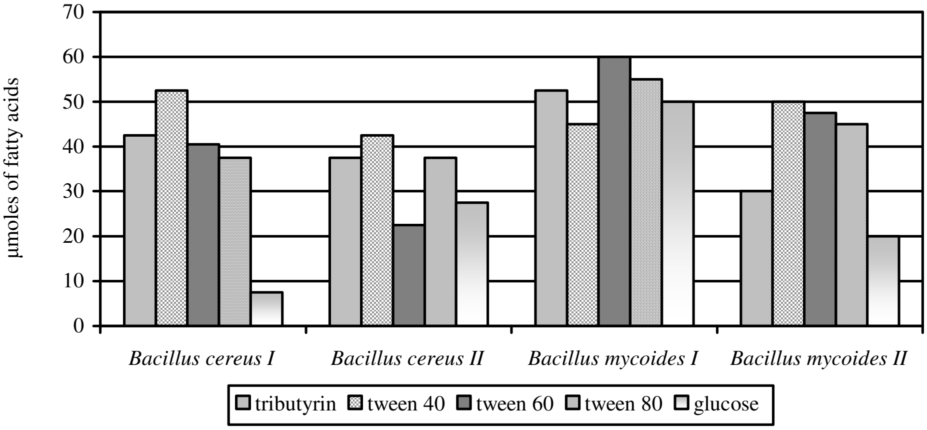

Fig. 2. Lipolytic activity of *B. cereus* and *B. mycoides* at pH 8

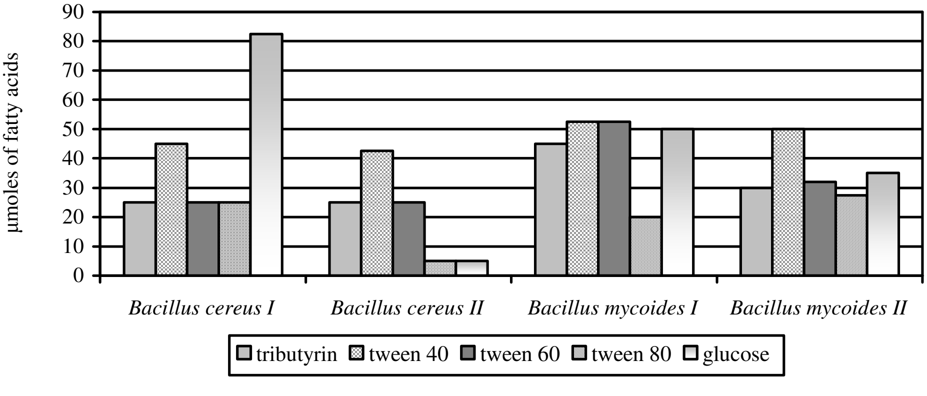

Fig. 3. Lipolytic activity of *B. cereus* and *B. mycoides* strains at 30°C

When analysing the influence of the temperature on the lipolytic activity, it was found that the highest amount of fatty acids was noted at 30 and 40°C (Figs. 3 and 4), and the lowest at 50 and 60°C. The highest values, for most of the strains, were obtained at 30°C on the medium with Tween 40, and the most active was the soil strain *B. mycoides* (52.5 µmol). The exception is the soil strain of *B. cereus*, which liberated 82.5 µmol on the medium with glucose (Fig. 3).

The amount of liberated fatty acids, on respective media, was slightly lower at 40°C, when compared with the amounts obtained at 30°C. Only the strains of *B. mycoides* A134 and G3, showed higher activity on the medium with glucose, liberating 87.5 and 42.5 µmol, respectively (Fig. 4).

The bacterial strains, at 40°C, used most favourably the fatty substrate in form of Tween 40, obtaining the highest values for the following strains of *B. mycoides:* A134 and G3, 52.5, noted as 52.5 and 45.0 µmol respectively. In the presence of Tween 80, examined strains liberated only 10 to 20 µmol, which proved their lowest lipolytic activity (Fig. 4).

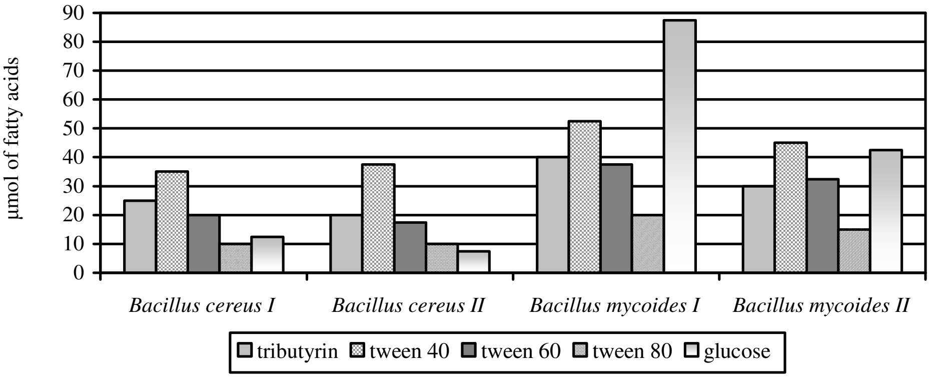

Fig. 4. Lipolytic activity of *B. cereus* and *B. mycoides* strains at 40°C

Summary and conclusion

The research proved significant diversity of lipolytic activity of *Bacillus cereus* and *Bacillus mycoides* strains, towards the source of carbon, pH and the temperature analysed in the experiment. Based on the obtained results following conclusions were drawn:

1. The highest amount of fatty acids was liberated by the strains at 30°C and pH equal to 8. Under these conditions the most favourable medium was with the addition of Tween 40, as the source of fatty substrate.
2. Strains under study were active even if there was no fatty substrate in the growth medium.
3. The most active were *B. mycoides* strains.
4. Individual strains of *B. cereus* and *B. mycoides* showed diversity in terms of liberated µmoles of fatty acids, which was influenced by the environment from which they were isolated.

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WPŁYW WARUNKÓW ŚRODOWISKA NA AKTYWNOŚĆ LIPOLITYCZNĄ SZCZEPÓW *Bacillus* sp.

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Abstrakt: Celem podjętych badań była ocena aktywności lipolitycznej szczepów *B. cereus* oraz *B. mycoides* w zależności od źródła węgla, pH oraz temperatury. Do badań wykorzystano 2 szczepy *Bacillus cereus* oraz 2 szczepy *Bacillus mycoides* wyizolowane z gleby i wody. Źródłem węgla w pożywkach były substraty tłuszczowe: tributyryna, Tween 40, Tween 60, Tween 80 oraz glukoza. Aktywność lipolityczną oznaczono w zakresie pH od 5 do 8 oraz w temperaturach od 30 do 60ºC. Oznaczenia aktywności lipolitycznej przeprowadzono metodą miareczkową, a wyniki podano jako ilość uwolnionych µmoli kwasów tłuszczowych. W przeprowadzonym doświadczeniu ilość uwolnionych µmoli kwasów tłuszczowych uzależniona była od rodzaju substancji tłuszczowej zawartej w podłożu, pH oraz temperatury. I tak, badane szczepy *B. cereus* oraz *B. mycoides* wykazywały najmniejszą aktywność przy pH 5 oraz 6, natomiast największą aktywność stwierdzono przy pH 7 i 8. W tych warunkach większość szczepów wykazywała aktywność lipolityczną nawet przy braku substratu tłuszczowego w podłożu. Najwięcej µmoli kwasów tłuszczowych uwolniono przy pH 8 na podłożu z dodatkiem Tween 40, a największe wartości (52,5 µmol) uzyskano dla glebowego szczepu *B. cereus*. Analizując wpływ temperatury na aktywność lipolityczną, stwierdzono, iż najwięcej µmoli kwasów tłuszczowych odnotowano w temperaturze 30 i 40ºC, a najmniej w 50 i 60ºC. Największe wartości dla większości szczepów uzyskano w temperaturze 30ºC na podłożu z dodatkiem Tween 40, gdzie najbardziej aktywny okazał się glebowy *B. mycoides* (52,5 µmol). Wyjątek stanowi glebowy *B. cereus*, który na podłożu z dodatkiem glukozy uwolnił aż 82,5 µmol. Uwzględniając wszystkie analizowane źródła węgla i parametry, należy stwierdzić, iż najaktywniejszymi były szczepy *B. mycoides*.

Słowa kluczowe: *Bacillus* sp., lipazy, tributyryna, Tween

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CONCENTRATION OF SELECTED PRIORITY SUBSTANCES IN KLODNICA RIVER CATCHMENTS

ZAWARTOŚĆ WYBRANYCH SUBSTANCJI PRIORYTETOWYCH W ZLEWNI RZEKI KLODNICY

Abstract: Results of research on concentration of selected priority substances in Klodnica River catchments were presented. To the research were chosen following priority substances: cadmium, mercury, anthracene, benzo[a]pyrene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene. Mercury was determed with the method of cold vapour atomic absorption spectrometry (CV AAS) using RA-915$^+$ analyzer with RP-91 attachment produced by Lumex. Cadmium was determed with the method of direct electrothermal atomic absorption (ET AAS) using AAnalyst 600 appliance produced by Perkin Elmer. PAHs were analysed with high-performance liquid chromatography method with fluorescence detection (HPLC-FLD) using liquid chromatograph HP 1050 produced by Hewlett Packard. The results of the research were compared with allowable concentration of priority substances in surface waters included in the proposal of UE concerning Environmental Quality Standards, and with requirements of the Polish legislation as the values determining water state indicators.

Keywords: priority substances, river catchments, screening study

Klodnica is the longest river flowing through the region of the Upper Silesia Industrial District (GOP). It is one of right-bank inflows of Odra river. It is over 75 km long, 40 km of which is situated in GOP. The river-head of Klodnica is situated in the southern part of Katowice, in Murckowski Forests. The river flows through the biggest cities of Upper Silesia, such as: Katowice, Ruda Slaska, Mikolow, Zabrze, Bytom, Gliwice and it ends its course in Kedzierzyn-Kozle flowing into Odra [1]. The most important inflows of Klodnica are right-bank: Bielszowicki Stream, Czarniawka Stream, Bytomka Stream, Drama Stream, Toszecki Stream and left-bank: Jamna Stream, Gieraltowicki Stream. In the course of the river three big barrage reservoirs are located: large Dzierzno Lake on Klodnica River, small Dzierzno Lake on Drama Stream and reservoir Plawniowice on Toszecki Stream. The biggest pollution discharges are drained to Klodnica in its main course, where the river and its inflows flow through densely populated and most industrialized avtive areas of Upper Silesia.

The main sources of pollution of Klodnica River are sewage discharge and that sewage flowing down from industrial and post-industrial areas, sewage flowing into river from the areas, where industrial landfills and dumping-grounds are located, discharges of mine waters that contain a lot of salt and sewages from region of cities and communies where there are no sewage treatment plant, or where sewage can not be refined in a sufficient way with the use of applied technology [2].

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Polish and European regulations in aspect of priority substances

One of main principles of The Water Framework Directive (WFD) is to achieve good chemical condition of waters, that is assessed by taking into consideration presence of substance that are proved to have or are highly probable to have a harmful influence on ecosystems and water organisms as well as on people's health (so called priority substances). A full list of priority substances can be found in annex X WFD [3]. A regulation by Minister of Environment on ways of classifying condition of uniform parts of surface waters, that came into force in 2008, contains limit values of markers of condition of waters [4]. Thanks to those markers it is possible to unequivocally interpret results of undertaken research. Through realisation of principles of WFD it is assumed that good chemical condition of waters will be achieved till 2015, establishing at the same time year 2025 as a deadline for elimination of substances recognised as priority dangerous substances from group of priority substances.

This paper presents results of screening research on content of chosen priority substances in catchment of Klodnica River. Two heavy metals: mercury and cadmium were chosen for research as well as compounds of group of polycyclic aromatic hydrocarbons: anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i] perylene, indeno[1,2,3-c,d]pyrene. Received contents of mercury, cadmium and above-mentioned PAHs in surface waters were compared with regulations of EU - Environmental Quality Standards (EQS) [5], and with regulations of Polish legislation [4]. Acceptable contents of mercury, cadmium and PAHs according to EQS and according to Polish legislation were presented in Table 1.

Table 1

Allowable concentrations of mercury, cadmium and selected PAHs by EQS [5], and by Polish legislation [4]

Priority substances	**EQS**		**(DzU 2008 Nr 162, poz. 1008)**	
	Annual average concentration [μg/dm^3]	**Maximum allowable concentration [μg/dm^3]**	**Annual average concentration [μg/dm^3]**	**Maximum value of concentration [μg/dm^3]**
Mercury and its compounds	0.05	0.07	-	0.07
Cadmium and its compounds	0.08÷0.25[1]	0.45÷1.5[1]	-	0.45÷1.50[2]
Anthracene	0.10	0.40	-	0.40
Benzo[a]pyrene	0.05	0.10	-	0.10
Σ Benzo[b]fluoranthene + Benzo[k]fluoranthene	0.030	not applicable	0.030	-
Σ Benzo[g,h,i]perylene + Indeno[1,2,3-c,d]pyrene	0.002	not applicable	0.002	-

[1] Depending on water hardness classes [5]

[2] Depending on water hardness classes [4]

\- No available data

Study of area

Researches on content of priority substances (mercury, cadmium and PAHs) in catchment of Klodnica River were conducted in four measurement sessions in 2008, with sampling frequency once a quarter. First session was conducted on 24th January 2008, second on 9th April 2008, third between 11th and 12th September 2008 and the fourth one on

11th December 2008. Eight measurement points were examined in the four sessions. The points were situated from the spring of Klodnica River in Brynow district of Katowice, through inlets of sewage and run-offs of sewage from sewage treatment plants placed in the course of the river next to mouth of most important inflows, right up to inlet of Klodnica River to barrage reservoir "Dzierzno Duze" and the inlet of the river to Odra River in Kedzierzyn-Kozle.

Methods of analysis

To determine concentration of mercury in waters of Klodnica River and its inflows a multifunctional analyser of mercury RA-915+ has been used. Its work is based on Zeeman atomic absorption spectroscopy with use of modulation with high polarisation of light, together with RP-91 attachment. The attachment is used to determine concentration of mercury in liquid samples with use of technique of "cold vapour". Limit of detection of the used method is 0,002 μg/dm^3. Cadmium in the waters was determined with technique of Electrothermal Atomic Absorption Spectrometry (ET AAS), with use of spectrometer AAnalyst 600 produced by Perkin Elmer. Limit of detection of the used method is 0.2 μg/dm^3. In order to determine concentration of PAHs the samples of water were extracted with SPE technique with use of octadecyl-(C_{18}) phase. Extracts were analysed with technique of high performance liquid chromatography with fluorescence detection (HPLC-FLD) with use of liquid chromatograph HP 1050 produced by Hewlett Packard. Limit of detection of used method is different depending on determined compound and is, as follows: anthracene 0.009 μg/dm^3, benzo[a]pyrene 0.003 μg/dm^3, sum (benzo[b]fluoranthene and benzo[k]fluoranthene) 0.004 μg/dm^3, sum (benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene) 0.003 μg/dm^3.

Results

Concentrations of mercury, cadmium and concentration of chosen PAHs for Klodnica River and its inflows that were obtained in four measurement sessions are shown in Tables 2 and 3.

Table 2

Concentrations of mercury and cadmium for Klodnica River and its side streams which were obtained in four measurement sessions [μg/dm^3]

Number of sampling point	**Hg [μg/dm^3]**				**Cd [μg/dm^3]**			
	Sesion I	**Sesion II**	**Sesion III**	**Sesion IV**	**Sesion I**	**Sesion II**	**Sesion III**	**Sesion IV**
„K1"	-	0.086	0.100	-	-	1.010	0.200	-
„D1"	-	0.016	-	-	-	<DL	-	-
„D2"	-	0.010	-	-	-	0.370	-	-
„D3"	-	0.048	-	-	-	0.220	-	-
„K4"	-	0.266	0.707	-	-	0.650	<DL	-
„K5"	0.137	0.182	0.458	0.108	<DL	0.500	0.380	0.510
„D4"	-	-	0.070	-	-	-	<DL	-
„K8"	-	0.236	-	-	-	0.330	-	-
„D5"	-	0.146	0.893	-	-	0.460	0.340	-
„D6"	-	-	0.069	-	-	-	<DL	-
„K9"	-	0.339	-	-	-	0.220	-	-

„D7”	0.163	0.190	0.084	0.083	<DL	<DL	0.220	<DL
„D8”	--	0.129	-	-	-	<DL	-	-
„D9”	-	0.189	0.296	-	-	0.650	0.300	-
„D10”	-	0.402	0.244	-	-	0.690	<DL	-
„D11”	-	-	0.052	-	-	-	<DL	-
„D12”	-	-	0.053	-	-	-	<DL	-
„D13”	-	0.354	-	-	-	<DL	-	-
„K10”	-	0.142	0.066	-	-	<DL	<DL	-
„K11”	0.131	0.337	0.075	0.369	0,600	0.590	<DL	<DL
„K14”	-	0.475	0.088	-	-	0.530	<DL	-
„K15”	-	0.179	-	-	-	<DL	-	-

Table 3

Concentrations of chosen PAHs for Klodnica River and its side streams that were obtained in four measurement sessions [µg/dm^3]

Number of sampling point	**Anthracene [µg/dm^3]**				**B[a]P^1 [µg/dm^3]**			
	Sesion I	**Sesion II**	**Sesion III**	**Sesion IV**	**Sesion I**	**Sesion II**	**Sesion III**	**Sesion IV**
„K1”	-	<DL	-	-	-	<DL	-	-
„D1”	-	<DL	-	-	-	<DL	-	-
„D2”	-	<DL	-	-	-	<DL	-	-
„D3”	-	<DL	-	-	-	<DL	-	-
„K4”	-	<DL	<DL	-	-	<DL	0.005	-
„K5”	<DL	<DL	<DL	0.011	0.003	<DL	<DL	0.015
„D4”	-	-	<DL	-	-	-	<DL	-
„K8”	-	<DL	-	-	-	<DL	-	-
„D5”	-	<DL	<DL	-	-	0.018	0.018	-
„D6”	-	-	<DL	-	-	-	<DL	-
„K9”	-	<DL	-	-	-	0.004	-	-
„D7”	<DL	<DL	0.012	<DL	0.007	0.006	0.003	0.014
„D8”	-	<DL	-	-	-	0.007	-	-
„D9”	-	<DL	<DL	-	-	<DL	<DL	-
„D10”	-	0.052	0.013	-	-	0.011	0.010	-
„D11”	-	-	<DL	-	-	-	<DL	-
„D12”	-	-	0.010	-	-	-	0.006	-
„D13”	-	<DL	-	-	-	0.003	-	-
„K10”	-	<DL	<DL	-	-	0.006	0.004	-
„K11”	<DL	<DL	0.012	0.031	0.004	<DL	<DL	0.058
„K14”	-	<DL	<DL	-	-	<DL	<DL	-
„K15”	-	<DL	-	-	-	<DL	-	-

Number of sampling point	**Σ B(b)F +B(k)F^2 [µg/dm^3]**				**Σ B(ghi)P +IP^3 [µg/dm^3]**			
	Sesion I	**Sesion II**	**Sesion III**	**Sesion IV**	**Sesion I**	**Sesion II**	**Sesion III**	**Sesion IV**
„K1”	-	<DL	-	-	-	<DL	-	-
„D1”	-	<DL	-	-	-	<DL	-	-
„D2”	-	<DL	-	-	-	<0.004	-	-
„D3”	-	<DL	-	-	-	<DL	-	-
„K4”	-	<DL	<DL	-	-	<DL	0.006	-
„K5”	0.004	<DL	<DL	0.021	0.002	0.004	<DL	0.018
„D4”	-	-	<DL	-	-	-	<DL	-
„K8”	-	<DL	-	-	-	0.004	-	-
„D5”	-	0.014	<DL	-	-	0.028	0.011	-
„D6”	-	-	<DL	-	-	-	<DL	-
„K9”	-	<DL	-	-	-	0.011	-	-
„D7”	0.009	0.011	<DL	0.018	0.010	0.008	0.004	0.022

„D8”	-	0.014	-	-	-	0.012	-	-
„D9”	-	<DL	<DL	-	-	<DL	<DL	-
„D10”	-	0.013	0.006	-	-	0.009	0.006	-
„D11”	-	-	<DL	-	-	-	<DL	-
„D12”	-	-	0.006	-	-	-	0.004	-
„D13”	-	0.007	-	-	-	0.005	-	-
„K10”	-	<DL	<DL	-	-	0.010	0.003	-
„K11”	0.005	<DL	0.006	0.078	0.007	0.004	0.010	0.091
„K14”	-	<DL	<DL	-	-	0.004	<DL	-
„K15”	-	<DL	-	-	-	<DL	-	-

[1] B[a]P - Benzo[a]pyrene

[2] B[b]F + B[k]F - Σ Benzo[b]fluoranthene + Benzo[k]fluoranthene

[3] B[g,h,i]P + IP - Σ Benzo[g,h,i]perylene + Indeno[1,2,3-c,d]pyrene

Points located on inlets and outlets of three sewage treatment plants draining treated sewage to Klodnica river were examined during third measurement session. Table 4 presents obtained contents of mercury, cadmium and PAHs on inlets of sewage and outlets of treated sewage from sewage treatment plants. Two of the examined sewage treatment plants were of mechanical-biological-chemical type and one was of mechanical-biological type. In all three cases we can notice a definite improvement of water quality concerning cadmium, mercury and PAHs in points located on outlets of examined sewage treatment plants.

Table 4

Concentrations of mercury, cadmium and concentrations of chosen PAHs, which were obtained on inlets and outlets of three sewage treatment plants draining treated sewage to Klodnica River [μg/dm^3]

Priority substances		**Sewage treatment plants mechanical-biological type**	**Sewage treatment plants mechanical-biological-chemical type**	**Sewage treatment plants mechanical-biological-chemical type**
Hg	Inlet of sewage [μg/dm^3]	0.458	0.476	0.283
	Outlet of treated sewage [μg/dm^3]	0.257	0.123	0.093
Cd	Inlet of sewage [μg/dm^3]	0.33	<DL	<DL
	Outlet of treated sewage [μg/dm^3]	<DL	<DL	<DL
Anthracene	Inlet of sewage [μg/dm^3]	<DL	0.010	0.066
	Outlet of treated sewage [μg/dm^3]	<DL	<DL	<DL
B[a]P	Inlet of sewage [μg/dm^3]	0.004	<DL	0.023
	Outlet of treated sewage [μg/dm^3]	<DL	<DL	<DL

Σ B[b]F +B[k]F	Inlet of sewage [µg/dm^3]	0.056	<DL	0.032
	Outlet of treated sewage [µg/dm^3]	<DL	<DL	<DL
Σ B[g,h,i]P +IP	Inlet of sewage [µg/dm^3]	<DL	<DL	0.023
	Outlet of treated sewage [µg/dm^3]	<DL	<DL	<DL

[1] B[a]P - Benzo[a]pyrene

[2] B[b]F + B[k]F - Σ Benzo[b]fluoranthene + Benzo[k]fluoranthene

[3] B[g,h,i]P + IP - Σ Benzo[g,h,i]perylene + Indeno[1,2,3-c,d]pyrene

Conclusion

- ✓ Concentrations of mercury, which were obtained in conducted researches in all point in four measurement sessions range from 0.01 to 0.893 µg/dm^3. According to Environment Quality Standards EQS [6], and under decree by Minister of Environment on ways of classifying condition of uniform parts of surface waters [8] the maximum permissible content of mercury in surface waters should not exceed 0.07 µg/dm^3. Concentrations of mercury did not exceed border markers only on leftside inflows of Klodnica River in points D1, D2 and D3. Concentration of mercury near to maximum permissible value was observed in points D4, D6 and D11. In the rest of examined points content of mercury considerably exceeds the permissible value.
- ✓ Concentrations of cadmium obtained in all examined point in four measurement sessions range from <0.2 to 1.01 µg/dm^3. Polish legislation as well as European Environment Quality standards accept increased content of cadmium up to 1.5 µg/dm^3 depending on class of water hardness. In 2007 Provincial Inspectorate for Environment Protection located fifteen points of operational monitoring [6] on Klodnica River. On this basis waters of Klodnica River in nine points were credited “V” class of quality - waters of bad quality, and in six points Klodnica River was credited “IV” class of quality - water of unsatisfying quality. Providing waters of Klodnica River belong to “IV” class of quality, then concentration of cadmium according to EQS [5] and Polish legislation [4] should not exceed 0.9 µg/dm^3. This condition was not fulfilled only in point K1. Providing waters of Klodnica River belong to “V” class of quality, then concentration of cadmium according to EQS [5] and Polish legislation [4] should not exceed 1.5 µg/dm^3. This condition was fulfilled in all examined points.
- ✓ Determined concentrations of PAHs in Klodnica River and its inflows for both measurement sessions range as follows: anthracene from <0.009 to 0.066 µg/dm^3, benzo[a]pyrene from <0.003 to 0.0180 µg/dm^3, sum (benzo[b]fluoranthene and benzo[k]fluoranthene) from <0.0045 to 0.032 µg/dm^3, sum (benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene) from <0.0025 to 0.0275 µg/dm^3. In majority of examined points concentration of PAHs was on level enabling its determination with used measurement method and comparing these concentrations with values of European Environment Quality standards EQS [5] as well as to limit values of concentration of priority substances described in decree by Minister of Environment [4]. Only few

measurement points located near collectors of mine waters from hard coal mines and points located on inlets of sewage treatment plants placed on Klodnica River slightly exceed permissible concentration. These are sum (benzo[b]fluoranthene and benzo[k]fluoranthene) and sum (benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene).

- ✓ The above research confirmed that there are three "hot points" [2], that is places of considerable excess of permissible concentrations of dangerous substances, in catchment of Klodnica River. First of them is point K4. In this point concentration of mercury was 0.707 $\mu g/dm^3$ in III measurement session and concentration of cadmium was 0.65 $\mu g/dm^3$ in II measurement session. Next „hot point" is point D5, where concentration of mercury was 0.893 $\mu g/dm^3$ in III measurement session and concentration of cadmium was 0.46 $\mu g/dm^3$ in II measurement session. In this point also small excess of PAHs were noticed. Third „hot point" is point D10. Concentration of mercury in this point was 0.402 $\mu g/dm^3$ and concentration of cadmium was 0.69 $\mu g/dm^3$.
- ✓ Examined were also points located near inlets of sewage and outlets of treated sewage from three sewage treatment plants working in catchment of Klodnica River. In all three cases comparing concentrations of pollution on inlets and outlets of sewage, both for mercury and cadmium as well as for PAHs, a considerable improvement of water quality on outlets of treated sewage can be observed.
- ✓ Conducted screening researches have proved, that in waters that are seriously anthropologically changed there may occur a problem of exceeding permissible concentrations of mercury, cadmium, sum (benzo[b]fluoranthene and benzo[k]fluoranthene) and sum (benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene), defined by European markers of environment and Polish legislation.

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ZAWARTOŚĆ WYBRANYCH SUBSTANCJI PRIORYTETOWYCH W ZLEWNI RZEKI KŁODNICY

Instytut Ekologii Terenów Uprzemysłowionych

Abstrakt: Przedstawiono wyniki badań zawartości wybranych substancji priorytetowych w zlewni rzeki Kłodnicy. Do badań wybrane zostały następujące substancje priorytetowe: kadm, rtęć, antracen, benzo[a]piren, benzo[b]fluoranten, benzo[k]fluoranten, benzo[g,h,i]perylen, indeno[1,2,3-c,d]piren. Rtęć oznaczana była techniką zimnych par absorpcyjnej spektrometrii atomowej (CV AAS) za pomocą analizatora RA-915$^+$ z przystawką RP-91 firmy Lumex, kadm oznaczano metodą absorpcyjnej spektrometrii atomowej z elektrotermiczną atomizacją próbki

(ET AAS) za pomocą urządzenia AAnalyst 600 firmy PerkinElmer, WWA analizowano techniką wysokosprawnej chromatografii cieczowej z detekcją fluorescencyjną (HPLC-FLD) przy użyciu chromatografu cieczowego HP 1050 firmy Hewlett Packard. Otrzymane wyniki badań porównano z dopuszczalnymi stężeniami substancji priorytetowych w wodach powierzchniowych zawartych w propozycji Unii Europejskiej dotyczących standardów jakości środowiska - Environmental Quality Standards (EQS) oraz z wymaganiami określanymi przez polskie prawodawstwo jako wartości graniczne chemicznych wskaźników jakości wód.

Słowa kluczowe: substancje priorytetowe, zlewnia rzeczna, badania screeningowe

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Jadwiga OPYDO[1]

DETERMINATION OF AVAILABLE FORMS OF THE MANGANESE IN SOILS BY STRIPPING VOLTAMMETRY

OZNACZANIE MANGANU PRZYSWAJALNEGO W GLEBACH METODĄ WOLTAMPEROMETRYCZNĄ

Abstract: Conditions of determination of manganese(II) in soils samples using differential pulse cathodic stripping voltammetry in an electrochemically enriched solution (DPCSV-EE) at hanging mercury drop electrode have been optimized. Manganese was extracted from soils with 1 mol·dm^{-3} HCl. 0.2 mol·dm^{-3} ammonia/ammonium chloride buffer (pH = 8÷9) was supporting electrolyte. Deposition time was 15÷30 s, deposition potential –1.7 V, initial potential –1.15 V and end potential –1.7 V vs. NEK. In the above conditions Mn(II) peak height vs. its concentration dependence is linear up to 7 · 10^{-5} mol·dm^{-3} Mn(II). The accuracy and precision of the proposed method are also satisfactory. The relative standard deviation for 90 ppm Mn(II) equals 12.8% and for 145 ppm equals 8.9%. The method has been applied to study the content of available forms of the manganese in soils from the vicinity of Zawiercie Steel Mill and Huta Glogow Smeltery.

Keywords: available manganese, stripping voltammetry, soils extracts

Manganese is a trace element, which plays a significant role in metabolizm of plants. Most importantly, it is involved in oxidation-reduction reactions [1]. Thus, the level of manganese in soils in agricultural areas needs to be monitored. Currently, in Poland available forms of manganese are determined with the use of spectrophotometric and spectrometric methods [2, 3].

Stripping voltammetry is one of more sensitive methods used in trace analysis. It has an advantage over typical methods like spectrometric ones because the cost of electroanalytical equipment is rather low. Many voltammetric procedures have been proposed for the determination of trace amounts of manganese: by adsorptive stripping voltammetry [4, 5], by differential pulse anodic stripping voltammetry [6, 7], by differential pulse voltammetry [8-10], by square wave voltammetry [11-14] and by differential pulse cathodic stripping voltammetry in an electrochemically enriched solution (DPCSV-EE) [15-17]. In the present paper possible application of DPCSV-EE method for determination of available forms of manganese in soil extracts was analyzed. Manganese were extracted from soil using 1 mol·dm^{-3} HCl. The method has been applied to study the degree of contamination of soils in the vicinity of Zawiercie Steel Mill and Huta Glogow Smeltery as well as Kornik reference area with manganese.

Experimental

Reagents

Hydrochloric acid, ammonia ("Suprapur", Merck), ammonium chloride (POCh, Poland). Ammonia + ammonium chloride buffer solution (pH 8÷9) prepared by mixing

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appropriate amounts of 0.4 mol·dm^{-3} NH_3 and 0.4 mol·dm^{-3} NH_4Cl. Standard solution of Mn(II) (1 g·dm^{-3}) were prepared from ampoules (Merck). Solutions with concentrations below 10^{-3} mol·dm^{-3} were prepared just before use. Water was doubly distilled in a quartz still.

Apparatus

Voltammetric measurements were carried out using a μAUTOLAB analyzer (ECO CHEMIE, Netherlands). A hanging mercury drop electrode (HMDE), having the surface of 3 mm^2, produced by Laboratorni Pristroje (Czech Republic), was the working electrode, KCl saturated calomel electrode (SCE), produced by Radiometer (Denmark), was the reference electrode, and Pt wire served as the counter electrode.

Procedures

Soil samples were taken from the top layer of soil: at the depth of 0÷20 cm for the copper mining area and Kornik and at the depth of 20÷40 cm for the Zawiercie. The soil was dried then homogenized and sieved through a mesh of 1 mm pore size. 10 g of soil was put into a 250 cm^3 bottle. 100 cm^3 of 1 mol·dm^{-3} HCl was added and the soil was shaken for 1 hr on a mechanical shaker. The extract was then filtrated.

1 to 3 cm^3 of the extract was put in a 25 cm^3 standard flask. The residue was diluted in 0.2 mol·dm^{-3} ammonia/ammonium chloride buffer solution. The pH was adjusted to 8÷9.

20 cm^3 of solution was transferred into a measuring vessel. After deaeration of the solution with purified nitrogen the manganese was deposited on the surface of electrode for 15 to 30 s (depending on the expected manganese content) at –1.70 V in stirred solution. 5 s after the stirrer, the potential was switched to the value –1.15 V and the reformed manganese(II) ions were determined by differential pulse cathodic voltammetry, within the potential range from –1.15 to –1.70. This cycle was repeated thrice. Mn(II) concentrations were determined using triple standard addition.

Results and discussion

Method of Mn(II) determination in ammonia/ammonium chloride buffer solution by differential pulse cathodic stripping voltammetry in an electrochemically enriched solution (DPCSV-EE) was prepared in the previous study [17]. It was used to assess the total manganese content in soils, ie manganese content in soil extracts obtained by using aqua regia extraction. The aim of the present study was to assess if previously prepared method allows to determine manganese available, ie manganese soluble in 1 mol·dm^{-3} HCl. Thus, influence of soil extract addition on analytic signal in determination of Mn(II) was checked.

This influence was analyzed by making a determination 2 · 10^{-5} mol·dm^{-3} Mn(II) in the presence of different amounts of soil extract. Experiments showed that maximum volume of the soil extract should not exceed 3 cm^3 (Fig. 1). With larger volumes of soil extract strong dampening of the manganese signal can be seen.

Using literature and present examination results optimal conditions for Mn(II) determination have been found.

- The base electrolyte: 0.2 mol·dm^{-3} ammonia/ammonium chloride buffer (pH = 8÷9)
- Deposition potential –1.7 V

- Initial potential –1.15 V
- End potential –1.7 V
- Deposition time 15÷30 s
- Amount of the soil extract added: 1 to 3 cm^3

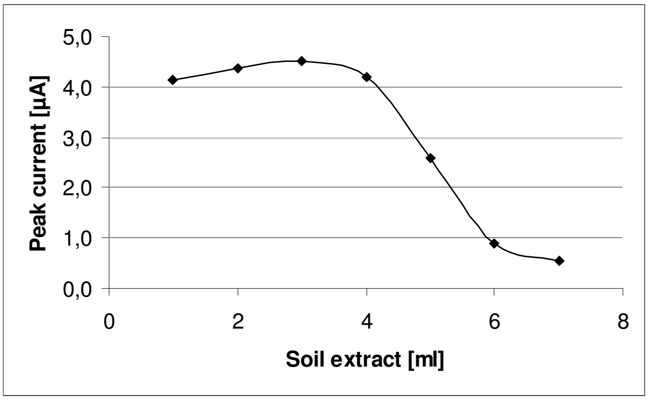

Fig. 1. Dependence of the peak current for the Mn(II) on the amount of soil extract added. Mn(II) concentration $2\cdot10^{-5}$ $mol\cdot dm^{-3}$. Supporting electrolyte 0.2 $mol\cdot dm^{-3}$ ammonia/ammonium chloride buffer (pH = 8÷9). Deposition potential –1.7 V. Initial potential –1.15 V. End potential –1.7 V. Deposition time 15 s

In the above conditions Mn(II) peak height vs its concentration dependence in the presence of 2 cm^3 of soil extract is linear up to $7\cdot10^{-5}$ $mol\cdot dm^{-3}$ Mn(II).

The developed method was statistically evaluated by estimating the precision and recovery manganese determined in the presence of soil extracts (Tab. 1). The recovery of added metal was very good and the precision was satisfactory (the relative standard deviation for 90 ppm Mn(II) equals 12.8% and for 145 ppm Mn(II) equals 8.9%).

Table 1

Recovery and precision of the determination of available forms of manganese in the analytical procedure

Series	Added [ppm]	Found [ppm]	Recovery [%]	S.D. [ppm]	R.S.D. [%]
I	90	88.3	98.1	11.3	12.8
II	145	136	93.8	12.1	8.9

Table 2

Manganese content in soil samples

Sampling regions	Depth [cm]	Research area	Mn available [ppm]	Mn total [ppm]
Copper mining area	0÷20	300 m south of Glogow Copper Smelter	82	106
	0÷20	600 m north of Glogow Copper Smelter	109	102
	0÷20	800 m north of Glogow Copper Smelter	85	166
	0÷20	3000 m north of Glogow Copper Smelter	36	33
Krakowsko-Czestochowska Upland	20÷40	2000 m north of Zawiercie Steel Mill	35	38
Kornik (reference area)	0÷20	Experimental Forest Zwierzyniec	82	134

The method has been applied to study the degree of contamination of soils in the vicinity of Zawiercie Steel Mill and Huta Glogow Smeltery with manganese (Tab. 2). For comparison results of determination of total manganese were presented in the last column [17].

Results show that manganese present in the examined soils poses no threat to plants and animals. According to Polish Branch Standard [3] available manganese content in soils equaled 9 to 110 ppm corresponds to soils of the III class of manganese content; such content is assessed as low.

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OZNACZANIE MANGANU PRZYSWAJALNEGO W GLEBACH METODĄ WOLTAMPEROMETRYCZNĄ

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Abstrakt: Przeanalizowano warunki oznaczania manganu(II) w próbkach gleb metodą woltamperometrii katodowej z elektrochemicznym wzbogacaniem (DPCSV-EE), w technice różnicowej pulsowej z użyciem wiszącej rtęciowej elektrody kroplowej. Mangan z gleb ekstrahowano roztworem 1 mol·dm^{-3} HCl. Elektrolitem podstawowym był 0,2 mol·dm^{-3} bufor amoniakalny (pH = 8÷9). Czas zatężania wynosił 15÷30 s, potencjał zatężania –1,7 V, potencjał startu –1,15 V, potencjał końcowy –1,7 V wzgl. NEK. W tych warunkach krzywa kalibracji jest prostoliniowa do 7 · 10^{-5} mol·dm^{-3} Mn(II). Dokładność i precyzja opracowanej metodyki są zadowalające (względne odchylenie standardowe dla 90 ppm Mn(II) wynosi 12,8%, a dla 145 ppm Mn(II) 8,9%). Opracowaną metodykę wykorzystano do analizy zawartości przyswajalnych form manganu w glebach, pochodzących z okolic Huty Miedzi Głogów i Huty Stali Zawiercie.

Słowa kluczowe: przyswajalny mangan, woltamperometria stripingowa, próbki gleb

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LATVIAN STUDENTS' UNDERSTANDING ABOUT THE ROLE OF WIDESPREAD NON-METALLIC COMPOUNDS IN ENVIRONMENTAL CHEMICAL PROCESSES

ROZUMIENIE ROLI NIEMETALI W PROCESACH CHEMICZNYCH ZACHODZĄCYCH W ŚRODOWISKU PRZEZ STUDENTÓW ŁOTEWSKICH

Abstract: The standard of professional high school education in Latvia envisages including environmental education in the contents of professional schools as a separate subject, however, we see in practice that there are not teaching materials in chemistry education and environmental education provided for professional schools. The problem is even more acute due to the fact that the time provided for teaching chemistry in professional schools is approximately 3 times shorter than that in secondary schools. The research shows us that approximately 40% of professional high school students have knowledge about the character of chemical processes taking place in the environment. One of the main reasons for this insufficiency and the lack of understanding is the absence of didactical teaching materials.

Keywords: environmental education, chemistry education, professional education, integration of education

The environment where humans live nowadays consists of several environmental compartments - atmosphere (the air we breathe), hydrosphere (the water we use), lithosphere (soil). The activities made by humans influence the chemical behaviour of those environmental systems; therefore, humans have to be aware of the consequences interfering in natural processes. The protection of the environment and the effectiveness of environmental management depend on both the educated specialists and the education of society on environmental subjects.

Environmental education is the teaching/learning methods based on the aspirations of society to form healthy environment and live in an environmentally friendly society according to nature; in the same time solving actual problems of developing society. In order to develop the basics of environmental education system we have to consider all educational levels (primary school, secondary, high, professional schools and life-long education).

The task of our research was to ascertain the understanding of professional school students in Latvia about ecological problems connected with chemical processes and chemical substances that are formed as a result of activities of humans in the environment and the influence on nature and human beings.

In this article we give the results of the first phase of our research taking place in Riga Technical University's (RTU) Liepaja branch.

Methods of approach in acquiring environmental education

Environmental studies as the component of interdisciplinary teaching/learning process

Environmental education is a branch that tends to avoid specialized approach. It forms a relationship "science - technology - environment - society" [1]. According to this view

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students acquire environmental studies in integrated way - as a part of chemistry, physics and other natural sciences in many countries all over the world:

- In Germany's secondary schools (Hauptschule, Realschule, Gymnasium) environmental studies are components of *biology, geography, social studies*. In addition *Chemistry, physics, religion* give considerable investment in environmental education [www-1].
- In Poland basic teaching plan foresees to include interdisciplinary course of environmental studies in all subjects [2].
- National education standard of Estonia foresees the integration of environmental education in the context of all subjects in comprehensive and vocational (professional) schools [www-2].
- In Russia the Federal rule known as "The Protection of the Environment" was adopted in 2002; it anticipates forming a complex ecological education. Ecological education is included both in comprehensive and professional schools as a part of *biology, law, society studies*. However, they study the basics of ecological education included in subjects "ecology", "the basics of ecology", "nature protection" in some public and private schools [www-3].

The positive aspect of this approach is that students acquire knowledge that reveals the processes taking place in the environment from the point of view of various science branches.

The lack in this approach is the fact that students study only the issues connected with processes in this subject (in physics - physical pollution, in chemistry - chemical pollution, in biology - biological pollution, in geography - the problems of the distinction of nature resources). Therefore it is possible that a student is not able to see all problems connected with the environment as a whole. The issues connected with the environment could repeat. For example, the effects of Chernobyl accident are discussed in all above-mentioned subjects (chemistry - nuclear reactions, physics - radioactivity, biology - mutation, geography - pollution transfer). Thus, being repeated, a student can lose interest and understanding about the importance of knowledge.

Environmental studies as a part of independent education structure

The second approach - the acquiring of environmental education as a separate subject, allows us to gain knowledge about a wider scope of ecological issues but not always it can connect the ecological problems with the branch that has caused it. This approach is used in studies in colleges [www-4; www-5].

In the teaching/learning structure in Latvia we use both approaches in comprehensive schools:

- In comprehensive education students acquire issues interdisciplinary. They are integrated in the contents of other science subjects - in chemistry, physics, biology, geography [www-6].
- In professional schools (vocational, technical, professional high schools) students study environmental science.

There are only few opportunities to accent the bond *chemistry ↔ environmental science* due to the lack of time, teaching materials and fewer teaching lessons in timetable in professional schools for acquiring chemistry and environmental science. The positive aspect is that students acquire environmental science systematically using a definite teaching plan.

Materials and methods

The first phase of our survey - students' understanding about environmental questions in connection with chemical processes took place in RTU Liepaja branch. It is a unique multistage teaching establishment in Latvia where students can start their studies in a professional school after having got basic education. Such students can continue their chosen studies in college programs, professional bachelor or academical bachelor programs. After acquiring these programs students have possibility to study in Master of Science programs.

A survey was conducted using closed type questions. This was necessary in order to collect information about students' understanding connected with chemical processes in the environment. One of the reasons for that, there are not teaching materials in chemistry and environmental sciences for professional school students. The survey included 190 RTU Liepaja branch students. We compared three independent groups (No. 1, No. 2, No. 3) knowledge in chemical processes in the environment.

- RTU Liepaja branch students who had received their secondary education in comprehensive high schools, where environmental issues are integrated in the chemistry course (No. 1).
- RTU Liepaja branch students who have finished RTU Liepaja branch professional school (No. 2) and have studied *Chemistry* and *Environmental Science*.
- Current RTU Liepaja branch students who studied subjects *Chemistry* and *Environmental Science* in the 2008/09 academic year (No. 3).

The survey questions were connected with the chemical nature of natural and anthropogenic processes taking place in the environment. The competence of RTU Liepaja branch students in environmental questions in connection with chemical processes was evaluated by analyzing answers to 15 questions presented in the survey. Students had to choose one from four given answers which was the more precise from their point of view. One of the answers was incorrect, two were only partly precise, but the 4th was completely correct. Coding answer variations and giving them points from 1-4, we received the answer Moda, which variated from 2.00 to 3.00 in most cases. It shows that students have mediocre level of understanding evaluating environmental problems. It allowed specifying more characteristic mistakes in students' answers.

We chose the group of the issues because there is a very strong anthropogenic influence on the planet and all nature resource users and producers of material values have to know the causes of this influence and the consequences of pollution.

The validity of the survey, credibility on the whole, its suitability for cultural environment was characterized by Kronbach-Alpha coefficient - "α", and a suitability of every question by coefficient "s". Since "α" was 0.43 in our case, we were able to consider that complex of given questions was suitable for students.

We worked on data with computer program Excel and SPSS used in social sciences. Statistics allowed us to conclude about both positive aspects and lack in students' knowledge and plan further strategy.

Results and discussion

We give the answers of respondents to the problems connected with various pollution forms of the environment with IVA and VA group non-metallic elements (carbon, silicon, nitrogen and phosphorus) compounds.

Nowadays a problematic issue is atmospheric pollution with sulphur and nitrogen oxides which cause acid rain. The main resources of anthropogenic origin of these compounds are fuel burning. 70% from all SO_2 waste comes from coal burning [3]. The pollution is not considerable both on the whole and considering one inhabitant in Latvia. However, if we speak about NO_2 pollution in USA and Western Europe 50% from NO_2 emission comes from cars while it is 70% in Latvia [4]. The potential source of acid rain in Liepaja is the plant "Liepajas Metalurgs". Therefore the first question to respondents was about their competence in non-metallic element oxides causing acid rain (1. question, $s = 0.712$). This question is significant both for all specialists nowadays who are connected with branches where there are emission of nitrogen and sulphur compounds and also every driver. Mean index in correct answers was 39.5%. The best results showed the respondent group (No. 2) - RTU Liepaja branch students who have finished RTU Liepaja branch professional high school and continue studies in university - 42.9%. Weaker results were showed by group No. 1 who studies in university after finishing a secondary school - 37.8% and group No. 3 who had studied both chemistry and environmental sciences - 39.8%. Most part of the respondents who gave incorrect answer thought that acid rain is caused by sulphur and carbon oxides - it gives proof of insufficient knowledge in the qualities of inorganic acids.

Respondents gave answers to the question about the influence of acid rain on objects made from marble and limestone (2. question, $s = 0.636$). It is known that acid rain damages architectural and historical monuments. The rain with sulphur causes corrosion in these objects. The damage of the monuments causes the loss of aesthetical values as well as a great material loss. Average in correct answers was 19.5%. The best knowledge in this question showed RTU Liepaja branch professional high school students (26.1% correct answers) who acquired subjects *Chemistry* and *Environmental sciences* in the year 2008/09. The weakest results had the group consisting of RTU Liepaja branch professional high school graduates - 5% correct answers. It can be explained with their bad knowledge of historical names of chemical compounds.

The 3rd question was formulated according to acid rain, $s = 0.722$ (rain water $pH = 2.0 \div 4.5$). The question was connected with the water clearness both in swimming places and the quality of drinking water because drinking water is taken from open reservoirs in many places in Latvia. As we see from data in Table 1 there are more correct answers comparing with other questions (average 60%). It can be explained with the fact both professional high school and university students have done laboratory works on pH in various solutions.

Nowadays an actual problem is eutrophication (over growing of reservoirs) caused by anthropogenic water pollution. This process is due to phosphoric compounds [5]. The 4th question ($s = 0.694$) was about chemical elements causing the growing up of reservoirs most of all. Correct answers were given by 38.2% of respondents. RTU Liepaja branch students studied in professional high school gave the most successful answers. Most of the incorrect authors thought that carbon compounds are the reason for eutrophication. Thus we can conclude that students' knowledge about chemical elements being in mineral fertilizers is poor. We can declare that the integration of chemistry and environmental sciences problems concerning IVA and VA group non-metallic elements role in nature processes in teaching/learning processes is insufficient.

Comparing competence coefficient k (k = correct answers/the whole number of answers), we received following results: 1. selection - k = 0.38, 2. selection - k = 0.38 and 3. selection - k = 0.39.

Thus we conclude that the level of students' knowledge about chemical processes in the environment does not depend on the educational establishment where they received secondary education.

Table 1

RTU Liepaja branch students survey results (right answers from the selected number of respondents)

Selection	**1. question**		**2. question**		**3. question**		**4. question**		**k**
	number	**[%]**	**number**	**[%]**	**number**	**[%]**	**number**	**[%]**	
No. 1 = 82	31	37.8	13	15.9	51	62.2	30	36.6	0.38
No. 2 = 20	9	42.9	1	5.0	12	60.0	9	45.0	0.38
No. 3 = 88	35	40.2	23	26.1	51	57.9	29	33.0	0.39
Total 190	75	39.5	37	19.5	114	60	68	38.2	

We plan to continue our research including students, teachers, lecturers from professional schools and high schools in Latvia.

Conclusions

In order to promote professional school students' understanding about the world as a unified and indivisible whole we need to teach subjects *Chemistry* and *Environmental science* with advised enactment.

1. The results of the survey showed that only approximately one third of RTU Liepaja branch students completely understand the chemical basics of processes taking place in the environment and are able to connect the knowledge gained in chemistry and environmental sciences lessons.
2. The analysis of the survey results showed that the quality of students' knowledge does not depend on the methods used in teaching/learning chemical processes in the environment; it is determined by students' ability to notice relationship, to reason and draw conclusions.
3. It is necessary to create new teaching materials that will effectively bond chemistry with environmental studies to ease work on acquiring knowledge about chemical processes in the environment.

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ROZUMIENIE ROLI NIEMETALI W PROCESACH CHEMICZNYCH ZACHODZĄCYCH W ŚRODOWISKU PRZEZ STUDENTÓW ŁOTEWSKICH

Abstrakt: Standardy wyższego kształcenia zawodowego w szkołach na Łotwie przewidują, że w programie nauczania tych szkół edukacja środowiskowa stanowi odrębny przedmiot nauczania. Jednak w praktyce brak jest materiałów dydaktycznych do prowadzenia w tych szkołach nauki chemii i edukacji środowiskowej. Problem ten jest jeszcze bardziej znaczący ze względu na czas przewidziany na nauczanie chemii w szkołach zawodowych, jest on około 3 razy krótszy niż w szkołach średnich. Wyniki badań pokazują, że tylko około 40% uczniów szkół zawodowych posiada wiedzę na temat charakteru procesów chemicznych zachodzących w środowisku. Jednym z głównych powodów tak niskiego poziomu nauczania i braku zrozumienia jest brak odpowiednich materiałów dydaktycznych.

Słowa kluczowe: edukacja środowiskowa, edukacja chemiczna, szkolnictwo zawodowe, nauczanie zintegrowane

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DETERMINATION OF HEAT EFFECTS DURING THE OXIDATION OF BIOMASS IN SUBCRITICAL WATER

WYZNACZANIE EFEKTÓW CIEPLNYCH REAKCJI UTLENIANIA BIOMASY W ŚRODOWISKU WODY PODKRYTYCZNEJ

Abstract: The search of alternative energy sources is one of the most important elements of sustainable development policy. The biomass is a valuable source of renewable energy but its transformation into biofuel needs a big amount of energy because many kinds of biomass have high hydrations level. The wet oxidation (in subcritical water conditions) of substances containing biopolymers gives a beneficial energetic effect of the process. We do not lose energy for the dehydration of biomass, which is directed towards the burning process. The waste containing cellulose (or another similar biopolymers) is a potential, valuable source of renewable energy. The wet oxidation process in subcritical water conditions can become an attractive alternative for the conventional burning processes.

Keywords: biomass, wet oxidation, subcritical water, renewable energy, cellulose, glucose, reaction calorimetry

We should treat our environment as borrowed from future generation and we should do everything to keep it in the same or better condition [1]. Nowadays the lack of energy sources is the growing problem. Trying to solve this difficult situation we should focus our attention on biomass as an alternative to fossil resources. It has always been a major source of energy for mankind and is presently estimated to contribute to 10÷14% of the world's energy supply. This form of renewable energy is widely used in the third world. It is necessary to increase the use of biomass in the Western world using the adequate methods. Biomass production can generate employment because of its availability in many countries [2].

Much attention should be focused on identifying suitable biomass species, which can provide highenergy outputs, to replace conventional fossil fuel energy sources. Due to global warming caused by excessive use of fossil fuel resource, renewable biomass resources should become more important in the future as alternatives to fossil resources. Together with the depletion of fossil resources and deterioration of our global environment, it will become more and more important to utilize biomass resources [3].

One of the most important and urgent problems in environmental management is waste treatment. A zero emission process for the efficient treatment of biomass waste should be developed. The conversion of biomass into energy, for example, fuels and chemicals, can be achieved in a number of ways. The most important problem is that resources should be environment-friendly utilized. The development of a process that could recover chemical resources from biomass wastes to control the circulation of carbon in biosphere is desirable [4]. The conversion of biomass into energy can be achieved by the application of a number of technologies. Each characterizes specific requirements, advantages and disadvantages.

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The technologies, which can convert biomass to sources of energy, should help to solve energy and environmental problems.

Biomass as a resource of energy

Biomass is a term for all organic material that stems from plants (including algae, trees and crops). One simple method is to define four main types of biomass: woody plants, herbaceous plants/grasses, aquatic plants, manures. Biomass is produced by green plants converting sunlight into plant material through photosynthesis and includes all land- and water-based vegetation, as well as all organic wastes [2]. Typically photosynthesis converts less than 1% of the available sunlight to store in chemical energy. The plant material is derived from the reaction between CO_2 in the air, H_2O and sunlight. The result is carbohydrates that form the building blocks of biomass. The biomass resources are also considered to be organic matter, in which the energy of sunlight is stored in chemical bonds. Chemical energy is released when the bonds between adjacent carbon, hydrogen and oxygen molecules are broken. It can be caused by digestion, combustion or decomposition. Biomass is also useful feed material for energy and chemical resources. It can be converted into three main types of product: electrical/heat energy, transport fuel and chemical feedstock.

Plant biomass is renewable, its combustion is neutral with regard to the carbon - dioxide cycle, as long as the usage and plantation are repeated under good control [5], and not only produces energy, but also chemicals [6]. For the reasons, we envision plant biomass as a future substitute for fossil fuels; it is necessary to find maximum utilization technology of biomass (especially cellulose biomass).

Cellulose - main component of biomass structure

Cellulose is one of the main components of biomass. It is the most abundant organic resource on the Earth [7]. Cellulose plant material represents an as-of-yet untapped source of fermentable sugars for significant industrial use [8]. Lignocelluloses or woody biomass are composed of carbohydrate of polymers - cellulose, lignin and smaller parts as extractives (acids, salts and minerals). The cellulose comprises typically two-thirds of the dry mass of plants. It is polysaccharid, which can be hydrolyzed to sugars and eventually be fermented to ethanol [9].

Cellulose $(C_6H_{10}O_5)_n$ (40÷60% of the dry biomass) is a linear polymer of cellobiose (glucose-glucose dimer). It consists of linear chains (1,4)-*D*-glucopyranose units, in which the units are linked 1-4 in β-configuration, with an average molecular weight around 100,000 [2]. The orientation of the linkages and additional hydrogen bonding make the polymer rigid and difficult to break. Unless very large excess of enzyme is used, the enzymatic digestibility of cellulose in native biomass is low (<20% yield) because of its structural characteristics [8].

In hydrolysis the polysaccharide is broken down to free sugar molecules by the addition of water. This is also called saccharification. The product, glucose - $C_6H_{12}O_6$, is a six-carbon sugar (hexose) [9]. Cellulose is a valuable renewable energy resource, and also glucose and its oligomers, which are obtained by its hydrolysis process, are expected to be valuable chemicals, food and feedstock [10].

Wet air oxidation (WAO)

The conversion of biomass into energy can be achieved by the application of a number of technologies. The really important feature in the convert reactions is water. Sub- and supercritical waters have unique features with respect to density, dielectric constant, ion product, viscosity, diffusivity, electric conductance and solvent ability. Two major reactions in sub- or supercritical water are: oxidation and hydrolysis [4].

Wet air oxidation (WAO) is an effective and extensively used industrial oxidation process to treat a variety of hazardous wastes and heavily polluted effluents [11]. The basic idea of the wet air oxidation process (Zimmeman and Diddams, 1960) is to enhance contact between molecular oxygen and the organic matter to be oxidised [12]. WAO is the oxidation of soluble or suspended oxidizable materials by using oxygen in aqueous phase, at high temperature (150÷350°C) and high pressure (50÷150 bar) [13]. In the ideal case, oxidation of organics compounds would only produce CO_2, H_2O, N_2 and metal oxide. However, in WAO processes, the oxidation is rarely complete and often leads to the production of refractory materials like acetic acid, which is the main compound [13]. The general pattern of oxidation compounds containing C, H, O atoms is described as follows:

organic materials → alcohols → aldehydes → carboxylic acids → carbon dioxide

and it is generally admitted that degradation mechanisms of organic materials is non-catalytic. The biggest advantage of WAO technology is that it is waste-free.

Calorimetric methods in kinetic reaction studies

The aim of our studies is determination heat effects during the oxidation of some kind of biomass using oxygen, increasing temperature and pressure. The study will present the results of non-catalytic WAO of glucose. We want to calibrate the method for our studies by using this simple substance, which heat of oxidation is known. Municipal wastes often contain cellulosic materials that originated from biomass. When cellulose could be converted to various saccharides by scission of glucoside bond in the consecutive reactions:

cellulose → oligosaccharides (cellotriose, cellobiose) → hexose (glucose)

Among these components, glucose is often considered as useful chemical resource that could be used as a starting material in chemical or biochemical reactions [4]. In our studies the really important reaction is oxidation of glucose:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \Delta H_r$$

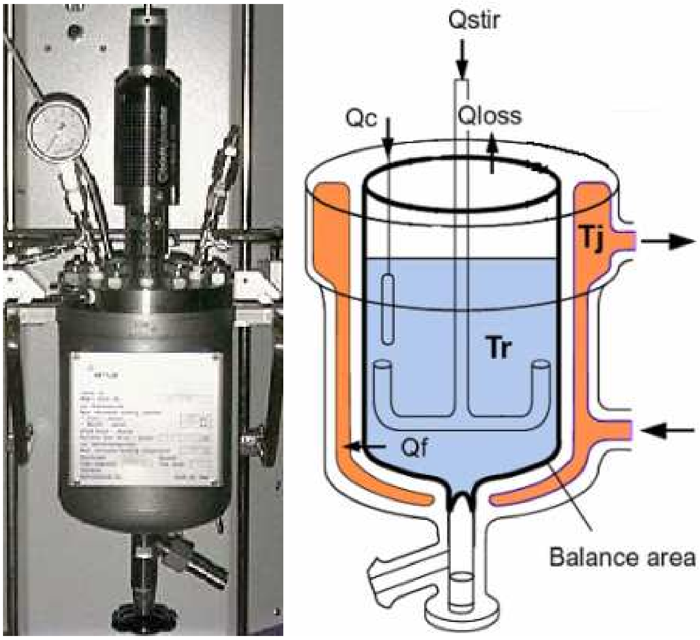
Calorimetry - one of the oldest methods known to science - is based on the fact that all chemical reactions and physical transformations generate or consume heat. The basis for calculation of accurate calorimetric data is the balancing of heat and mass flow.

The calorimetric measurements, which are researching in calorimetric reactor RC1, will be used for these studies. A Mettler-Toledo reaction calorimeter with 2 dm^3 jacketed batch reactor equipped with a gas type stirrer was used in this study as a pressure reactor (Fig. 1).

The calorimetric principle applied in RC1 apparatus is based on a continuous measurement of the temperature difference between the reactor content (T_r) and the heat transferred to fluid in the outer jacket (T_j). At any given time, the heat transferred between the fluid in the jacket and the reactor content, q_{flow}, is given by:

$$q_{\text{flow}} = UA(T_r - T_j)$$

Where U is the heat transfer coefficient and A is the heat exchange area. These parameters are determined by the calibration process.



A B

Fig. 1. Calorimetric reactor RC1 made by Mettler Toledo Company: A) photo, B) diagram

We applicated 700 g samples of glucose solutions to the reactor, in different conditions of temperature (from 20 to 200°C) and pressure. Applicated solutions concentrations of glucose ranged from 1000 to 2000 ppm. The same experiments were carried out for water (in the same conditions) for creating baseline.

Results

Our first calculations rest on calorimetric rules. The reaction of enthalpy is determined by integrating the peak of the heat generation rate Q_r. In the evolution we define the start and end point of possible reaction and its baseline (we use a pure water as baseline). Between the start and end pairs for the reaction limits, the integral is calculated as the area between Q_r curve and the baseline Q_b (Fig. 2).

Overall heat generated during the process ΔH_r was estimated from the following equation:

$$\Delta H_r = \int_S^E (Q_r - Q_b)\,dt$$

where:

ΔH_r - reaction enthalpy [J/mol],
Q_r - heat generation rate, calculated from the sum of the selected heat flow terms [W],
Q_b - baseline for Q_r [W].

As an area between recorded thermal curves Q_r and base line Q_b.

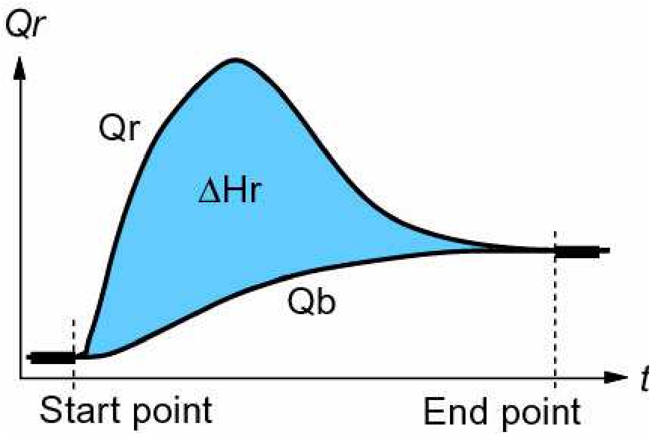


Fig. 2. Heat flow curves of possible reaction and its baseline

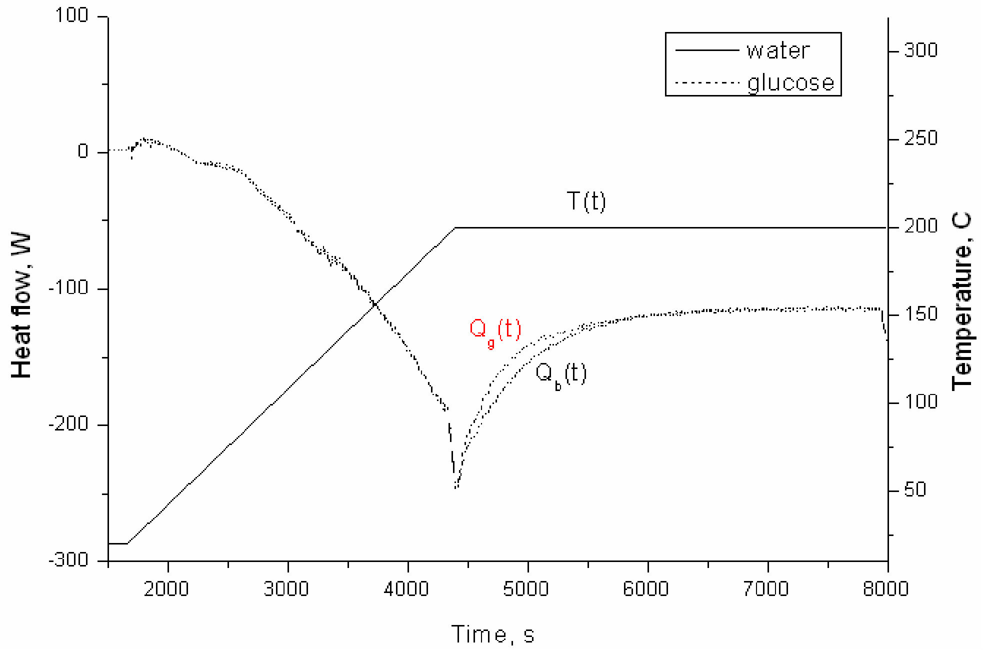


Fig. 3. Changes of water and glucose solution heat flow in RC-1 reactor (C_0 = 1198 ppm of glucose)

Our first results, which rest on these rules, show curves of water (baseline-Q_b) and glucose solution (Q_g) heat flow in time and temperature (Fig. 3). We do not observe any reaction during the first 4500 s of the reaction. When the temperature reaches 200°C, we can see the area between q_g curve and the baseline curve (Q_b). It is result of heat effects. The area between these curves is heat of glucose decomposition in wet oxidation reaction. We can calculate decreasing of total organic carbon (TOC) in glucose solution, too. The area converted into decreasing TOC unit gives us minor heat of reaction.

Basing on the diagram (Fig. 1B) we can create heat flow balance for our studies:

$$Q_r = Q_{flow} + Q_m + Q_{loss}$$

where:
Q_r - heat generation rate of chemical or physical reaction [W],
Q_{flow} - heat flow through the reactor wall - calibration power [W],
Q_m - heat accumulation by the reaction mass and insert,
Q_{loss} - heat flows through the reactor head assembly (radiation, conduction).

This method is suitable for the studies of heat processes. It can be used for the wet air oxidation studies. Due to technique described above we can calculate both heat effect of process and deduce the kinetics and mechanism of oxidation reaction.

The kinetic model, suggested in these studies, will be used for the simulation of installations' work producing using energy in the process of biopolymers' oxidation in subcritical water. We do not lose energy for the dehydration of biomass, which is directed towards the burning process. The waste containing cellulose (or another similar biopolymers) is a potential, valuable source of renewable energy.

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WYZNACZANIE EFEKTÓW CIEPLNYCH REAKCJI UTLENIANIA BIOMASY W ŚRODOWISKU WODY PODKRYTYCZNEJ

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Abstrakt: Poszukiwanie alternatywnych źródeł energii jest jednym z ważniejszych elementów polityki zrównoważonego rozwoju. Biomasa jest w tym kontekście cennym źródłem energii odnawialnej, jednak ze względu na fakt, iż wiele rodzajów biomasy ma wysoki stopień uwodnienia, przekształcenie jej w biopaliwo wymaga dużych nakładów energetycznych. Stosując utlenianie substancji zawierających biopolimery w warunkach wody podkrytycznej (tzw. mokre utlenianie), uzyskujemy korzystny efekt energetyczny procesu, gdyż nie tracimy energii na odwadnianie strumieni kierowanych do spalania. W tym ujęciu odpady zawierające celulozę (lub inne podobne biopolimery) są potencjalnym, cennym źródłem energii odnawialnej, a proces utleniania w warunkach wody podkrytycznej może stać się atrakcyjną alternatywą dla konwencjonalnych procesów spalania.

Słowa kluczowe: biomasa, mokre utlenianie, woda podkrytyczna, energia odnawialna, celuloza, glukoza, reakcja kalorymetryczna

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and Grzegorz ŁAGÓD[3]

ANAEROBIC AND AEROBIC TREATMENT OF WASTEWATER OF MILK PLANTS

OCZYSZCZANIE ŚCIEKÓW MLECZARSKICH W WARUNKACH TLENOWO-BEZTLENOWYCH

Abstract: Wastewater of milk plants contain high concentrations of inorganic and organic pollutants such as suspended solids - 2000 g/m^3; COD - 3700 g/m^3; BOD$_{20}$ - 2500 g/m^3. The disadvantage of aerobic biological dairy wastewater treatment plant (WWTP) technologies is the high costs of electricity for the air supply to aeration tanks, high rate filters and others. High concentrations of organic matters cause the overload and the bulking of activated sludge and perturb the work of secondary sedimentation tanks. In this work the effective stage anaerobic-aerobic treatment technology of milk plants wastewater is proposed. Treatment plants were equipped with the carriers in order to increase the concentration of biomass in bioreactors. Microorganisms of the activated sludge at this stage of treatment are attached to the proposed load carriers made of artificial fibers of the VIYA (Eyelash) type. The high efficiency of anaerobic-aerobic method in section bioreactors with carriers for immobilization of microorganisms was observed. Due to the design of bioreactors section and the single wastewater pass through the system the microorganisms biocenosis was formed in bioreactors separate sections. The formed biocenosis are typical for occurred conditions and the sewage quality in given section. The wastewater is being treated gradually and the growth of microbial biomass decreases due to the formed, so-called, "bioconveyer". The system of microorganisms based on the food relation type "predator-victim" reducing the excess of biomass is formed in the particular elements of WWTP system. The outflowing activated sludge may be characterized by quick sedimentation, small amounts, high ash content (up to 60%) and significant dewatering.

Keywords: milk plants wastewater, anaerobic-aerobic treatment, microorganisms immobilization, bioreactor, bioconveyer

Wastewaters generated in milk plants contain high concentrations of inorganic and organic pollutants such as: suspended solids - 2000 g/m^3; COD - 3700 g/m^3; BOD$_{20}$ - 2500 g/m^3 [1-3]. As a result, dairy wastewater treatment is a complicated task. Its solution requires considerable financial funds [4]. Milk plant wastewater treatment is conducted with the use of biological methods [5]. For example, in aeration tanks, in high rate filters, in circulative oxidizing channels or in two step schemes with aeration tanks.

The disadvantages of aerobic biological wastewater treatment technologies of milk plants are: the high costs of electricity in the aeration tanks of air supply, high rate filters and the other above-mentioned treatment plants; the high growth of surplus activated sludge biomass difficult to dewatering and needs a process of stabilization. Instability of the work of aeration tanks is caused by the periodic and seasonal operation of the dairy industry (in summer the average flow rate can be increased up to 100 m^3/day or more, and in winter it can be decreased to 20 m^3/day). High concentrations of organic matters cause the

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overload and the bulking of activated sludge and violate the work of the secondary sedimentation tanks.

In this work the gradual anaerobic-aerobic treatment technology of milk plants wastewater was proposed. The studied treatment plants were equipped with the carriers made of the artificial fibers in order to increase the concentration of biomass in bioreactors. Microorganisms of activated sludge at this stage of treatment are attached to the proposed carriers [6-8].

Description of experiment and results

The study was conducted on the laboratory installation (Fig. 1). It consists of two anaerobic and three aerobic sequentially connected bioreactors.

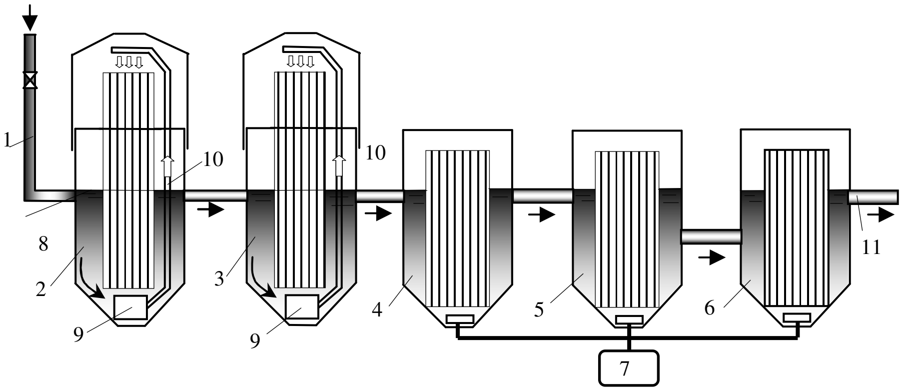

Fig. 1. Scheme of laboratory installation: 1 - supplying of model solution; 2, 3 - anaerobic bioreactors (sect. No. 1 and sect. No. 2); 4, 5, 6 - aerobic bioreactors (sect. No. 3, sect. No. 4 and sect. No. 5); 7 - microcompressor; 8 - fibers carriers installation; 9 - pump; 10 - recirculation pipe; 11 - treated water pipe

Table 1

Characteristics of used model solutions in the anaerobic-aerobic treatment

Name	**Concentration of organic matters [g in 100 g of milk whey]**				**Concentration of organic matters [kg/m^3]**	**Dilution [-]**	**Concentration of organic matters in model solution [kg/m^3]**
	proteins	**lipids**	**carbo-hydrates**	**Σ**			
Model solution 1	1.0	-	3.5	4.5	45	1:9	4.5
Model solution 2	0.6	0.4	0.11	1.11	11.1	1:2	3.7
Model solution 3	0.6	0.4	0.11	1.11	11.1	1:1	5.55
Model solution 4	0.6	0.1	4.0	4.7	47	1:9	4.7

Model solution was prepared for a milk plants sewage simulation (Tab. 1). This solution was supplied to the first anaerobic bioreactor and then passed through the following anaerobic and aerobic bioreactors. Because solutions were prepared from milk, so they contain almost all the ingredients of dairy wastewater.

The mixing of reacting masses in the anaerobic bioreactors was made by means of the pumps at the bottom and recirculation pipe with holes for water sprinkling. Air was supplied with microcompressor to the aerobic sections to maintain dissolved oxygen concentrations within range of 1.6÷2.5 g/m^3. The carriers of loading were made of artificial fibers of the VIYA (Eyelash) type were installed into bioreactors for the immobilization of biomass [8]. The design parameters of the laboratory installation are presented in Table 2.

Table 2

Design parameters of laboratory installation

Parameter	**Total**	**Section number**				
		1	**2**	**3**	**4**	**5**
Volume [cm^3]	3925	785	785	785	785	785
Diameter of carrier fiber [mm]	-	3	3	3	3	3
Length of fiber [m]	-	0.12	0.12	0.12	0.12	0.12
Number of fibers	-	260	255	220	80	70
Volume that fibers occupier [cm^3]	-	281	275	238	86	76
Part of section volume that fibers occupier	-	0.48	0.47	0.3	0.11	0.1

At the beginning of the work of laboratory installation the activated sludge from Kyev WWTP “Bortnitskaya” was carried to the bioreactors to increase biomass.

Figure 2 presents the dependence of total nitrogen (N_{tot} - N) to the operation time. A significant reduction of total nitrogen has been observed on 45th day of work. Treatment effects of model solutions from total nitrogen were noted, as follows: in section No. 3 - 61.4%, in section No. 4 - 73.8%, in section No. 5 - 82.3%.

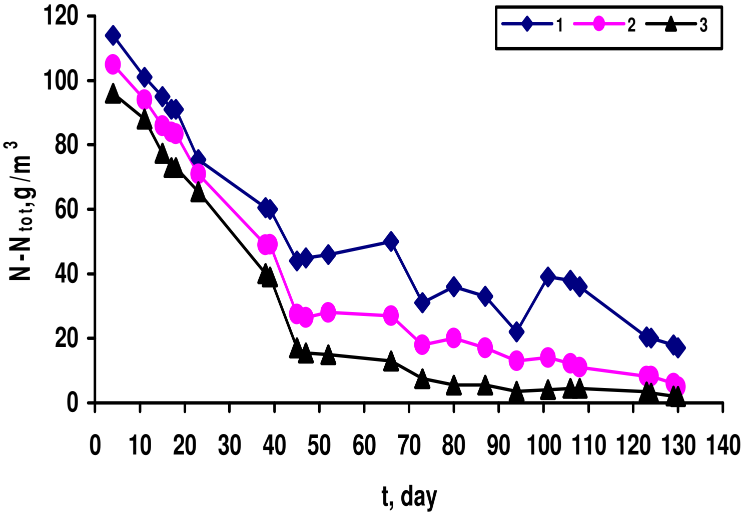

Fig. 2. Dependence of total nitrogen (N_{tot} - N) from the operation time: 1 - in section No. 3; 2 - in section No. 4; 3 - in section No. 5

Figure 3 presents the dependence of COD in model solutions to the operation time after anaerobic and aerobic treatment: after bioreactor No. 2 and bioreactor No. 5. The high degree of wastewater purification was observed. Concentrations of organic matters due to COD in the treated wastewater were: after bioreactor 2 - 300 g/m^3; after bioreactor 5 - 20 g/m^3.

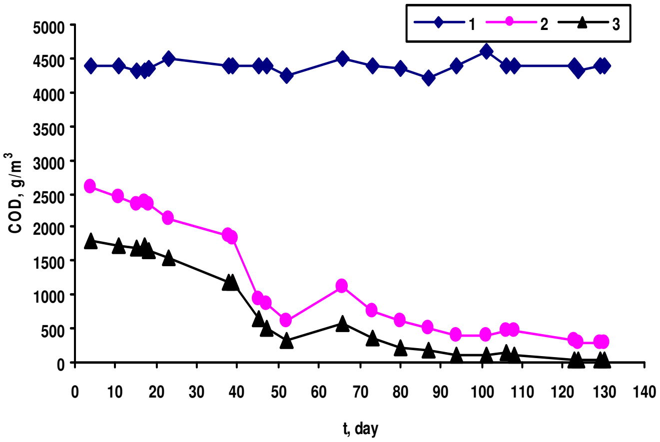

Fig. 3. Dependence of COD in model solutions after anaerobic and aerobic treatment from the operation time: 1 - inlet water; 2 - after bioreactor 2; 3 - after bioreactor 5

Discussion

The results of the laboratory research show the high degree of dairy wastewater treatment. The following degrees of reduction were achieved for the pollutants indicator: COD - 86.7÷93%, total nitrogen - 96.9÷97.9%.

The high efficiency of anaerobic-aerobic method in section bioreactors with carriers for microorganism immobilization was observed. Due to the design of bioreactors section and the single flow of wastewater through them the microorganisms biocenosis was formed in separate bioreactors sections. This is typical for studied conditions and the quality of the sewage in given section. The wastewater is treated gradually and the growth of biomass of microorganisms decreases due to the formed so-called "bioconveyer" [8]. The system of microorganisms on the food type "predator- victim" is formed in a chain of sections, thus it reduces the excess of biomass. The higher trophic level of predator decreases the number of its forms [9].

The results of our biological studies show the presence of large number of microorganisms such as *Bodo*, *Vorticella microstoma*, *Arcella discoides*, *Oligoheta* and others in section No. 3. These microorganisms are typical to highly polluted water environment with large concentration of organic matters as a result of anaerobic destruction of proteins, carbohydrates and others. The small flagellata are consumed by the infusoria, rotifers, predatory infusoria and others (section No. 5) in examined wastewater purification process from organic matters.

The concentration of organic matters and biomass of bacteria decreased in section of bioreactors. At the end of purification process quantity of excess biomass is small therefore its self-oxidation and self-regulation as a result of consumption by higher levels organisms of trophic chains. The decrease of biomass concentration from 3.8 (in section No. 1) to 1.5 g/g carrier (in section No. 5) in water clarification process was observed.

Summary and conclusions

On the basis of our studies it is possible to make the following conclusions:

- With the purpose of microorganisms biocenosis formation on the principle of “bioconveyer” the application of sectional flow scheme installation for anaerobic and aerobic purification is recommended. The growth of microorganisms biomass reduction resulted from consumption in the food chain “predator - victim” was formed in sections: from bacteria and small flagellata to predatory infusoria, rotifers, worms and other microorganisms.
- It is possible to arrange a fiber loading of the VIYA type in bioreactors to increase the concentration of microorganisms in anaerobic and aerobic purification plants. This loading is characterized by a large surface area and is able to attach a large quantity of microorganisms.
- The use of attached biomass for biological wastewater treatment allows to stabilize bioreactors work conditions. Because of that the changing the quality of input parameters (pollution concentrations) and discharges during prolonged operation time do not affect the performance quality of treated wastewater at the outlet from sewage treatment plants with selection and immobilization of microorganisms biomass.
- Anaerobic-aerobic method of treatment allows to effectively purify milk plants wastewater. The achieved concentrations of pollutants in treated water are COD - 20 g/m^3, and total nitrogen - 2÷3 g/m^3.
- The output concentration of suspended solids in treated water from laboratory plants was 5÷10 g/m^3. Suspended solids settle quickly and form dense sludge layer.
- As a result of presented anaerobic-aerobic wastewater treatment of milk plants a small amount of sludge is formed, which dewaters well.

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OCZYSZCZANIE ŚCIEKÓW MLECZARSKICH W WARUNKACH BEZTLENOWO-TLENOWYCH

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Abstrakt: Ścieki z przemysłu mleczarskiego cechują się dużą koncentracją zarówno nieorganicznych, jak i organicznych zanieczyszczeń, przykładowo stężenie zawiesiny ogólnej może osiągać poziom 2000 g/m^3; ChZT - 3700 g/m^3; BZT$_{20}$ - 2500 g/m^3. Stąd też technologia biologicznego oczyszczania tego typu ścieków w warunkach tlenowych niesie za sobą wysokie koszty energii elektrycznej, związane przede wszystkim z dostarczeniem dużej ilości powietrza do komór biorekcji. Duża koncentracja związków organicznych powoduje często przeciążenie osadu czynnego oraz jego pienienie, co prowadzi do zakłóceń w pracy osadników wtórnych. Prezentowana praca przedstawia tlenowo-beztlenową technologię biologicznego oczyszczania ścieków z przemysłu mleczarskiego. Przedstawiona została pilotowa instalacja oczyszczania ścieków wyposażona w nośniki służące zwiększeniu koncentracji biomasy w bioreaktorze. Mikroorganizmy osadu czynnego na biologicznym stopniu oczyszczania ścieków są przytwierdzane do wspominanych nośników wykonanych z włókien typu VIYA (Rzęsa). Pozwala to osiągnąć zwiększoną skuteczność beztlenowo-tlenowej metody oczyszczania ścieków przy obecności biomasy immobilizowanej. Poprzez wykonanie poszczególnych sekcji bioreaktorów uzyskano możliwość oczyszczania ścieków przy ich jednorazowym przepływie poprzez układ, w którym biocenoza mikroorganizmów w każdej z sekcji odpowiada specyficznym warunkom technologicznym oraz jakości dopływających ścieków. W prezentowanym układzie technologicznym ścieki są oczyszczane stopniowo, zaś przyrost biomasy maleje ze względu na oddziaływanie „bionośników”. W kolejnych ogniwach sekcji wytwarza się specyficzna zależność pokarmowa typu „drapieżnik-ofiara”, która pozwala zredukować ilość osadu nadmiernego. Odpływający z układu osad czynny szybko sedymentuje i łatwo ulega redukcji uwodnienia.

Słowa kluczowe: ścieki mleczarskie, oczyszczanie tlenowo-beztlenowe, immobilizacja mikroorganizmów, bioreaktor, bionośnik

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COMPARATIVE STUDY OF THE SEED EXTRACTS OF *Apiaceae* PLANTS IN ULTRASONIC FIELD CONDITIONS

BADANIA PORÓWNAWCZE EKSTRAKTÓW Z NASION ROŚLIN Z RODZINY *Apiaceae* W WARUNKACH POLA ULTRADŹWIĘKOWEGO

Abstract: There are many bioactive compounds with known effects on microorganisms (fungi, bacteria, viruses) in plants of the *Apiaceae* family. The properties of selected extracts obtained with classical method, and their antifungal activities were described in previous works. In this paper, the results of a study concerning the properties of the following seed extracts are described: parsley (*Petroselinum crispum*), common hogweed (*Heracleum sphondylium*), hogweed Sosnowsky (*Heracleum Sosnowsky*), ground-elder (*Aegopodium podagraria*), dill (*Anethum Graveolens*), lowage (*Levisticum officinale*) and cow parsley *(Anthriscus sylvestris)*. These extracts were obtained with the use of ethyl acetate as an extractant, at room temperature in ultrasonic bath. The analysis of the extracts was carried out on GC-MS an Agilent 6890N gas chromatograph. Antioxidant content in the extracts was measured with two different methods: with the use of ascorbic acid or linoleic acid in the first method and *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) in the second method. The obtained results showed that the extracts obtained with the proposed method contain more native agents so their biological activity should be higher.

Keywords: *Apiaceae*, seeds extracts, antioxidant activity, DMPD

There are many bioactive compounds with known effects on microorganisms (fungi, bacteria, viruses) in plants of the *Apiaceae* family. The properties of selected extracts obtained with classical method, and their antifungal activity (to *Fusarium culmorum*, *Botrytis cinerea* and *Alternaria alternata*) were described in previous paper [1]. In this work, the results of a study concerning the properties of the following seed extracts are described: parsley (*Petroselinum crispum*), common hogweed (*Heracleum sphondylium*), hogweed Sosnowsky (*Heracleum Sosnowsky*), ground-elder (*Aegopodium podagraria*), dill (*Anethum Graveolens*), lowage (*Levisticum officinale*) and cow parsley (*Anthriscus sylvestris*).

All the chemicals used were of the analytical grade. The *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride (DMPD), ascorbic acid, linoleic acid, pyrogallol and all analytical grade solvents, were purchased from Sigma Aldrich. Iron(II) chloride was purchased from Fluka Chemical Co. Plant material was ground to powder in order to obtain extracts.

Ethyl acetate extracts were obtained by extracting the plant material: 24 hours at room temperature (maceration); 30 hours in Soxhlet apparatus; half an hour at room temperature in ultrasonic bath (35 kHz). The solvent was removed in a vacuum evaporation. GC-MS analyses of extracts were performed with the use an Agilent 6890N series gas chromatograph equipped with an Agilent 5975C inert XL MSD (Agilent Technologies). A vaporization injector in the split mode (1:50) at 270°C, with a HPMS5 capillary column

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was used. The oven temperature was programmed from 50 to 140°C and the rate of temperature increase was 10°C/min. At 140°C the analysis was performed isothermally after 2 min; then the temperature was increased to 250°C with the same rate as previously. At 250°C the analysis was performed isothermally after 15 min.

High purity helium was used as the carrier gas. The identity of each compound was determined by means of a comparison of its spectral data with the data from the Wiley library spectral bank (G1035B; Rev D.02.00; Agilent Technologies).

Antioxidants content in the extracts was measured with two different methods with the use of ascorbic acid/linoleic acid in the first method [2] and *N*,*N*-dimethyl-*p*-phenylene-diamine (DMPD) in the second method [3]. The total phenolate compounds content in the extracts was determined by a colorimetric assay, using the Folin-Ciocalteu reagent and pyrogallol as a standard [4].

The lowest efficiency of the extraction was obtained when the maceration at room temperature was used. This group of extracts was not used in our following consecutive investigations.

The efficiencies of extractions performed in ultrasonic bath (USBE) were higher in all the cases, than the efficiencies of extractions performed for a long time, at high temperature in Soxhlet apparatus. The efficiency of the extraction was from 6.3% for ground-elder to 35.6% for hogweed Sosnowsky. In high temperature extraction, some of compounds were decomposed (unsaturated compounds and glycosides, with discharge aglycone with considerably smaller molecular mass). In comparison with the extracts made in Soxhlet apparatus, the extracts obtained in ultrasonic baths (USBE) did not include at all, or included only a very small quantity of not-cumarin phenols. The corresponding values for cow parsley amount to 2.4 and 1.8%, for hogweed Sosnowsky - 1.1 and 0.2%, also less cumarins and furanocumarins (for hogweed Sosnowsky 52.2 and 20.6%, and for lowage - 20.8 and 2.2%). At the same time, more terpenes and unsaturated compounds with different chemical nature were found. In the case of extracts from common hogweed and hogweed Sosnowsky - the presence of *n*-octyl alcohol and octyl esters was detected.

The received results showed that extracts, obtained with the proposed method, are characterized by more primeval properties; in consequence, they should have a higher biological activity. This effect was especially observed in the extracts of hogweed Sosnowsky and common hogweed seeds, including more *n*-octyl alcohol and octyl acetate than the plant extracts obtained in Soxhlet apparatus. The research works conducted by the Department of Plant Protection of Agricultural University in Krakow show that the fungistatic activity of this substances is high. This effect is confirmed by the retardation of growth of fungi *Fusarium culmorum, Botrytis cinerea, Alternaria alternate,* with oil from hogweed Sosnowsky seeds, which includes a lot of *n*-octyl alcohol and octyl ester. A strong inhibition of the tested fungi was also observed in nutrient solution with additives of this substances [5].

The total content of phenols in the obtained extracts and their antioxidative activity were determined, in order to observe the relation between these quantities and the biological activity of plant extracts, which were described in numerous publications [6]. Antioxidant content in the extracts was measured with two methods, based on different mechanisms of chemical process - according to the well-established information of extracts' composition. In DMPD method a transfer of hydrogen atom occurs, characteristic of phenolic compounds. In the case of the use of linoleic acid and ascorbic acid, there occurs a transfer of electron in non-enzymatic formation of peroxides [7, 8].

It was found that the antioxidative capacity, which is determined by the complexion DMPD with Fe^{3+} is decreased. It is connected with the presence of phenols and conform with the results of total content determination. The antioxidative properties of extracts obtained with both methods, studied by means of inhibition of not enzymatic oxidation of linoleic acid give similar results, or even higher, in the case when the USBE method was used. It is connected with a bigger content of terpenes and other unsaturated compounds. According to the experimental works of Jeng-Leun Mau et al [9], all the compounds of isoprene structure reveal inhibitive effect in relation to peroxidation of linoleic acid. Moreover, terpenes are in synergetic relation to other antioxidants which are present in extracts.

To recapitulate, it should be supposed that the extracts received with the described method, have to be characterized by the same or a higher fungistatic activity. This conclusion will be subject to further investigations.

Acknowledgments

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BADANIA PORÓWNAWCZE EKSTRAKTÓW Z NASION ROŚLIN Z RODZINY *Apiaceae* W WARUNKACH ULTRADŹWIĘKOWYCH

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Abstrakt: W roślinach z rodziny *Apiaceae* występuje wiele substancji znanych ze swej aktywności biologicznej w stosunku do mikroorganizmów (grzybów, bakterii i wirusów). We wcześniejszych badaniach opisano właściwości wybranych ekstraktów otrzymanych metodą klasyczną, ich skład i aktywność w stosunku do grzybów patogennych. W tej pracy przedstawiono właściwości ekstraktów otrzymanych z nasion: pietruszki *(Petroselinum crispum*), barszczu zwyczajnego (*Heracleum sphondylium*), barszczu Sosnowskiego (*Heracleum Sosnowsky*), podagrycznika (*Aegopodium podagraria*), kopru ogrodowego (*Anethum Graveolens*), lubczyku (*Levisticum officinale*) i trybuli (*Anthriscus sylvestris*) przez ekstrakcję octanem etylu w temperaturze pokojowej w łaźni ultradźwiękowej. Analizę otrzymanych ekstraktów prowadzono metodą GC-MS (Agilent 6890N). Właściwości antyutleniające oznaczano dwiema metodami: z udziałem kwasu askorbinowego lub linolowego oraz w obecności

N,*N*-dimetyl-*p*-fenylenodiaminy (DMPD). Uzyskane rezultaty pokazały, że ekstrakty otrzymane proponowaną metodą mają bardziej pierwotne właściwości, a co za tym idzie, powinny wykazywać większą aktywność biologiczną.

Słowa kluczowe: *Apiaceae*, ekstrakty z nasion, aktywność antyoksydacyjna, DMPD

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DISTRIBUTION OF TRACE ELEMENTS IN LAKE SEDIMENTS: A SCIENTOMETRIC ANALYSIS

PIERWIASTKI ŚLADOWE W OSADACH JEZIORNYCH - ANALIZA SCIENTOMETRYCZNA

Abstract: This paper examines and compares research activities in the field of lake sediment analysis for the period 2000-2009. The scientometric data is collected from 79 articles published in major journals included in the Science Citation Index (SCI). The most commonly used journals were Science of the Total Environment, Chemosphere, Microchemical Journal, Applied Geochemistry and Environment International. The trace elements which are analyzed in most cases are Pb, Cu, Ni, Cr, Co, Mn and Zn. None of countries show research effort activity that would be outstandingly higher than the majority, although some of them, eg Sweden, The United States, Canada and China have shown activity above the average level.

Keywords: trace elements, scientometric analysis, sediments

Water body sediments and their characterization from viewpoint of trace element and especially heavy metal concentrations are important ecosystem and environmental quality assessment indicators exactly in long-term significance. Sediment pollution is caused by natural and anthropogenic influence. The physical and chemical characteristics of sediments are a major component in determining the habitat (eg fish, invertebrates and others) quality for aquatic biological organisms as well as indirectly also human health.

The paper examines and compares research activities in the field of trace element analysis in lake sediments for the period 2000-2009.

Materials and methods

Scientific papers analyzed

The scientometric data is collected from 79 articles published in major journals included in the Science Citation Index and Science Direct databases. Only papers about trace metals in lake sediments were included in the analysis database. The journal with most published papers on the particular theme was *Science of the Total Environment*, followed by *Environment International* and *Applied Geochemistry* (Fig. 1).

Results and discussions

Scientometric studies of published papers about trace elements in lake sediments in the last ten years have shown quite even distribution of annual publications - 6 to 7 papers per year with a slight increase in years 2007 and 2008. As seen below (Fig. 2) year 2009 shows a decrease in numbers of published papers but this could mostly be due to the fact that the calendar year was not yet finished in the time of preparation of this study.

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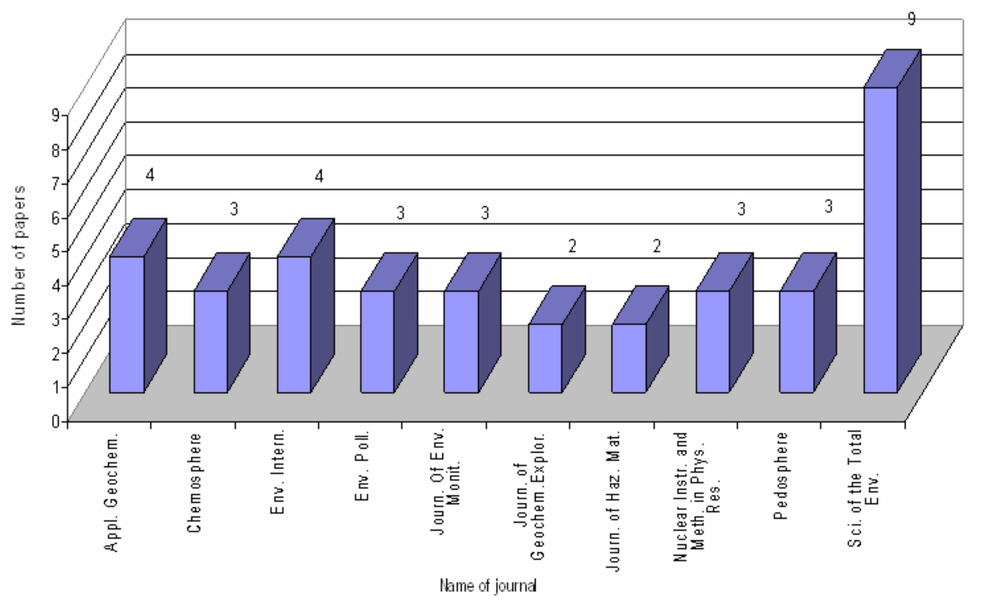

Fig. 1. 10 journals with most published papers on subject of trace metals in lake sediments from 2000-2009

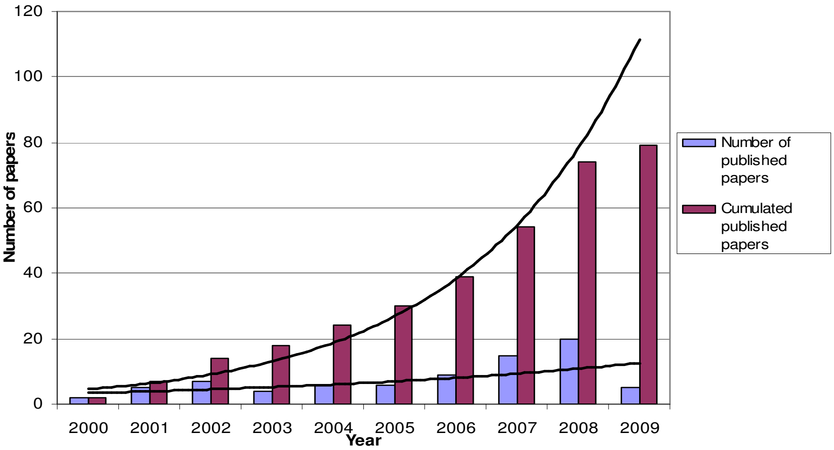

Fig. 2. Number of published papers per year on topics related to trace elements in lake sediments

Most commonly analyzed elements

The papers that have been written in the recent years could be arranged elementwise according to the amount of work carried out on it. Using this criterion, Figure 3 shows that the most studied elements in lake sediments are Zn, Pb, Cu, Cr, Cd, Mn and Ni [1]. The noted trace elements evidently sufficiently characterize sediment pollution level.

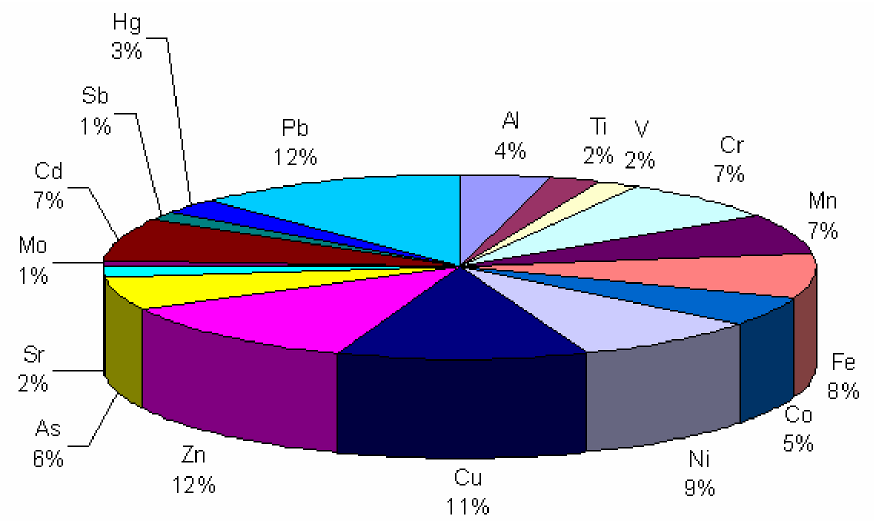

Fig. 3. Distribution of published works on the distribution of elements in lake sediments, as a function of elements involved

Sample collection specifications

Sample collection for analysis can be divided into two major groups according to the goal of the study - deep core samples (>50 cm, reaching 193 cm and more) if study is focused on geochronology or palaelohydrology [2, 3] and shallow core samples (<50 cm) or just the upper layer of sediments if the study focuses on metal composition, distribution, impact assessment or other similar objectives [4-6]. It can be noted that no certain depth of analysis is used by majority of researchers - every individual scientist chooses this parameter by their own technical and scientific possibilities. Naturally, also different geographical, climatic and hydrological aspects are taken into account, as well as the rate of sedimentation in each particular research site.

Treatment and methods of analysis of collected samples

As general methods for analysis of trace elements, three most common can be pointed out - inductively coupled plasma mass spectrometry or atomic emission spectrometry (ICP-MS/AES), atomic absorption spectrometry (AAS) and X-ray fluorescence. In majority of cases HNO_3, HCl and HF in different proportions have been used to acidify the collected samples for further analysis [7, 8]. In separate cases instrumental methods of analysis have been used as well (analyzing collected samples without further pretreatment or dissolving) but this is a distinct minority - only 15÷20% of total research studies have utilized such an approach. This fact could indicate both increased reliability and simplicity of chemical analysis methods.

Regional distribution of studies

None of countries show research effort activity that would be outstandingly higher than the majority, although some regions, eg North America have shown activity above the average level. In past few years Chinese scientists have shown increased activity in researching this field as well.

Conclusion

Scientometric analysis of published scientific papers on the subject of trace elements in lake sediments in years 2000-2009 have shown that journal with most published articles on this topic is Science of the Total Environment (9 papers), while most commonly studied elements were Zn, Pb, Cu, Cr, Cd, Mn and Ni. Depths of sediment layers collected for samples varied greatly amongst analyzed papers starting from 2 cm and going up to 193 cm and more. Undoubtedly predominant methods of sample analysis were ICP-MS/AES, AAS and X-ray fluorescence.

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PIERWIASTKI ŚLADOWE W OSADACH JEZIORNYCH - ANALIZA SCIENTOMETRYCZNA

Abstrakt: W pracy przedstawiono analizę scientometryczną wyników badań osadów dennych pobranych z jezior w latach 2000-2009. Analizowano wyniki badań, które były przedstawione w 79 artykułach publikowanych na łamach najważniejszych czasopism zawartych w Science Citation Index (SCI). Najczęściej cytowane były czasopisma: Science of Total Environment, Chemosphere, Microchemical Journal, Applied Geochemistry oraz Environment International. W większości prac przedstawiono wyniki oznaczeń pierwiastków śladowych, m.in.: Pb, Cu, Ni, Cr, Co, Mn i Zn. W żadnej z prac nie stwierdzono zbyt dużych stężeń, chociaż w niektórych krajach, np. w Szwecji, Stanach Zjednoczonych, Kanadzie i Chinach, stwierdzone stężenia były większe od wartości średniej.

Słowa kluczowe: pierwiastki śladowe, analiza pomiarów naukowych, osady

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EVALUATING THRESHOLD RADIAL AND ADAPTIVE FILL SECONDARY SAMPLING METHODS FOR CONTAMINATED SOIL SAMPLING EFFICIENCY

OCENA SKUTECZNOŚCI OPRÓBKOWANIA WTÓRNEGO METODAMI UZUPEŁNIANIA PROMIENIOWEGO I WYPEŁNIANIA ADAPTACYJNEGO W BADANIACH ZANIECZYSZCZENIA GLEB

Abstract: The purpose of the work was to compare effectiveness of common secondary sampling methods for assessing the distribution of soil pollution. The study case is based on an example of assessing the spatial distribution of soil contamination with lead in Slawkow area (Upper Silesian Industrial Region). This comparison was made in regard to both precision of the spatial estimation and minimization the cost of measuring campaign. The special attention was given to the often applied secondary sampling designs such as threshold radial (also known as adaptive cluster sampling) and adaptive fill sampling. These two methods were tested in typical municipal and suburban environment in Slawkow area. The work contains also detailed statistical and geostatistical analysis of the above-mentioned contamination, and elaboration of series of its spatial distributions using numerous alternative sampling designs. The determined sampling plans make it possible to find compromise between ecological and financial aspects. A combination of the obtained results with the legal regulations in force concerning concentrations of heavy metals in soils are the basis for reliably estimation the ecological hazard arising from the soil contamination with lead in the Slawkow area. The results of performed analyses show that better efficiency in terms of cost and precision of measuring campaign gives rather coarser preliminary sampling design followed by appropriate secondary sampling then use the one-stage very dense measuring grid.

Keywords: geostatistics, heavy metals, soils, ecological risk, secondary sampling designs

The choice of appropriate sampling design is essential in different soil related surveys. This arises from the fact, that our knowledge on soil in their natural state is never fully known and collecting samples as well as laboratory analysis is expensive and time consuming, especially when investigations are performed on large areas. Secondary sampling is very important stage of many environmental studies, which can significantly improve the analysis by relatively low cost. Secondary sampling design can refine a model, get a deeper insight into studied phenomenon, clarify situation and thus make right decision.

The goal of the work was a study-case based evaluation of effectiveness of commonly used secondary spatial sampling designs such threshold radial design (also known as adaptive cluster sampling) and adaptive fill design for delineation of the extent of the area polluted with heavy metals.

Site description and data collection

Study area was located in Slawkow city and its vicinity (Upper Silesian Industrial Region). The studies were performed using soil samples from the archives of the Polish Geological Institute [1]). A satellite view of study area with sample point locations (exhaustive data set) were shown in Figure 1.

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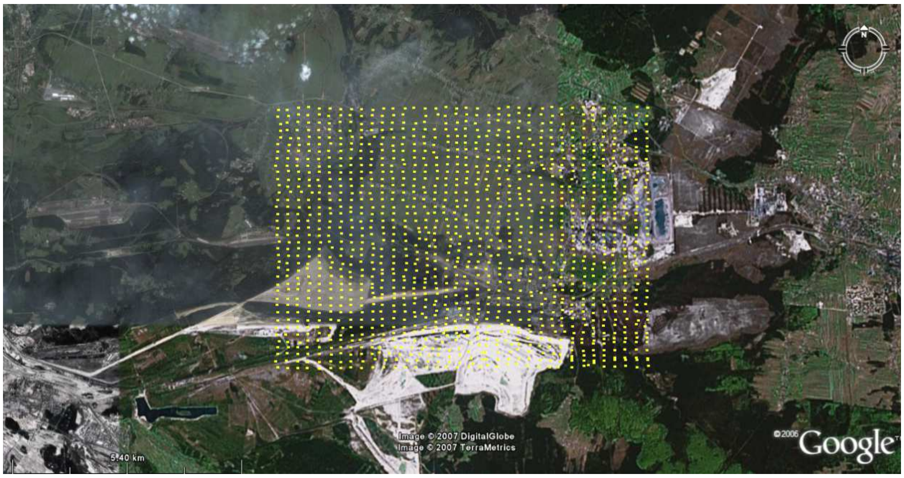

Fig. 1. Satellite view of Slawkow with sample point locations (exhaustive data set)

Secondary sampling strategies and geostatistical methods

In order to limit the time consuming analyses we focused in our work on two commonly used secondary sample designs [2, 3]:

1. Threshold Radial (also known as Adaptive Cluster Sampling) which is a straightforward secondary sample design that places samples in a radial pattern around existing data points that exceed a decision threshold. Threshold radial can be useful in situations where one have a lot of very low or undetected samples and one or two very high measurements.

2. Adaptive Fill Design

In this sample design, samples are placed in the largest spatial gaps among data points. Unlike Threshold Radial, this design gives no regard to the measured values, only their relative positions. A set of new sample candidates is defined by a grid (much like a spatial model) that overlays the data points and acknowledges site boundaries, polygons, and whether layers are active or not. From this set of N candidates the first winning location is simply that value which has the maximum distance to its closest neighbor. The design searches for the second location among the remaining candidates by comparing with the N+1 locations. If there are ties among the two locations, then the tie breaker method is used. The process repeats until one of the following becomes true:

- The total number of samples has been located
- There are no remaining candidates
- No remaining candidate satisfies the minimum distance constraint.

To study soil contamination with lead, it was necessary to obtain the spatial distribution of lead concentrations in soils, as well as the spatial distribution of estimation errors. This was done using geostatistical methods. The ordinary kriging was selected as the most appropriate technique for our analysis. Ordinary kriging is the most effective linear estimator as it assumes that the average value of the estimation error equals zero, and thus minimises the variance of the estimation error [4-10]. The series of spatial distribution of lead concentrations in soils were produced. In the first step preliminary coarse sampling designs (systematic or random) were chosen from the very dense exhaustive data set shown in Figure 1. These designs were treated as pre-information for subsequent sampling. Then above-described spatial distributions were created by careful variogram modeling and kriging technique. At this stage all modeled spatial distributions of lead concentrations in soils were validated using cross-validation methods [11]. Each of the data points was individually removed from the data set, and after that, its value was modeled and subsequently compared with the measured one. Next, the scatter plots of estimated values versus the measured ones were calculated. Using these scatter plots several estimation errors were carefully calculated. Furthermore, modeled spatial distributions were validated using the true values taken from exhaustive data set. Another important measure of sampling design was the total cost of measuring campaign calculated on the basis of the cost of single measurement. By comparing the quality of the spatial distribution with the total cost of measurements it was possible to evaluate the efficiency of the sampling grid under investigation. Then, it was decided what type of secondary sample design should be applied, and how many additional measurements should be used. This allowed for significant reduction of uncertainty by relatively low cost. Then all above-described analyses were repeated once or twice. For clarity multistage sampling process was abbreviated. For instance, the abbreviation "radial_100_100_rand" means, that preliminary sample grid was random, and furthermore 100 additional points were added using threshold radial sampling technique.

Analyses were performed using Arc Gis software (namely, FIELDS, Geostatistical Analyst and Spatial Analyst components) [12].

Results and discussion

Below, in Figures 2 and 3, as well as Figures 6 and 7, exemplary sampling designs are presented (eg systematic and random preliminary sampling design followed by threshold radial and adaptive fill secondary samplings) with the spatial distribution of prediction errors (root mean square prediction error-RMSE).

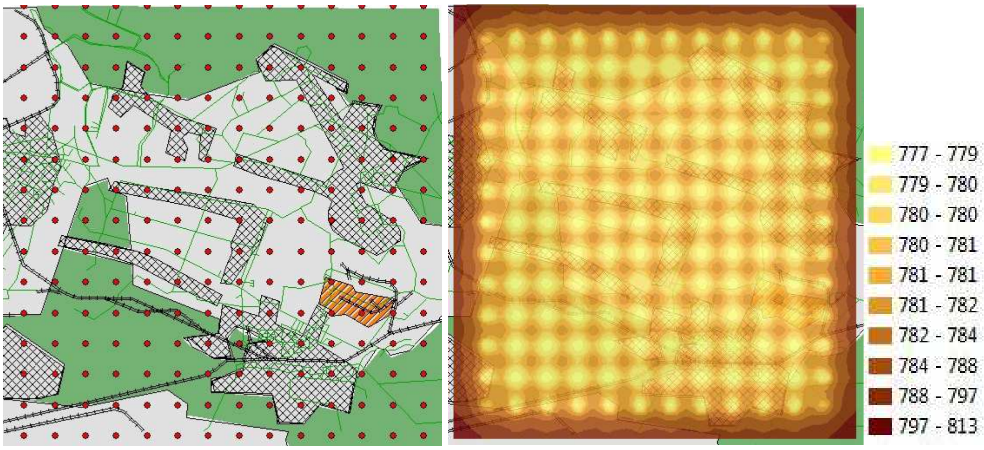

Fig. 2. Preliminary systematic sampling design of 200 observations (left) with the spatial distribution of RMSE (right)

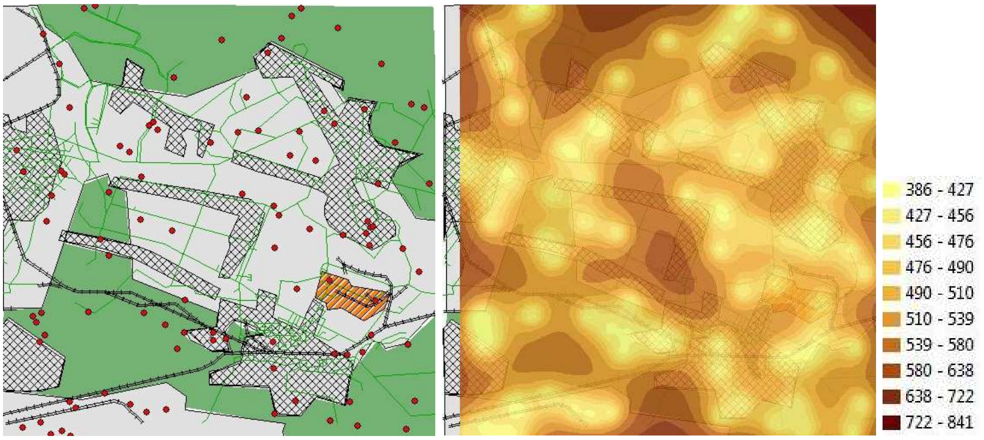

Fig. 3. Preliminary simple random sampling design of 100 observations (left) with the spatial distribution of RMSE (right)

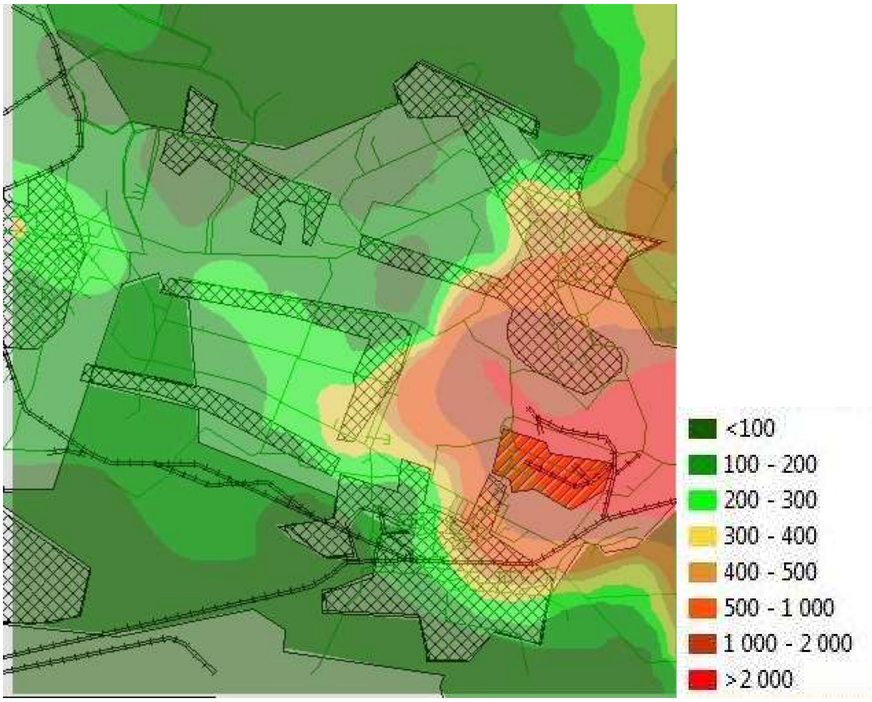

Fig. 4. Spatial distribution of lead contamination in [mg/kg] made on basis sampling design from Figure 2

Figures 4 and 5 show spatial distributions of lead contamination obtained on basis sampling from the Figures 2 and 3, respectively. In Figure 8 root mean square standardized prediction error as a function of cost of measuring campaign is presented for numerous different preliminary and secondary sampling designs.

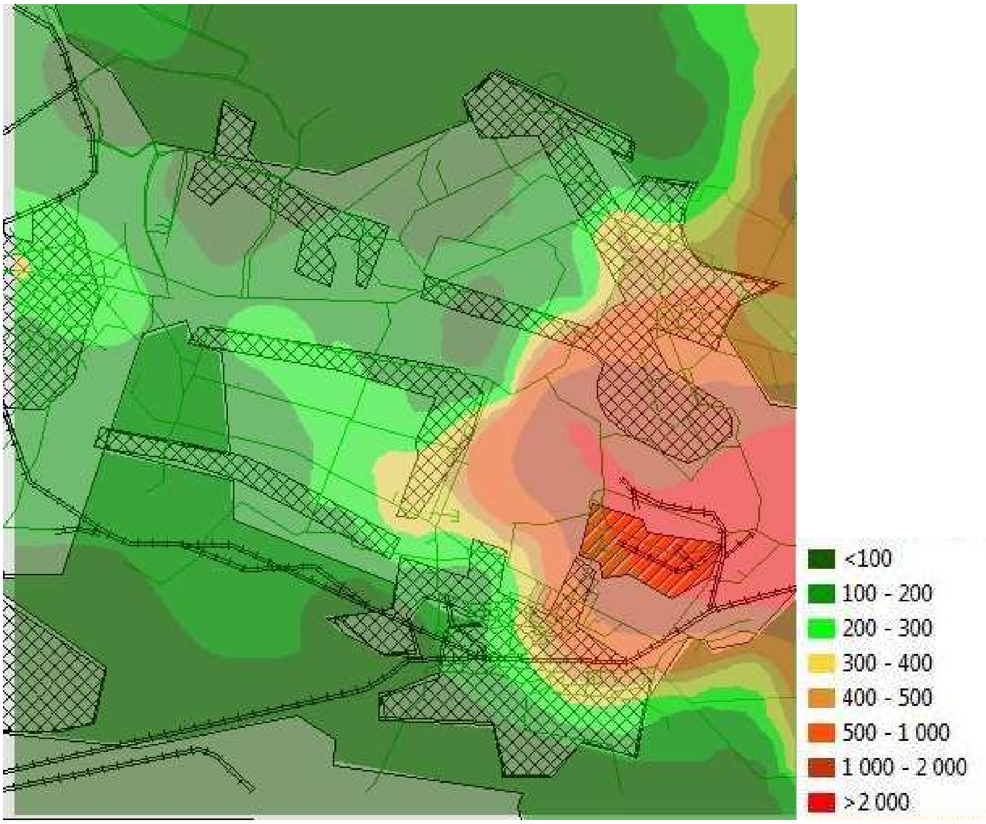

Fig. 5. Spatial distribution of lead contamination in [mg/kg] made on basis sampling design from Figure 3

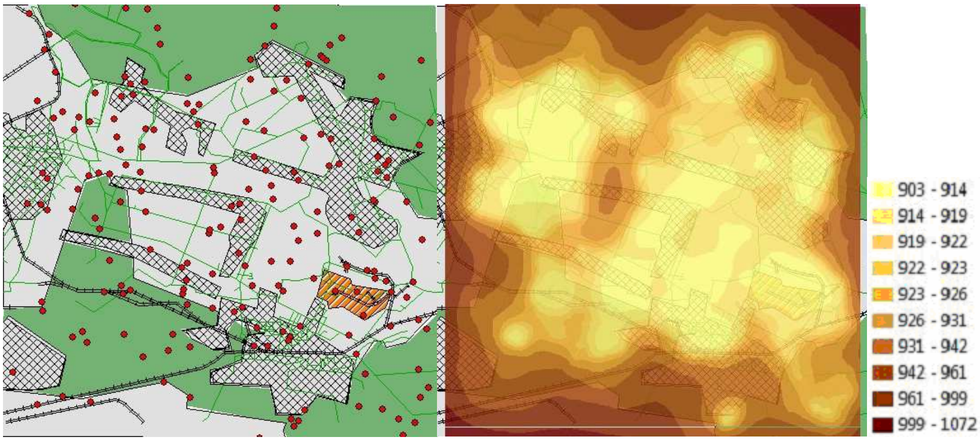

Fig. 6. Optimisation of sampling design from Figure 3 (random_100) with 100 additional sampling points (left) with with the spatial distribution of RMSE (right)

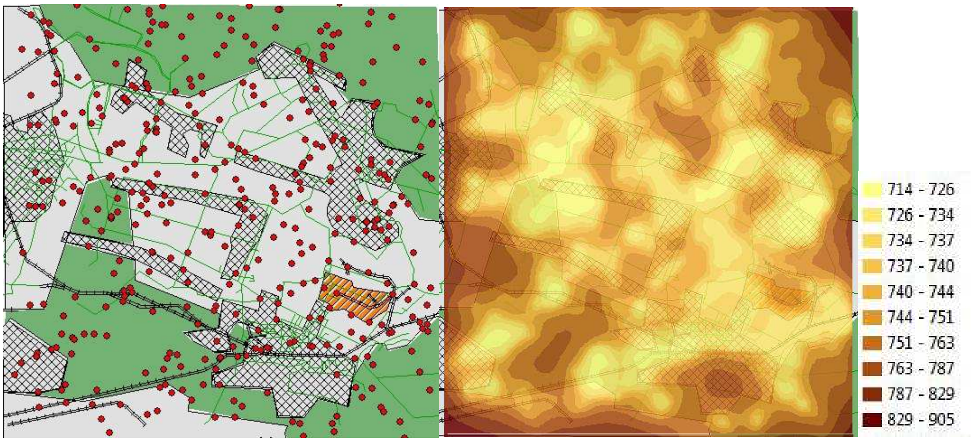

Fig. 7. Optimisation of sampling design from Figure 3 (random_100) with 200 additional sampling points (left) with the spatial distribution of RMSE (right)

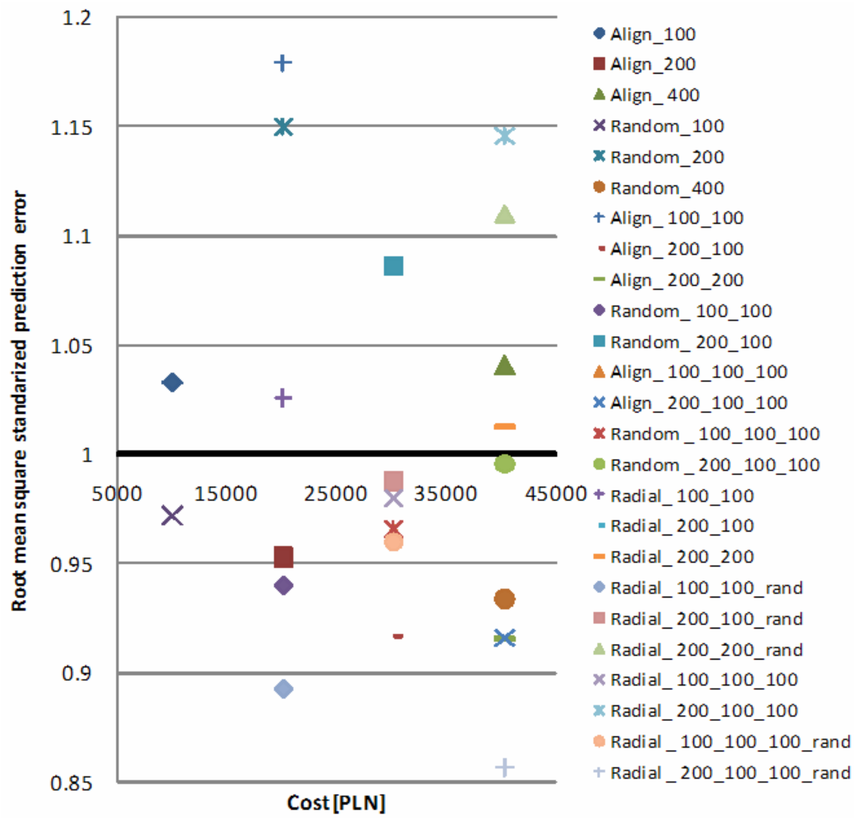

Fig. 8. Root mean square standardized error as a function of cost of measuring campaign calculated for different preliminary and secondary sampling designs

At first glance, the above-shown results seem to be rather ambiguous. However, detailed analysis of estimation errors for many multistages sampling designs showed that much better results in terms of cost and precision give coarser preliminary sampling designs followed by appropriate chosen secondary sampling than use of the one-stage very dense measuring grid. This result arises from the fact that although mostly used dense regular sampling grids, are relatively precise, but in the same time they are very costly. While preliminary random sampling designs give unbiased results, but in general, are less precise than systematic sampling designs. Multi-stage sampling allows for treating the intermediate results as the pre-information for subsequent sampling. This make it possible to better control and tune the whole sampling process according to circumstances occurring during sampling campaign.

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OCENA SKUTECZNOŚCI OPRÓBKOWANIA WTÓRNEGO METODAMI UZUPEŁNIANIA PROMIENIOWEGO I WYPEŁNIANIA ADAPTACYJNEGO W BADANIACH ZANIECZYSZCZENIA GLEB

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Abstrakt: Celem pracy było porównanie efektywności wybranych metod opróbkowania dodatkowego, wykonywanego w celu wyznaczenia rozkładu zanieczyszczenia gleby. Studium przypadku dotyczyło wyznaczania rozkładu przestrzennego zanieczyszczenia gleby ołowiem w okolicach Sławkowa (Górnośląski Okręg Przemysłowy). Głównymi kryteriami efektywności metod opróbkowania dodatkowego były oceny dokładności rozkładu przestrzennego oraz koszty kampanii pomiarowej. Szczególną uwagę zwrócono na często stosowane opróbkowanie dodatkowe metodami uzupełniania promieniowego (zwanego również adaptacyjnym opróbkowaniem klastrowym) oraz wypełniania adaptacyjnego. Te dwie metody były przetestowane w typowym miejskim i podmiejskim środowisku, na terenie Sławkowa i w jego okolicach. Praca zawiera również statystyczną i geostatystyczną analizę omawianego zanieczyszczenia gleby, określenie jego ciągłości przestrzennej, a także wyznaczenie serii rozkładów przestrzennych zanieczyszczenia gleby ołowiem z wykorzystaniu wyżej wymienionych metod opróbkowania dodatkowego. Wyznaczone sieci pomiarowe pozwoliły na znalezienie kompromisu pomiędzy aspektem ekologicznym a finansowym. Rezultaty analizy statystycznej i geostatystycznej wraz z obowiązującymi uregulowaniami prawnymi dotyczącymi zawartości metali ciężkich w glebie są podstawą do rzetelnego określenia potencjalnego ryzyka ekologicznego wynikającego z zanieczyszczenia gleb ołowiem w okolicach Sławkowa. Rezultaty wykonanych analiz pokazują, że lepszą skuteczność określaną kosztem i precyzją kampanii pomiarowej daje rzadsze opróbkowanie wstępne uzupełnione odpowiednim opróbowaniem dodatkowym niż jednoetapowa kampania pomiarowa z gęstą siecią pomiarową.

Słowa kluczowe: geostatystyka, metale ciężkie, ryzyko ekologiczne, opróbkowanie dodatkowe