SOCIETY OF ECOLOGICAL CHEMISTRY AND ENGINEERING

ECOLOGICAL CHEMISTRY AND ENGINEERING A

CHEMIA I INŻYNIERIA EKOLOGICZNA A

Vol. 19 No. 3

OPOLE 2012

EDITORIAL COMMITTEE
Witold Wacławek (Society of Ecological Chemistry and Engineering, PL) – Editor-in-Chief
Marina V. Frontasyeva (Joint Institute for Nuclear Research, Dubna, RU) – heavy metals and radionuclides
Vasil Simeonov (University of Sofia, BG) – monitoring
Maria Wacławek (Opole University, PL) – alternative energy sources
Barbara Wiśniowska-Kielian (University of Agriculture, Kraków, PL) – agricultural chemistry

PROGRAMMING BOARD
Witold Wacławek (Society of Ecological Chemistry and Engineering, PL) – Chairman
Jerzy Bartnicki (Meteorological Institute – DNMI, Oslo-Blindern, NO)
Mykhaylo Bratychak (National University of Technology, Lviv, UA)
Bogusław Buszewski (Nicolaus Copernicus University, Toruń, PL)
Eugenija Kupcinskiene (University of Agriculture, Kaunas, LT)
Bernd Markert (International Graduate School [IHI], Zittau, DE)
Nelson Marmiroli (University, Parma, IT)
Jacek Namieśnik (University of Technology, Gdańsk, PL)
Lucjan Pawłowski (University of Technology, Lublin, PL)
Krzysztof J. Rudziński (Institute of Physical Chemistry PAS, Warszawa, PL)
Manfred Sager (Agency for Health and Food Safety, Vienna, AT)
Mark R.D. Seaward (University of Bradford, UK)
Pavlina Simeonova (Bulgarian Academy of Sciences, Sofia, BG)
Petr Škarpa (Mendel University of Agriculture and Forestry, Brno, CZ)
Piotr Tomasik (University of Agriculture, Kraków, PL)
Roman Zarzycki (University of Technology, Łódź, PL)
Małgorzata Rajfur (Opole University, PL) – Secretary

STATISTICAL EDITORS
Władysław Kamiński (Technical University, Łódź, PL)
Zbigniew Ziembik (Opole University, Opole, PL)

LANGUAGE EDITOR
Ian Barnes (University of Wuppertal, Wuppertal, DE)

EDITORIAL OFFICE
Opole University
ul. kard. B. Kominka 6, 45–032 OPOLE, PL
phone: +48 77 455 91 49
email: waclawek@uni.opole.pl

SECRETARY
Małgorzata Rajfur
phone: +48 77 401 60 42
email: mrajfur@o2.pl

Copyright © by
Society of Ecological Chemistry and Engineering, Opole

Ecological Chemistry and Engineering A / Chemia i Inżynieria Ekologiczna A
is partly financed by Ministry of Science and Higher Education, Warszawa

ISSN 1898–6188

CONTENTS

VARIA

SPIS TREŚCI

VARIA

DOI: 10.2428/ecea.2012.19(03)020 ECOL CHEM ENG A. 2012;19(3):191-201

Jolanta KOZŁOWSKA-STRAWSKA[1]

FAT CONTENT AND FATTY ACID COMPOSITION IN OILSEED RAPE GROWN IN THE LUBELSKI REGION UNDER DIFFERENT LEVELS OF SOIL SULPHUR FERTILITY

ZAWARTOŚĆ TŁUSZCZU I SKŁAD KWASÓW TŁUSZCZOWYCH W NASIONACH RZEPAKU UPRAWIANEGO W WARUNKACH ZRÓŻNICOWANEJ ZASOBNOŚCI GLEB LUBELSZCZYZNY W SIARKĘ

Abstract: A new problem arising in plant production is the increasing deficit of sulphur in Polish soils. This can lead to low-levels in yield for many crops, along with deterioration in the quality of harvested plant samples. Therefore, the purpose of this study was to evaluate the relationship between soil fertility and sulphur in the Lubelski region, in addition to certain quality characteristics of spring rapeseeds. The research samples consisted of soil and plant samples taken from the Lubelski region. The soil samples were selected taken into consideration their agronomic category and sulphate sulphur content. The spring rape was chosen as a test crop and was harvested at full maturity. The test plants were selected because of their high nutritional requirements for sulphur. The results of this study indicate that the quantity of sulphur in the soils of the Lubelski region was characterised by a large variability, ranging in quantity from the low to very high. This affected the fat content in seeds and the fatty acid composition. The lowest fat content was characterised as rapeseed harvested in the localities of high or very high sulphur content in the soil. In addition, the percentage of oleic acid clearly increased with increasing soil fertility in the S-SO_4. This content in terms of its valuable health benefits, essential fatty acids, clearly decreases with increasing sulphur content in the soil in a form directly available to plants. There was no clear relationship between the soils sulphur fertility in the Lubelski region and the share of undesirable erucic acid from the perspective of nutritional point of view.

Keywords: spring oilseed rape, fat content, fatty acid composition, soil fertility in the S-SO_4

Sulphur, in addition to nitrogen, phosphorus, and potassium, is considered as an essential nutrient for the proper growth and development of living organisms. It is part of the composition of many important compounds, the lack of which causes disturbance in plant development as well as holding a possible disease threat for humans and animals [1, 2].

---

[1] Department of Agricultural and Environmental Chemistry, University of Life Science in Lublin, ul. Akademicka 15, 20–950 Lublin, Poland, phone: +48 81 445 60 18, email: jolanta.kozlowska@up.lublin.pl

Despite this importance, until the early 1980s, European countries did not pay too much attention to this. Determining fertilizer needs for crops were also not taken into account. This was connected with the fact that in most parts of our continent the sulphur balance was positive. The positive balance of this component is primarily SO_2 emitted in significant quantities into the atmosphere [3–5].

The excessive sulphur compounds adversely affected the health of ecosystems and the growth and development of crops. The scale of devastation was so great that several actions to reduce the amount of SO_2 emitted into the atmosphere were taken [6].

A reduction of sulphur deposits from the atmosphere and a decrease in the quantity of this ingredient put together with mineral fertilizers have led to a shortage of sulphur in plant production [2, 4, 5].

Studies conducted in our country have shown that about 57 % of Polish soil contains a natural sulphur level, which indicates a low fertility of this valuable nutrient [4, 7]. An absence of this component can be expected especially in lighter, usually acidified mineral soils, located far away from industrial centres [8, 9].

In the case of oil seed crops, the lack of sulphur usually leads to a reduction in the percentage of fat in the seeds along with changes in fatty acids [5, 10, 11]. This is due to the relatively high nutritional requirements of rape in relation to its sulphur content. It was assumed that with an average volume yield, the plants uptake 30 to 60 kg $S \cdot ha^{-1}$ [3, 4, 12].

Consequently, the purpose of this study, based on field preliminary results and studies of literature, was an attempt to assess some characteristics of the seed quality for spring rape grown under different sulphur soil conditions of the Lubelski region.

Materials and methods

The study was performed based on soil and plant samples taken from the villages of the Lubelski province: Nosow, Halasy and Jablonna Majatek. The selection of soils for the studies took into account their agronomic category separated on the basis of silt and clay fraction content (diameter fraction < 0.02 mm). Each individual category was characterised by its agronomic soil content of silt and clay fraction: very light to 10 %, light 11–20 %, average 21–35 %, heavy over 35 % (Table 1).

Table 1

Granulometric composition of the soil samples

No. attempt	Locality	Content of each fraction [%]			Granulometric groups
		1.0–0.1 mm	0.1–0.02 mm	< 0.02 mm	
1	Nosow	53	26	21	sandy silty clay
2	Nosow	60	25	15	light loamy sand
3	Halasy	45	29	26	slightly silty clay
4	Jablonna-Majatek	15	49	36	clay dust

Another criterion taken into account when choosing soil samples for the study was the sulphate sulphur content in the soil (Table 2).

Table 2

$S\text{-}SO_4$ content in the soil samples

No. attempt	Locality	$S\text{-}SO_4$ [mg · kg^{-1}]
1	Nosow	21.0
2	Nosow	8.5
3	Halasy	52.5
4	Jablonna-Majatek	24.5

The soil samples were taken from a soil layer to a depth of 0–20 cm. From each locality, the five soil samples were collected and air-dried. The dried soil samples were triturated in a porcelain mortar and sieved through a 1 mm mesh. The analysis was then averaged over the sample resulting from mixing the primary samples and then the results were determined by:

– granulometric composition with areometric Casagrande's method modified by Pruszynski,

– sulphur sulphate – nephelometric according to the formula of Bardsley and Lancaster [13].

The plant samples (spring rape) were taken from the above-mentioned soil agronomic categories. The test plants were selected because of high nutritional requirements for sulphur by *Brassicaceae* plants. The plant samples were collected in accordance with established criteria for collecting and preparing of plant samples for agricultural chemical analysis.

The dried plant samples were crushed by mills to obtain a sample in the dust form, with as much of a uniform particle diameter as possible. The prepared averaged samples of rapeseeds were marked as:

– fat-extraction method in the Soxtec HT-6,

– fatty acids by gas chromatography (Unicam gas chromatograph).

This work includes the results obtained from preliminary field tests and literature studies on the influential effect of sulphur fertilization on rapeseed according to its quantity and quality of yield.

Results and discussion

Sulphate sulphur usually constitutes not more than several percent to the total soil sulphur. However, this form is the most important for agriculture and ecology because it determines the state of plants supply with this valuable nutrient [14].

An analysis of soil samples taken from the Lubelski region showed that the $S\text{-}SO_4$ content in the examined soil samples ranged from low to very high fertility (Table 3).

The assessment of soil pollution by sulphur at a depth of 0–20 cm usually is made by taking into account its natural content, depending on the type of soil, and it features including four levels of this chemical element content:

– I^{o} – low content,

– II^o – average content,
– III^o – high content,
– IV^o – very high content (very high pollution).

Levels: I, II and III determine the natural content of sulphur forms in different soils, while level IV – content increased due to anthropogenic [15].

Table 3

Evaluation of soil sulphur fertility in S-SO_4 levels in Lubelski region

No. attempt	Locality	Fertility level
1	Nosow	II^o
2	Nosow	I^o
3	Halasy	IV^o
4	Jablonna-Majatek	I^o

The natural, low fertility of sulphur-sulphate soils were characterised with samples taken from Nosow (locality 2) and Jablonna-Majatek. While the number of S-SO_4 in the soil sample originating from the Nosow village was 2.9-times lower compared with the sample taken near Jablonna-Majatek at a level of 8.5 mg S-$SO_4 \cdot kg^{-1}$ soil (Table 2). Such amount of sulphur sulphate was found within agricultural soils of the Lubelski region, but it was almost 2-times lower than the average characteristic for this region amounting to 16.5 mg S-$SO_4 \cdot kg^{-1}$ soil [14]. This problem was also highlighted by other authors, who suggest that 64 % of Lubelski region soils contain less S-SO_4 than for the average for the whole region [7, 14].

The increased content of sulphate sulphur was identified in soil samples collected in Nosow (locality 1). However, the amount of S-SO_4 only slightly exceeded the average content from soil of the Lubelskie region and agricultural farmlands throughout the country (the average for Poland is – 17.9 mg S-$SO_4 \cdot kg^{-1}$ soil) [16].

Sulphur, besides nitrogen, phosphorus, and potassium, are considered as essential nutrients for the proper growth and development of living organisms. This stems from its role and participation in the metabolic processes of living organisms. Hence, an adequate supply for plants with S-SO_4 affects their normal development, and consequently the yield and quality of harvested plant materials [16, 17].

Among the cultivated crops, the greatest demand for sulphur is from rape and *Brassicaceae* plants. Rape uptakes an average of 1.5 to 2 kg S $\cdot$ 100 kg^{-1} seeds and straw, and the greatest demand for this nutrient is usually in spring from the start of the growing season until the end of florescence. Such large nutritional requirements for rape can often exceed the amount of available sulphur in the soil [2, 18, 19].

An analysis of plant material collected at designated points in the Lubelski region shows a clear relationship between the level of soil sulphur fertility, and the amount of spring rapeseed yield (Table 4).

The highest yields of oilseed rape within the limits of 2.07–2.41 1000 kg $\cdot$ ha^{-1} was observed in the Jablonna-Majatek village and Nosow (locality 2). In these localities, the level of sulphur sulphate in the soil was determined as a natural level. This may indicate that plants grown in these two test points, along with other climatic factors and cultivation used sufficient amounts of S-SO_4 to allow an appropriate yield.

Table 4

Yields of spring oilseed rape grown under different conditions of soil sulphur fertility

No. attempt	Locality	Yield [1000 kg · ha^{-1}]	
		straw	seeds
1	Nosow	3.76	1.54
2	Nosow	5.08	2.07
3	Halasy	0.78	0.32
4	Jablonna-Majatek	5.89	2.41

Slightly lower yields of spring rapeseeds were found in the Nosow (locality 1). The amount of seeds produced here were 1.3–1.6 times lower compared with the yield obtained in Jablonna-Majatek and Nosow (locality 2).

The least favourable conditions for growth and development of oilseed rape was found in Halasy, where the amount of S-SO_4 in the soil was determined as a result of increased antropogenic impact. In the analysed locality, the spring rapeseed yield was only 0.32 1000 kg · ha^{-1} and was 4.8–7.5 times lower than in the other experimental points. This may indicate that both a deficiency and excess of sulphur in crop production negatively affects the growth and yield of crops. Plants grown under excessive environmental conditions of sulphur usually uptake it in large quantities [20, 21]. In such conditions, the amount of organic sulphur in the dry weight of plants increases only slightly, and as a result the remaining quantity of this component accumulates in various tissues in the form of sulphates. These processes may affect the ionic imbalance in plants in addition to the violation of cell sap buffer systems. This in turn influences the amount and quality of harvested crops [7, 22].

The level of S-SO_4 soil fertility in soil of Lubelski region also affected the quality of the harvested plant material. The main criterion in assessing the quality of rapeseeds was their content of fat and protein and the presence of antinutritional substances, such as glucosinolates. The content of these compounds depends mainly on the variety, but the environmental conditions (weather, soil) and agronomic also play a role. Among the agronomic factors on the chemical composition of rapeseed, fertilization has the greatest impact. An important macroelement in nutrition of brassicacea plants is sulphur [23]. A deficiency in sulphur strongly influences the ability to acquire nitrogen from the soil, and hence the biosynthesis of protein nitrogen compounds. In addition, sulphur fertilization increases the percentage of fat and vegetable fats involved in *essential fatty acids* (EFA), thereby improving the nutritional value of oil. It should be noted, that too higher dosage will usually increase the sulphur content of alkenyl glucosinolates in rapeseed, which may worsen the nutritional value of the solvent extracted meal [24–26].

In the conducted research, the fat content of spring rapeseeds developed within 44.70–45.38 % (Table 5).

Table 5

Overall fat content in rapeseed

No. attempt	Locality	Fat content [%]
1	Nosow	45.38
2	Nosow	45.06
3	Halasy	45.35
4	Jablonna-Majatek	44.70

Standards adopted in 2007 by the *Polish Central Crop Variety Research Centre* (COBORU), specifies that the optimal fat content in dry matter of spring oilseed rapeseed should be at 45.0 % [27].

The highest content of fat were measured for spring rapeseeds collected in Nosow (locality 1) and Halasy, where the level of soil fertility of sulphur sulphate was determined as the average or had increased due to antropogenic impact. The lowest percentage of fat was found in the seeds from plants grown in Jablonna-Majatek. The amount of fat found there was at a level of 44.70 % and was slightly lower than the value adopted by COBORU as optimal.

The varied content of sulphur sulphate in the soils of the Lubelski region has also been reflected in the composition of fatty acids in seeds of spring oilseed rape (Table 6).

Table 6

Changes in fatty acid composition in seeds of spring rape

No. attempt	Percentage of fatty acids in the crude fat [%]											
	12:0	14:0	16:0	16:1	18:0	18:1 cis	18:2	18:3 Σ	20:0	20:1	22:0	22:1
1	—	0.08	4.45	0.22	1.94	58.97	22.26	10.30	0.36	1.13	0.30	—
2	—	0.06	4.53	0.11	1.99	57.97	22.51	10.55	0.56	1.41	0.31	—
3	—	0.06	4.52	0.14	1.86	59.08	22.19	10.26	0.53	1.06	0.29	—
4	0.03	0.07	4.61	0.29	1.95	58.20	22.22	10.44	0.55	1.19	0.39	0.07

Explanation of table: (–) – no value; Σ – total acids 18:3 alpha and gamma 18:3; 12:0 – capric acid; 18:3 – α-linolenic acid; 14:0 – myristic acid; 18:3 – γ-linolenic acid; 16:0 – palmitic acid; 20:0 – arachidic acid; 16:1 – palmitoleate acid; 20:1 – eicosenoic acid; 18:0 – stearic acid; 22:0 – behenic acid; 18:1 – oleic acid; 22:1 – erucic acid; 18:2 – linoleic acid.

The content of rapeseed fatty acids in oil with varying levels of saturation determines its relevance and use in human nutrition [28].

For cooking and the production of biofuels purposes, the desirable content of oil should be with high oleic acid ($C_{18:1}$) to over 75 % and with a reduced content of polyunsaturated linolenic acid ($C_{18:3}$) – less than 4 %.

Salad dressings oil, and oils typically used as a liquid part of the carcass to produce margarines should be characterised by increased linoleic acid content ($C_{18:2}$) up to 26 % and with reduced content of linolenic acid – below 4 %.

The oil contains large amounts of saturated fatty acids such as lauric ($C_{12:0}$) and palmitic ($C_{16:0}$) can be used in the manufacture of margarine. Short sequence saturated fatty acids may also be a valuable raw material for the cosmetics industry, baking, confectionery and food industry.

In the production of anti-foaming thickeners for solid lubricants and factices, the most desirable are rape varieties with very high erucic acid ($C_{22:1}$) above 90 %. It should be noted however, that the demand for this type of oil is quite low [10].

In spring, rapeseeds collected from the Lubelski region, the share of oleic acid in relation to the total content of all detected fatty acids ranged between 57.97–59.08 % (Table 6). The highest content of this acid were characterised by rapeseed harvested in Halasy. In this locality, the amount of S-SO_4 in the soil samples was very high and amounted to 52.5 mg S-$SO_4 \cdot kg^{-1}$ soil. In contrast, the lowest percentage of oleic acid was observed in dry seeds from Nosow (locality 2), where the amount of sulphate sulphur in the soil was low and stood at 8.5 mg S-$SO_4 \cdot kg^{-1}$ soil. In the other two localities, the share of acid in the raw fat stood at 58.20 % and 58.97 %. This may indicate that the increase in sulphur content in the soil in a form directly available to plants was accompanied by oleic acid content in seeds of spring rape. This is also confirmed by the earlier studies Kaczor and Kozlowska [8]. This is undoubtedly a major impact on the quality of harvested vegetable fat. The high content of oleic acid ($C_{18:1}$) in rape oil is relatively close to the prized olive oil. In addition, the high content of oleic acid in rapeseed oil is also healthy, because it decreases the amount of LDL fraction (*Low-Density-Lipoprotein*), which are considered the most dangerous in the development of atherosclerosis [29].

Another point of view was presented in the work of Krauze and Bowszys [4] and Szulc et al [28]. They point out that the fertilization of various sulphur compounds can lead to a decrease in fatty acid content for the polyunsaturated fatty acids.

In terms of health benefits, rapeseed oil is also appropriate as an important part in vegetable fat and other essential fatty acids (EFA). They are an important component of all membranes of the body and are involved in the regulation of vital functions by increasing the levels of prostaglandins. In addition, this affects the activity of metyloglutanylo-CoA reductase, which in turn affects the reduction of cholesterol synthesized by the liver [28, 30].

Among the essential fatty acids found in rapeseed oil linoleic acid ($C_{18:\,2}$) and alpha-and gamma-linolenic acid ($C_{18:3}$) are considered noteworthy (Table 6).

The share of linoleic acid in crude fat was between 22.22–22.51 % and was hardly dependant on the fertility of soil for sulphur sulphate in the Lubelski region. At the same time, it is worth highlighting, that the highest acid content of the seeds were in rapeseed from Nosow (locality 2), although the soil samples from this village reported the lowest content of S-SO_4, compared with other soil tests. Similar dependencies were also found in the case of linolenic acid ($C_{18:3}$) where the percentage of crude fat was between 10.26–10.55 %.

Due to stipulated quality requirements that are set for rapeseeds; in determining the fatty acid content we should also consider the content of undesirable erucic acid ($C_{22:1}$), in terms of nutritional value. In accordance with accepted standards, the contribution of erucic should not exceed 2 % [31]. None of the analysed samples were found to exceeded the standard. A small quantity of the fatty acid was observed only in the dry weight of rapeseeds coming from Jablonna-Majatek. In the analysed sample, the level of erucic acid in the crude fat was only at 0.07 %. Similar observations were also made by Kaczor and Kozlowska [8], which in their study found no effect on the sulphur fertilisation part of erucic acid in rapeseed oil.

Among other fatty acids found in spring rapeseeds, the percentage of palmitic acid ($C_{16:0}$) was at 4.45–4.61 %. The highest percentage share of that acid were characterised by rapeseed grown in Jablonna-Majatek, where the amount found in the soil S-SO_4 was determined as low.

The percentage of stearic acid ($C_{18:0}$) in the crude fat also, to some extent, depends on the sulphur sulphate soil fertility in the Lubelski region. The highest content of this acid at 1.94–1.99 % was found in spring rapeseeds coming from Nosow (locality 1 and 2) and Jablonna-Majatek. The lowest level of stearic acid was recorded in rapeseed grown in Halasy. The contribution of this fatty acid in crude fat was at 1.86 %. It should be noted that in the soil samples originating from this locality, the amount of S-SO_4 was very high and amounted to 52.5 mg · kg^{-1}. It can be assumed therefore that such a high level of sulphur in the form available to plants directly affects the quality of harvested vegetable fat.

The eicosenoic acid content ($C_{20:1}$) ranged between 1.06–1.41 %. The largest decrease in the percentage of this acid was also found in samples of rapeseed collected in Halasy. In our study, the quantity of plant fatty acid was 1.1–1.3 times lower compared with the values found in rapeseed grown in other localities.

In contrast to the participation of these fatty acids, the lowest percentage of acid, arachidic ($C_{20:0}$) was found in spring rapeseeds, which were collected in Nosow (locality 1). In the plant material from this locality, the quantity of the fatty acid was 0.36 % while the S-SO_4 content in the soil was defined as the average.

In all samples of rapeseed grown under different conditions of soil fertility with the sulphur sulphate, a small percentage of behenic acid ($C_{22:0}$) was recorded. It levelled off at 0.29-0.39 %.

The palmitoleate acid content ($C_{16:1}$) also to some extent depends on the S-SO_4 in the soil material originating from the Lubelski region. The highest share of that acid was characterised by rapeseed collected from Jablonna-Majatek and Nosow (locality 1). In the case of plant samples collected in Nosow (locality 2), where the amount of S-SO_4 in the soil was low and amounted to only 8.5 mg S-SO_4 · kg^{-1} soil, the percentage of palmitoleate acid was the lowest and amounted to 0.11 %.

In the collected oil low levels of myristic acid ($C_{14:0}$) were found which amounted to 0.06–0.08 %.

Concerning the effect of the sulphur sulphate content in the soil on the quantity and quality of oil obtained from seeds of spring rape, it should be stressed that this was just one of many factors influencing the quality parameters of *Brassicaceae* plants. One cannot forget very important factors in the cultivation of oilseed rape, cultivar characteristics, climatic conditions (humidity, temperature) and other agronomic treatments [1, 10].

Conclusions

The results of preliminary studies to determine the relationship between sulphur sulphate soil fertility in the Lubelski region, and selected characteristics of rapeseed quality permit us to draw the following conclusions:

1. The sulphate sulphur soil fertility of the Lubelski region was clearly differentiated from the low fertility (Nosow – locality 2 and Jablonna-Majatek) by high (Nosow – locality 1) to very high (Halasy).
2. The level of fertility in the analysed soil in S-SO_4 had an influence on the yield and the total fat content in seeds of spring rape.
3. The amount of fat found in the seed samples was within the range for optimum values set by the Polish Central Crop Variety Research Centre.
4. The highest content of fat was characterised by a spring oilseed rapeseed grown in Nosow (locality 1) and Halasy, where the amount of sulphate sulphur in the soil was defined as high or very high fertility.
5. The amount of sulphate sulphur in soil samples also had an influence on the quality of the vegetable fat that was influencing the percentage of individual fatty acids in the crude fat.
6. The percentage of oleic acid ($C_{18:1}$ cis) clearly increased with increasing soil fertility in the sulphur absorbed by the plant and reached the highest value in Halasy, where the level of soil fertility in S-SO_4 was determined as level IV (very high fertility).
7. The content of essential fatty acids clearly reduces with increases of S-SO_4 content the soil. The highest percentages of these acids were found in seed samples collected from the locality with a natural amount of sulphur sulphate in soils (locality 2 and 4).
8. A high sulphate sulphur content in the analysed samples of soil resulted in a decrease in the percentage of fatty acids such as: stearic ($C_{18:0}$), eicosenoic ($C_{20:0}$) and behenic ($C_{22:0}$).
9. There were no relationships between the fertility of sulphur soil in Lubelski region, and participation in terms of arbitrary values of dietary erucic acid ($C_{22:1}$).
10. In addition to the soil's fertility in S-SO_4 to changes in total fat content and the percentages of individual fatty acids, other factors most probably also had an influence including, stress, varied varieties, climatic conditions, and other agrotechnical should be emphasised.

References

[1] Tańska M, Rotkiewicz D. Wpływ różnych czynników na jakość nasion rzepaku. Rośl Oleis. 2003;29:595-616.

[2] Wielebski F, Wójtowicz M. Problemy nawożenia rzepaku siarką w Polsce i na świecie. Rośl Oleis. 2000;21(2):449-463.

[3] Bloem EM. Schwefel-Bilanz von Agrarökosystemen unter besonderer Berücksichtigung hydrologischer und bodenphysikalischer Standorteigenschaften. Landbauforschung Völkenrode. Sonderheft. 1998;192:1-156.

[4] Krauze A, Bowszys T. Wpływ terminu nawożenia siarką rzepaku jarego Star na plon nasion oraz zawartość siarki i tłuszczu. Rośl Oleis. 2001;21(2):285-290.

[5] Podleśna A. Nawożenie siarką jako czynnik kształtujący metabolizm roślin uprawnych i jakość płodów rolnych. Pamięt Puław. 2005;139:161-174.

[6] Marska E, Wróbel J. Znaczenie siarki dla roślin uprawnych. Folia Univ Agric Stetin 204 Agricultura. 2000;81:69-76.

[7] Motowicka-Terelak T, Terelak H. Siarka w glebach Polski – stan i zagrożenia. Warszawa: PIOŚ. Bibl Monit Środow. 1998.

[8] Kaczor A, Kozłowska J. Wpływ nawożenia siarką i wapnowania na ogólną zawartość tłuszczu i skład kwasów tłuszczowych w nasionach roślin krzyżowych. Zesz Probl Post Nauk Roln. 2002;482:245-250.

[9] Lipiński W, Motowicka-Terelak T, Terelak H. Sulphur cocentration in the agricultural soils of the Lublin region and Poland. Acta Agrophys. 2001;52:161-165.

[10] Bartkowiak-Broda I, Wałkowski T, Ogrodowczyk M. Przyrodnicze i agrotechniczne możliwości kształtowania jakości nasion rzepaku. Pamięt Puław. 2005;139:7-25.

[11] Jankowski K, Budzyński W, Symanowski A. Effect of sulfur on the quality of winter rape seeds. J Elementol. 2008;13(4): 521-534.

[12] Jain DK, Gupta AK. Response of mustard to sulphur through gypsum. Recycling of plant nutrients from industrial processes. 10th Int Sympos of CIEC., Procedings. 1996;159-374.

[13] Boratyński K, Grom A, Ziętecka M. Badania nad zawartością siarki w glebie. Cz I Rocz Glebozn. 1975;26(3):121-139.

[14] Lipiński W. Wybrane czynniki kształtujące występowanie siarki w glebach użytków rolnych Lubelszczyzny. Fol Univ Agric Stetin Agricult. 2000;81(204):77-81.

[15] Motowicka-Terelak T, Terelak H, Witek T. Liczby graniczne do wyceny zawartości siarki w glebach i roślinach. IUNG. 1993;P(53):15-20.

[16] Motowicka-Terelak T, Terelak H. Siarka w glebach i roślinach Polski. Folia Univ Agric Stetin. 204 Agricultura. 2000;81:7-16.

[17] Haneklaus S, Bloem E, Schnug E. Sulphur in agroecosystems. Fol Univ Agric Stetin Agricult. 204 Agricultura. 2000;81:17-31.

[18] Szulc P, Piotrowski R, Drozdowska L, Skinder Z. Wpływ nawożenia siarką na plon i akumulację związków siarki w nasionach rzepaku jarego odmiany Star. Fol Univ Agric Stetin Agricult 204 Agricultura. 2000;81:157-162.

[19] Wielebski F. Nawożenie różnych typów odmian rzepaku ozimego siarką w zróżnicowanych warunkach glebowych. I. Wpływ na plon i elementy struktury plonu nasion. Rośl Oleis. 2006;27:265-282.

[20] Dechnik I, Gliński J, Kaczor A, Kern H. Rozpoznanie wpływu kwaśnych deszczy na glebę i roślinę. Probl Agrofiz. 1990;60:3-70.

[21] Motowicka-Terelak T, Dudka S. Degradacja chemiczna gleb zanieczyszczonych siarką i jej wpływ na rośliny uprawne. IUNG. 1991;5-93.

[22] Kaczor A. Ion balance in ordinary cocksfoot sprinkled with acid fall and limed with dolomite. Zesz Probl Post Nauk Roln. 1994;413:161-166.

[23] Wielebski F. Nawożenie różnych typów odmian rzepaku ozimego siarką w zróżnicowanych warunkach glebowych. II. Wpływ na jakość i skład chemiczny nasion. Rośl Oleis. 2006;27:283-297.

[24] Figas A, Drozdowska L, Sadowski Cz. Zależność między nawożeniem siarką a zawartością glukozynolanów i zasiedleniem nasion rzepaku jarego 'Margo' przez Alternaria brassicae. Acta Sci Polon. Agricultura. 2008; 7(3): 43-52.

[25] Tańska M, Rotkiewicz D. Wpływ różnych czynników na jakość nasion rzepaku. Rośl Oleis. 2003;24:595-616.

[26] Wielebski F, Muśnicki Cz. Zmiany ilościowe i jakościowe u dwóch odmian rzepaku ozimego pod wpływem wzrastających dawek siarki w warunkach kontrolowanego niedoboru siarki. (Doświadczenie wazonowe). Rocz AR Poznań. 1998;51(303):129-147.

[27] COBORU. Wyniki porejestrowych doświadczeń odmianowych. Rzepak ozimy, rzepak jary 2007. Porejestrowe doświadczenia odmianowe. 56, 20.

[28] Szulc P, Wejnerowska G, Drozdowska L, Gaca J. Wpływ nawożenia siarką na zmianę zawartości wybranych kwasów tłuszczowych w nasionach rzepaku jarego. Biul Magnezol. 2001;6(1):72-79.
[29] Jerzewska M, Ptasznik S. Ocena występujących na rynku krajowym olejów rzepakowych pod względem zmienności składu kwasów tłuszczowych. Rośl Oleis. 2000;21(2): 557-568.
[30] Spasibionek S. Znaczenie mutagenezy w tworzeniu nowych genotypów roślin oleistych o zmiennym składzie kwasów tłuszczowych. Rośl Oleis. 2002;23(2):533-546.
[31] PN-90/R-66151.: Rośliny przemysłowe oleiste – Ziarno rzepaku i rzepiku podwójnie ulepszonego. NKP 091 ds. Nasiennictwa Rolniczego i Produktów Rolnych. 1990, 3 pp.

ZAWARTOŚĆ TŁUSZCZU I SKŁAD KWASÓW TŁUSZCZOWYCH W NASIONACH RZEPAKU UPRAWIANEGO W WARUNKACH ZRÓŻNICOWANEJ ZASOBNOŚCI GLEB LUBELSZCZYZNY W SIARKĘ

Katedra Chemii Rolnej i Środowiskowej
Uniwersytet Przyrodniczy w Lublinie

Abstrakt: Celem podjętych badań była próba oceny zależności pomiędzy zasobnością gleb Lubelszczyzny w siarkę a niektórymi cechami jakościowymi nasion rzepaku jarego. Materiał badawczy stanowiły próbki glebowe i roślinne pobrane z terenu województwa lubelskiego. Wybór gleb do badań uwzględniał ich kategorię agronomiczną oraz zawartość siarki siarczanowej. Rośliną testową był rzepak jary zbierany w fazie pełnej dojrzałości. Wybór rośliny do badań podyktowany był jej dużymi wymaganiami pokarmowymi w stosunku do siarki. Wyniki przeprowadzonych badań wskazują, że zasobność gleb Lubelszczyzny w siarkę cechowała duża zmienność, począwszy od zasobności niskiej do bardzo wysokiej. Rzutowało to na ogólną zawartość tłuszczu w nasionach oraz na skład kwasów tłuszczowych. Najmniejszą zawartością tłuszczu charakteryzowały się nasiona rzepaku zbieranego w miejscowościach o podwyższonej lub bardzo wysokiej zawartości siarki w glebie. Również procentowy udział kwasu oleinowego wyraźnie zwiększał się wraz ze wzrostem zasobności gleb w S-SO$_4$. Zawartość, cennych z punktu widzenia walorów zdrowotnych, niezbędnych nienasyconych kwasów tłuszczowych wyraźnie jednak malała w miarę zwiększania się w glebie zawartości siarki w formie bezpośrednio dostępnej dla roślin. Nie stwierdzono natomiast wyraźnej zależności pomiędzy zasobnością gleb Lubelszczyzny w siarkę a udziałem niepożądanego z punktu widzenia żywieniowego, kwasu erukowego.

Słowa kluczowe: rzepak jary, zawartość tłuszczu, skład kwasów tłuszczowych, zasobność gleb w S-SO$_4$

DOI: 10.2428/ecea.2012.19(03)021 ECOL CHEM ENG A. 2012;19(3):203-211

Krzysztof GONDEK[1]

CONTENTS OF SULPHUR, TOTAL PROTEIN, METHIONINE AND CYSTEINE IN SPRING WHEAT BIOMASS AFTER FERTILIZATION WITH SEWAGE SLUDGE

ZAWARTOŚĆ SIARKI, BIAŁKA OGÓLNEGO, METIONINY I CYSTEINY W BIOMASIE PSZENICY JAREJ PO NAWOŻENIU OSADAMI ŚCIEKOWYMI

Abstract: The aim of conducted experiments was determining the effect of fertilization with municipal sewage sludge on the contents of sulphur, total protein, methionine and cysteine in spring wheat biomass. The research was conducted in a three-year field experiment on Stagnic Gleysol soil with granulometric composition of heavy silt loam.

On the basis of obtained results it was found that sulphur contents in wheat grain and straw on the treatments where fertilization was applied did not differ significantly. Applied fertilization with mineral fertilizers and farmyard manure and sewage sludge did not lead to any marked changes in weighted average contents of methionine and cysteine in spring wheat grain.

Keywords: spring wheat, sewage sludge, sulphur, total protein, methionine, cysteine

Adequate amount of nutrients supplied to plants and ensured optimal soil conditions are the main element determining production of expected yield of satisfactory biological and technological value. Wheat grain quality is to the greatest extent determined genetically, but the environmental and agrotechnological conditions, including fertilization, are also very important.

A comparison of the effect of natural and organic fertilizers with mineral fertilizer activity shows a greater influence of mineral fertilization on chemical composition of plants. When organic materials of waste origin, among others sewage sludge, are used for fertilization, one may expect their beneficial influence not only on the crop yield but also on its biological value [1].

Under soil and climatic conditions of Poland nitrogen is the element, which among the fertilizer components decisively influences the quantity and quality of obtained

---

[1] Department of Agricultural and Environmental Chemistry, University of Agriculture, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 4346, fax: +48 12 662 4341, email: rrgondek@cyf-kr.edu.pl

yields [2]. However, soil fertility and therefore its productivity is determined also by the content of bioavailable forms of other nutrients, including sulphur. The necessity to fertilize plants with sulphur is due in the first place to this element participation in nitrogen metabolism, which is better utilized at sufficient plant supply in sulphur [3]. The share of this element in forming such amino acids as methionine and cysteine, which are crucial components of proteins, is no less important.

The investigations were conducted to assess the content of sulphur, total protein, methionine and cysteine in spring wheat biomass after its fertilization with municipal sewage sludge.

Material and methods

The assessment of applied fertilization effect on the contents of sulphur, total protein, methinone and cysteine in spring wheat biomass was conducted in a three-year field experiment on ploughland situated 10 km west of Krakow (49°59.625′N; 19°41.910′E). The soil from the area of the experiment was classified as Stagnic Gleysol with granulometric composition of heavy silt clay. Before the experiment outset the basic soil properties were determined using the methods commonly applied by agricultural chemistry [4] and the obtained values have been presented in Table 1.

Table 1

Same properties of farmyard manure and sewage sludge used in experiment and soil before establishment of experiment

Property	Unit	FYM	SSI	SSII	Soil
pH H_2O	[-]	8.23	6.23	6.57	5.94
Dry matter	[$g \cdot kg^{-1}$]	226	297	258	—
Total N	[$g \cdot kg^{-1}$ d.m.]	34.0	26.2	41.6	1.59
Total P		12.8	8.2	22.3	72*
Total K		21.8	1.92	1.26	298*
Total S		4.76	9.66	6.96	0.41

* Content of available forms [$mg \cdot kg^{-1}$ d.m.].

The experiment was set up using randomized blocks methods and the area of fertilized plots was 30 m^2 (5 m · 6 m). The experimental design comprised 6 treatments in four replications: non-fertilized soil (0), soil fertilized with mineral fertilizers (NPK), soil fertilized with mineral fertilizers with sulphur supplement (NPKS), soil fertilized with pig *farmyard manure* (FYM), soil fertilized with municipal sewage sludge from mechanical-biological treatment plant in Krzeszowice (SSI) and soil fertilized with municipal sewage sludge from biological treatment plant in Czernichow (SSII). The sewage sludge and farmyard manure were applied once in the first year of the experiment. Basic chemical properties were determined in the sewage sludge and farmyard manure [5] and the obtained results were shown in Table 1.

Prior to the experiment outset the field was limed to make the soil pH meet the requirements stated in the regulation on municipal sewage sludge [6]. The liming was conducted using hydrated lime, according to 0.5 of hydrolytic acidity. The following year in spring, after completing basic cultivation measures farmyard manure and sewage sludge were spread on the surface of the plots and ploughed (to the depth of 20 cm). Supplementary doses of mineral fertilizers which were used two weeks later were mixed with the soil by means of aggregate cultivator and harrow. Nitrogen dose supplied with the fertilizer was 110.0 kg N · kg^{-1}. Phosphorus and potassium were supplemented to equal level supplied with fertilization on all treatments (except the control), phosphorus to 58.6 kg P · ha^{-1} as a single superphosphate and potassium to 120.0 kg K · ha^{-1} as 60 % potassium salt. Because of a considerable influence of sulphur on biological value of crop yield and great amounts of this element supplied to the soil with farmyard manure and municipal sewage sludge, an additional treatment was introduced to the experimental design, in which sulphur fertilization was conducted against the background of mineral fertilization with nitrogen, phosphorus and potassium. Sulphur was supplied with ammonium sulphate dosed 20.0 kg S · ha^{-1}. On treatments where basic and supplementary doses of mineral fertilizers were used, nitrogen was supplied as ammonium nitrate.

In order to avoid species changeability under the conditions of the experiments, each year the test plant was spring wheat, 'Jagna' c.v. Assumed plant density was 485 plants per 1 m^2. In order to supplement N, P, K nutrients and S but only on the treatment where sulphur was supplied in a mineral form, in the second and third year of the investigations the identical doses were applied as in the first year, but exclusively as mineral fertilizers, N – ammonium nitrate, P – single superphosphate, K – 60 % potassium salt and S – as ammonium sulphate. Wheat vegetation period depended on the weather conditions and lasted 117 days in the first year, 106 days in the second and 107 days in the third. Wheat was harvested at full maturity. During vegetation chemical treatment was applied to protect the plantation against weeds and fungal diseases. In order to determine wheat grain yield under field conditions, the plants were gathered from the 4 m^2 area of each plot separately. After ear threshing, the obtained grain and straw yields were dried (at 70 ^{o}C) and dry mass content was determined. In dried and milled plant material sulphur content was assessed after a sample mineralization in concentrated nitric acid [7], with ICP-AES method on JY 238 Ultrace apparatus. Methionine and cysteine concentrations were assessed after oxidating hydrolysis with formic acid followed by sample hydrolysis with 6 mol · dm^{-3} HCl solution conducted by means of ninhydrin method on Ingos AAA-400 analyzer.

Chemical analysis of the experimental material was conducted in four replications, while initial materials (farmyard manure, sewage sludge and soil) were analyzed in two replications and a reference sample NCS DC 733448 (China National Analysis Center for Iron & Steel) was attached to each analytic series.

A two-way analysis of variance (factors: fertilization x years) was conducted on the results obtained for grain and straw yields, whereas one-way ANOVA was conducted for sulphur, protein and amino acid contents (factor: fertilization) in totally randomized design using f-Fisher test. The significance of differences between arithmetic means was verified basing on the homogenous groups determined by t-Tukey test on significance level $p < 0.05$. All statistical computations were made using Statistica PL package [8].

The weather conditions (rainfall and temperature) during the experiment were presented in Tables 2 and 3.

Table 2

Monthly and periodic precipitation totals in study years [mm]

Year	Month						Σ
	III; Mar.	IV; Apr.	V; May	VI; Jun.	VII; Jul.	VIII; Aug.	Jan.; I – Dec.; XII
2005	20.7	49.1	61.3	40.6	113.4	102.6	597.5
2006	60.1	56.5	51.9	89.1	14.1	104.1	567.9
2007	61.1	15.4	51.7	72.1	71.0	76.4	830.4

Table 3

Mean daily air temperature in study years [^{o}C]

Year	Month						Mean
	III; Mar.	IV; Apr.	V; May	VI; Jun.	VII; Jul.	VIII; Aug.	Jan.; I – Dec.; XII
2005	–0.2	6.8	11.4	14.4	17.6	15.4	6.8
2006	0.2	5.6	10.9	15.0	18.6	15.6	7.5
2007	6.0	8.5	15.2	18.4	19.4	19.0	9.2

The weather course during the period of the experiment did not differ between the years. The highest rainfall amount (total from March to August) characterized the year 2005 (1st year of the experiment). A higher annual temperature was registered in 2007, whereas average temperature during spring wheat vegetation (March to August) generally did not differ.

Results and discussion

Spring wheat grain yields registered in the experiment on the fertilized treatments in the first year were generally lower in comparison with the yields obtained on the same treatments in two subsequent years of the experiment (Table 4).

Table 4

Yield of grain spring wheat [$Mg \cdot ha^{-1}$ d.m. ± SD; n = 4]

Objects	1st year	2nd year	3rd year
0	$2.53^{ab} \pm 0.15$	$1.93^{a} \pm 0.29$	$2.55^{ab} \pm 0.12$
NPK	$4.13^{bcde} \pm 0.54$	$4.81^{de} \pm 0.52$	$4.30^{de} \pm 0.40$
NPKS	$4.05^{bcde} \pm 1.01$	$4.05^{bcde} \pm 1.10$	$5.07^{e} \pm 0.28$
FYM	$3.36^{abcd} \pm 0.19$	$4.01^{bcde} \pm 1.16$	$5.39^{e} \pm 0.36$
SSI	$3.83^{bcde} \pm 0.32$	$4.83^{de} \pm 0.62$	$4.70^{de} \pm 0.44$
SSII	$3.40^{abcd} \pm 0.92$	$4.60^{de} \pm 0.98$	$4.42^{de} \pm 0.13$

Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Tukey test.

Additional sulphur fertilization in a mineral form (NPKS) did not cause any marked increase in wheat grain yield in comparison with the yields gathered on the treatments where farmyard manure (FYM), municipal sewage sludge (SSI, SSII) or nitrogen, phosphorus and potassium were applied in mineral form (NPK).

Greater diversification was noted for yields of spring wheat straw (Table 5).

Table 5

Yield of straw spring wheat [$Mg \cdot ha^{-1}$ d.m. ± SD; n = 4]

Objects	1st year	2nd year	3rd year
0	$3.01^{ab} \pm 0.56$	$2.03^{a} \pm 0.16$	$3.48^{b} \pm 0.30$
NPK	$4.91^{de} \pm 0.47$	$4.64^{cde} \pm 0.57$	$4.36^{cd} \pm 0.14$
NPKS	$5.65^{e} \pm 0.99$	$4.38^{cd} \pm 0.66$	$4.71^{cde} \pm 0.41$
FYM	$4.21^{bcd} \pm 0.21$	$4.61^{cde} \pm 0.37$	$4.71^{cde} \pm 0.30$
SSI	$3.99^{bcd} \pm 0.28$	$4.67^{cde} \pm 0.69$	$4.65^{cde} \pm 0.14$
SSII	$3.86^{bcd} \pm 0.74$	$4.63^{cde} \pm 0.59$	$4.62^{cde} \pm 0.26$

Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Tukey test.

Irrespective of applied fertilization significantly greatest straw yields were gathered in the first year of the experiment on the treatment where mineral fertilization with nitrogen, phosphorus and potassium and sulphur (NPKS) was conducted. In the two subsequent years straw yields on the treatments where only mineral fertilization was used (NPK, NPKS) were higher (from 5 % to 22 %) in comparison with yields from the same treatments harvested in the first year. On the treatments where wheat was fertilized with farmyard manure (FYM) or municipal sewage sludge (SSI, SSII) in the second and third year of the experiments between 9 % and 20 % increase in straw biomass yield was obtained in comparison with the amount of straw harvested on these treatments in the first year.

Proper and rational fertilization affects the amount and improves biological and technological value of plant yields [9]. Unfavourable production conditions in Poland compared with the adjacent countries pose a severe obstacle in generating high yields of good quality. However, it is possible to improve soil fertility through proper agrotechnical measures, including fertilization. According to Kwiatkowski et al [9] application of intensive cultivation measures on winter wheat plantations, irrespective of cultivar, allowed to reach over 72 % higher grain yields in comparison with yields from protected crops tended in a limited way. According to Lipa [10] limited mineral fertilization and lower herbicide doses may cause a decline in yields in result of worsening of most cereal stand and yield structure elements (number of ears per m^2, TGW). In the presented experiments, grain or straw yields obtained on the treatments where fertilization with farmyard manure (FYM) or municipal sewage sludge (SSI, SSII) was conducted and approximate to these produced on mineral fertilizer treatments were obtained in the second year of the experiment. It shows that also the kind of applied fertilization has a significant influence on plant yielding. Apart from agrotechnical measures, the important factor limiting yielding of both winter and spring wheat forms is soil pH [11, 12].

No matter which fertilization was applied, sulphur concentrations both in the vegetative (straw) and generative (grain) parts of wheat were significantly higher than this element content assessed in wheat biomass from the non-fertilized treatment – (0) (Table 6). No marked differences were registered between sulphur content in wheat biomass fertilized with this element mineral form and S content in the biomass from the treatments where farmyard manure (FYM) or municipal sewage sludge (SSI, SSII) were used.

Table 6

Weighted mean (from three years) content of sulphur in biomass of spring wheat [g · kg^{-1} d.m. ± SD; n = 4]

Objects	Grain	Straw
0	$1.29^{a} \pm 0.06$	$0.92^{a} \pm 0.03$
NPK	$1.44^{b} \pm 0.04$	$1.12^{b} \pm 0.03$
NPKS	$1.41^{b} \pm 0.07$	$1.14^{b} \pm 0.05$
FYM	$1.44^{b} \pm 0.08$	$1.01^{ab} \pm 0.15$
SSI	$1.46^{b} \pm 0.02$	$1.12^{b} \pm 0.04$
SSII	$1.37^{ab} \pm 0.04$	$0.99^{ab} \pm 0.10$

Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to the Tukey test.

As reported by Kaczor et al [13] sulphur content depends on the plant development stage, its organ but also on fertilization. Increase in sulphur content resulting from fertilization with this element was noted by McGrath and Zhao [14] and Zhao et al [15]. It has been also reflected by the results of presented experiments. A diversification in sulphur contents was also noted between generative (grain) and vegetative (straw) wheat parts. Such dependency was not confirmed either by the Author's investigations or by Boreczek [16]. Despite the fact that wheat belongs to a group of plants with relatively low sulphur requirements, this element deficiency in soil may lead to lower nitrogen utilization [11].

Total (for three years) amounts of sulphur taken up with wheat grain and straw yield were on a similar level, whereas the differences between individual treatments were not statistically significant (Table 7).

Table 7

Total (from three years) uptake of sulphur with biomass of spring wheat [kg · ha^{-1} ± SD; n = 4]

Objects	Grain	Straw	Σ
0	$9.0^{a} \pm 0.6$	$7.8^{a} \pm 0.6$	$16.8^{a} \pm 0.4$
NPK	$19.0^{b} \pm 1.0$	$15.6^{bc} \pm 1.6$	$34.6^{b} \pm 2.4$
NPKS	$18.7^{b} \pm 3.0$	$16.9^{c} \pm 2.3$	$35.6^{b} \pm 4.9$
FYM	$18.4^{b} \pm 1.5$	$13.6^{bc} \pm 2.2$	$32.0^{b} \pm 2.3$
SSI	$19.5^{b} \pm 1.2$	$14.9^{bc} \pm 0.5$	$34.4^{b} \pm 0.9$
SSII	$17.0^{b} \pm 2.3$	$13.0^{b} \pm 2.1$	$30.0^{b} \pm 4.4$

Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to the Tukey test.

The highest amounts of sulphur (total amounts for grain and straw) were absorbed by wheat on the treatments where sulphur was supplied in mineral form (NPKS). In comparison with this treatment, sulphur quantities absorbed by wheat fertilized with farmyard manure or sewage sludge were between 3 % and 16 % lower.

Total protein content (N content multiplied by 6.25) in spring wheat grain from fertilized treatments was significantly higher than assessed in wheat grain on the treatment where no fertilization was applied (0) (Table 8). The lowest quantities of total protein were assessed in wheat grain receiving mineral fertilizers, without sulphur supplement (NPK) and in wheat grain from the treatment where farmyard manure (FYM) was used, however the results were not corroborated by the research conducted by Filipek et al [1]. On the treatments where wheat was fertilized with municipal sewage sludge (SSI and SSII), total protein contents were comparable with values assessed in wheat grain from the treatment on which mineral fertilization but with sulphur supplement (NPKS) was used. Total protein contents determined in spring wheat grain from these treatments were comparable to assessed by Filipek et al [1] in winter wheat grain fertilized with farmyard manure and sewage sludge from a dairy plant, but in only the limed series.

Table 8

Weighted mean (from three years) content of total protein [g · kg^{-1} d.m. ± SD; n = 4] and cysteine and methionine in proteins of grain of spring wheat [g · kg^{-1} proteins ± SD; n = 4]

Objects	Total protein	Cysteine	Methionine
0	$142.6^a \pm 4.13$	$12.7^a \pm 0.4$	$7.32^a \pm 0.28$
NPK	$158.2^b \pm 4.89$	$12.7^a \pm 0.5$	$7.11^a \pm 0.27$
NPKS	$160.8^b \pm 3.18$	$12.7^a \pm 0.4$	$7.16^a \pm 0.31$
FYM	$158.8^b \pm 8.04$	$12.2^a \pm 1.5$	$7.31^a \pm 0.69$
SSI	$161.2^b \pm 3.74$	$12.6^a \pm 0.7$	$7.59^a \pm 0.61$
SSII	$160.3^b \pm 3.51$	$12.4^a \pm 0.3$	$7.69^a \pm 0.24$

Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to the Tukey test.

According to Kwiatkowski et al [9] protein content in wheat grain is conditioned in the first place by the level of agrotechnical measures, by which not only optimal fertilization level is meant.

Cysteine content in spring wheat grain protein did not differ significantly between treatments (Table 8). Still, wheat grain protein from the treatments on which fertilization with farmyard manure (FYM) and municipal sewage sludge (SSI and SSII) was applied, contained between 0.8 % and almost 4 % less of this amino acid than the content assessed in wheat grain protein from the treatments where only mineral fertilizers (NPK, NPKS) were used, but also in comparison with the content assessed in grain protein on the treatment which was not receiving any fertilization (0).

An opposite dependence was observed for methionine content in grain protein. The content of this amino acid was the lowest in wheat grain protein from the treatments on which exclusively mineral fertilizers (NPK and NPKS) were applied. Methionine contents in wheat grain protein fertilized with farmyard manure (FYM) or municipal sewage sludge (SSI and SSII) were between 2.5 % and almost 8 % higher in comparison with the content assessed in grain protein on the treatments on which mineral fertilizers were applied.

Limited or unbalanced fertilization generally causes a decrease in plant yields, but also worsening of most elements of stand structure and quality of obtained biomass. This situation may be aggravated by increasingly frequent signals about among others sulphur deficiency in the soils of Europe, which significantly affects the improvement of biological properties of plant yields [17]. Fertilization with sewage sludge did not cause any marked changes in weighted average for three years cysteine and methionine content assessed in grain protein of wheat fertilized with mineral materials. According to Dubetz and Gardiner [18] the content of protein and amino acids change visibly under the influence of increasing nitrogen doses. In the Author's own investigations the level of nitrogen fertilization was not diversified, whereas the application of moderate dose of this element to the soil in mineral fertilizers and organic materials (farmyard manure, sewage sludge) contributed to stabilization of the content of studied amino acids in spring wheat grain protein.

Proper balancing of amino acids in feed doses with animal requirements is crucial not only for utilization of the most precious element, *ie* feed protein but also because of decreasing the amount of nitrogen excreted with urea in case of any amino acid excess in comparison with needs. The analyzed spring wheat grain, irrespective of applied fertilization, met the animal nutritional requirements with respect to methionine and cysteine contents [19].

Conclusions

1. No significant differences were registered between sulphur content in the biomass of wheat fertilized with this element mineral form and this element content in wheat biomass from the treatments where farmyard manure or sewage sludge were used as fertilizers.
2. Applied fertilization did not diversify total protein content in spring wheat grain.
3. Fertilization of spring wheat with municipal sewage sludge did not cause any notable changes of weighed average content of the analyzed amino acids.

References

[1] Filipek T, Fidecki M, Harasim P. Ann UMCS, Sec E. 2004;59(4),1925-1931.
[2] Ciećko Z, Żołnowski AC, Krajewski W. Zesz Probl Post Nauk Roln. 2006;513,55-62.
[3] Millard P, Gordon AH, Richardson AJ, Chesson A. J Sci Food Agric. 2006;40,305-314.
[4] Lityński T, Jurkowska H, Gorlach E. Analiza chemiczno-rolnicza. Warszawa: Wyd PWN; 1976.
[5] Baran S, Turski R. Ćwiczenia specjalistyczne z utylizacji odpadów i ścieków. Lublin: Wyd. AR w Lublinie; 1996.
[6] Rozporządzenie Ministra Środowiska z dn. 27 sierpnia 2002 r. w sprawie komunalnych osadów ściekowych. DzU. 2002; nr 134, poz 1140.
[7] Ostrowska A, Gawliński A, Szczubiałka Z. Metody analizy i oceny gleby i roślin. Warszawa: Wyd. IOŚ; 1991.

[8] Stanisz A. Przystępny kurs statystyki w oparciu o program Statistica PL na przykładach z medycyny. Kraków: Wyd. Statsoft Polska; 1998.
[9] Kwiatkowski C, Wesołowski M, Harasim E, Kubecki J. Pamięt Puław. 2006;142,277-286.
[10] Lipa J. Pamięt Puław. 2004;135,241-259.
[11] Kocoń A. Pamięt Puław. 2005;139,55-64.
[12] Brodowska MS. Acta Agrophys. 2003;1(4),617-622.
[13] Kaczor A, Brodowska MS, Kowalski G. Ann UMCS, Sec E. 2004;59(4),1847-1853.
[14] McGrath SP, Zhao FJ. J Agric Sci. 1996;162,53-62.
[15] Zhao FJ, Hawkesford MJ, Warrilow AGS, McGrath SP, Clarkson DT. Plant Soil. 1996;81,317-327.
[16] Boreczek B. Fragm Agron. 2001;4,118-133.
[17] Flaete NES, Hollung K, Ruud L, Sogn T, Faergestad EM, Skarpeid HJ, Magnus EM, Uhlen AK. J Cereal Sci. 2005;4,357-369.
[18] Dubetz S, Gardiner EE. J Plant Nutr. 1980;2(5),517-523.
[19] Kujawiak R (red). Racjonalne żywienie zwierząt. Poznań: Wyd. Polskie SANO; 1996.

ZAWARTOŚĆ SIARKI, BIAŁKA OGÓLNEGO, METIONINY I CYSTEINY W BIOMASIE PSZENICY JAREJ PO NAWOŻENIU OSADAMI ŚCIEKOWYMI

Katedra Chemii Rolnej i Środowiskowej
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem przeprowadzonych badań było określenie wpływu nawożenia komunalnymi osadami ściekowymi na zawartość siarki, białka ogólnego, metioniny i cysteiny w biomasie pszenicy jarej. Badania przeprowadzono w 3-letnim doświadczeniu polowym na glebie opadowo-glejowych o składzie granulometrycznym gliny ciężkiej pylastej.

Na podstawie uzyskanych wyników badań stwierdzono, że zawartość siarki w ziarnie i słomie pszenicy oraz zawartość białka w ziarnie z obiektów, w których zastosowano nawożenie nie różniła się istotnie. Zastosowane nawożenie nawozami mineralnymi, a także obornikiem i osadami ściekowymi nie spowodowało istotnych zmian w średniej ważonej zawartości metioniny i cysteiny w ziarnie pszenicy jarej.

Słowa kluczowe: siarka, pszenica jara, osady ściekowe, białko ogólne, metionina, cysteina

DOI: 10.2428/ecea.2012.19(03)022 ECOL CHEM ENG A. 2012;19(3):213-221

Zofia SPIAK[1], Urszula PISZCZ[1]
and Tomasz ZBROSZCZYK[1]

MODIFICATION OF SULFUR CONTENT IN *Miscanthus x giganteus* UNDER DIFFERENT NITROGEN AND POTASSIUM FERTILIZATION

ZMIANY ZAWARTOŚCI SIARKI W MISKANCIE OLBRZYMIM POD WPŁYWEM ZRÓŻNICOWANEGO NAWOŻENIA AZOTEM I POTASEM

Abstract: The purpose of the presented work was to evaluate the changes in the sulfur content in the aboveground parts of *Miscanthus x giganteus* under the influence of applying incrementally increasing doses of nitrogen and potassium. Field experiments were conducted using the split-plot method on light, sandy soil. Varying doses of nitrogen-based fertilizers were used: 100 kg N; 150 kg N and 200 kg N · ha^{-1}, with potassium 83 kg K and 124 kg K (100 kg and 150 kg K_2O). Plant samples were taken from 2007 to 2009 during the months of June through October. Throughout the vegetation period the sulfur content in the studied Miscanthus was twice as high in the leaves as in the stems. The highest amount of sulfur was found in young plants. By the end of the vegetation period the sulfur content in the leaves fell by about 30 % and in the stems by 60 %. None of the studies found that fertilizing with nitrogen significantly modified the sulfur content in Miscanthus. However, it was determined that the sulfur content was significantly higher in the stems and leaves of the Miscanthus fertilized with higher doses of potassium. Throughout the three-year study period increasingly higher amounts of sulfur were observed in the aboveground parts of Miscanthus at all the field trial locations.

Keywords: *Miscanthus x giganteus*, nitrogen and potassium fertilizing, sulfur content, vegetation period

Despite its significant presence in the environment, sulfur is becoming an increasingly deficient ingredient in agrocenoses [1, 2]. A lack of sulfur makes it impossible to obtain high plant yields, because of the significant role sulfur plays in key physiological processes. More and more often prophylactic crop fertilization is being recommended for every kind of plant group, since even if there is a high content of sulfur in the soil, it does not usually have a toxic effect on plants. The purpose of growing energy plants is to obtain as much biomass as possible within a given set of quality parameters. In

---

[1] Department of Plant Nutrition, Wroclaw University of Environmental and Life Sciences, ul. Grunwaldzka 53, 50–375 Wrocław, Poland, phone: +48 71 320 56 05, email: zofia.spiak@up.wroc.pl

numerous studies the yields of different energy plants were compared, and their chemical content and the quality of the ash was analyzed [3–7]. The chemical content of energy plants depends to a high degree on the granulometric category of the soil [8, 9], fertilizing especially young plants, and soil fertility [10–12]. The chemical content of Miscanthus is dependent on the volume of the obtained yield, and above all, the timing of plant harvesting [13–15].

The sulfur content in the biomass of Miscanthus used for combustion should be as low as possible and stay below 0.08 % S because of the possibility of an adverse impact on energy-producing equipment [16, 17]. Miscanthus should not be fertilized with fertilizers containing sulfur, because in comparison with butterfly or cabbage plants, grasses show less need for sulfur, and its removal is connected to a much higher degree to the grain yield rather than the straw yield. Refraining from using sulfur during fertilization even with a sulfur deficiency in the soil does not mean there will be a low content of this element in plant tissues, since plants can absorb sulfur oxide from the atmosphere.

The purpose of the conducted study was to evaluate the changes in sulfur content in the aboveground parts of *Miscanthus x giganteus* throughout the vegetation period and evaluate the effect of applying fertilizers with the elements that most greatly modify the mass of vegetative parts – nitrogen and potassium.

Materials and methods

A multiyear testing field was set up in 2004 in light soil, categorized as a weak rye complex, using the method of randomly drawn subblocks with two variables. In the study two levels of potassium fertilization (83 and 124 kg K $\cdot$ ha^{-1}) and three levels of nitrogen fertilization (100, 150 and 200 kg N $\cdot$ ha^{-1}) were tested. Potassium was in the form of a 60 % potassium salt, and nitrogen was used in the form of urea. At all testing plots phosphorus fertilization was applied in the amount of 26 kg P $\cdot$ ha^{-1}, in the form of granular triple superphosphate. An exact description of the experiment, sampling methodology, soil and atmosphere conditions, yields, and the chemical content of Miscanthus can be found in the monograph on Miscanthus [15].

The evaluation of the sulfur content dynamics was conducted in the years 2007–2009. Plant samples were taken five times during the vegetation period beginning in June through October in the first ten days of each month. Twenty plants were cut from the field plots, their mass was determined, and they were separated into leaves and stems. During the last sample taken in October the proportion of the leaf mass and the stem mass in the Miscanthus yield was determined. The sulfur content in the plant samples was determined by the nefelometric method of Butters-Chenery.

A statistical analysis with the application of variance analysis for the random subblock method was applied to the obtained results. Then, after determining the significance of the differences the results were evaluated with the help of the confidence interval (LSD = 0.05), based on multiple tests of the Duncan range.

Results and discussion

During the studies the sulfur content in the Miscanthus leaves varied from 0.65 to 1.79 g S · kg^{-1}, while lower sulfur concentrations were found in the stems, which ranged from 0.19 to 1.40 g S · kg^{-1} d.m. (Tables 1 and 2). The highest sulfur content was found in the leaves and stems in young plants intensively fertilized with nitrogen and potassium.

Table 1

Sulfur content [g · kg^{-1}] in the leaves of *Miscanthus x giganteus* during vegetation

Month	Dose of K	Dose of N	Years			Mean 2007–2009
			2007	2008	2009	
June	83	100	1.36	1.19	1.58	1.38
		150	1.30	1.22	1.40	1.31
		200	1.47	1.85	1.48	1.60
	124	100	1.41	1.33	1.71	1.48
		150	1.34	1.72	1.62	1.56
		200	1.33	1.72	1.79	1.62
July	83	100	1.25	1.08	1.47	1.27
		150	1.23	1.15	1.33	1.24
		200	1.32	1.70	1.33	1.45
	124	100	1.32	1.24	1.62	1.39
		150	1.22	1.61	1.50	1.44
		200	1.20	1.59	1.66	1.48
August	83	100	1.05	1.09	1.29	1.14
		150	0.96	1.24	1.18	1.12
		200	0.76	1.04	1.05	0.95
	124	100	1.26	1.13	1.24	1.21
		150	1.11	1.24	1.18	1.17
		200	1.10	1.09	1.14	1.11
September	83	100	1.02	1.24	1.24	1.16
		150	0.96	1.03	1.15	1.05
		200	0.93	1.01	1.21	1.05
	124	100	1.13	1.31	1.25	1.23
		150	1.06	1.19	1.52	1.26
		200	1.04	1.04	1.19	1.09
October	83	100	0.65	0.91	1.23	0.93
		150	0.91	0.90	1.27	1.03
		200	0.97	0.92	1.09	0.99
	124	100	0.82	1.22	1.30	1.11
		150	0.96	1.04	1.29	1.10
		200	1.09	1.02	1.15	1.09

Table 1 contd.

Month	Dose of K	Dose of N	Years			Mean 2007–2009
			2007	2008	2009	
Mean values						
June			1.37	1.51	1.60	1.49
July			1.26	1.39	1.48	1.38
August			1.04	1.14	1.18	1.12
September			1.02	1.13	1.26	1.14
October			0.90	1.00	1.22	1.04
LSD $\alpha_{0.05}$			**0.08**	**0.15**	**0.11**	**0.06**
	83		1.07	1.17	1.29	1.18
	124		1.16	1.30	1.41	1.29
LSD $\alpha_{0.05}$			**0.05**	**0.10**	**0.07**	**0.04**
		100	1.12	1.17	1.39	1.23
		150	1.10	1.23	1.34	1.23
		200	1.12	1.30	1.31	1.24
LSD $\alpha_{0.05}$			**n.s.**	**n.s.**	**n.s.**	**n.s.**
Years			1.12	1.23	1.35	1.23
LSD $\alpha_{0.05}$			**0.05**			—

n.s. – not significant difference.

During the beginning period of plant growth in June, the sulfur content in the stems was on average about 64 % of the sulfur content in the leaves. Towards the end of the vegetation period the sulfur content in the stems was about 37 % of the sulfur content in the Miscanthus leaves. In Malinowska's studies [11] during the first year of planting sugar Miscanthus it was determined that the amount of N, P, K was four times higher in the leaves and the amount of K and Mg was two times higher in the stems.

Over the period of vegetation and plant growth a decrease in the sulfur content in the plant tissues was observed. On the average the sulfur content in the Miscanthus leaves dropped during the study period by 0.45 g S $\cdot$ kg^{-1}, which was 30 % of the initial value. From June to October the drop in the sulfur content in the stems was even higher, 59 % (0.60 g S $\cdot$ kg^{-1} d.m.). Similar dependencies – decreases in the sulfur content in the aboveground parts of Miscanthus throughout the vegetation period – were also determined in the studies with different Miscanthus clones done by Kalembasa et al [18]. However, in their studies the sulfur content in Miscanthus annuals decreased from the beginning of June through September, but later in October increased.

The determined sulfur content in the Miscanthus leaves was higher than in the stems of this plant. They also absorb much more moisture. For this reason it does not seem appropriate to use them for making into pellets [16]. At the end of the Miscanthus vegetation the leaves contained on average of 1.23 g S $\cdot$ kg^{-1}. This is a small amount compared with the organic waste materials such as manure – 4.9 g S $\cdot$ kg^{-1}, or a mushroom floor – 16.9 g S $\cdot$ kg^{-1} [19]. However, because of limited access to organic waste materials, Miscanthus leaves can become a precious source of sulfur and other nutritional elements at farms which have an opportunity to obtain Miscanthus leaves and use them for fertilization in addition to organic waste.

The time of harvesting the Miscanthus also has a significant impact, not only on the sulfur content, but also on the dry mass content, moisture of the collected biomass, and the ash content [14]. In numerous studies it was determined that in the period from October through March the N, P, K and Mg content also dropped by almost half [13, 20].

In our experiment the decrease in the sulfur content in the aboveground parts of Miscanthus took place throughout the vegetation period, but it should be noted that both in the leaves and the stems an especially significant drop in the sulfur concentration took place in the first two months of vegetation – June and July. In each of the study years the decrease was statistically significant, and the average sulfur content in August was a 25 % decrease from the value found in the Miscanthus leaves at the beginning of the vegetation period and a 47 % decrease from the beginning value in the stems.

Despite the known interdependencies between sulfur and nitrogen, fertilizing the Miscanthus in the experiment with different doses of nitrogen did not significantly modify the sulfur content for any of the study years in either the leaves or stems (Tables 1 and 2). The mean sulfur content at all plots fertilized with nitrogen was similar. Increasing the nitrogen dose in the second year of studies from 100 to 200 kg N · ha^{-1} only caused a slight difference in the sulfur content in the leaves from 1.17 to 1.30 g · S · kg^{-1} and in the stems from 0.59 to 0.63 g · S · kg^{-1}; however, those tendencies were not confirmed statistically.

It should be added that fertilizing with nitrogen did not have any significant impact on the shooting mass created in this period [15].

A stronger factor impacting the sulfur content in the Miscanthus leaves and stems turned out to be potassium fertilizing (Tables 1 and 2). Increasing the dose of this element from 83 kg K to 124 kg K · ha^{-1} caused a significant increase in the sulfur content of the Miscanthus biomass in each of the analyzed periods of plant growth.

In the Miscanthus leaves the average annual increase in the sulfur content was 0.12 g S · kg^{-1}, which corresponded to a 10 % increase in the concentration of this element. This was a uniform increase in the three consecutive years: 9 %, 12 % and 10 %, respectively. An increase in the sulfur content as the Miscanthus plantation aged was also observed in the stems of the plant (Table 2). The increase was pronounced, 0.25g · S (66 %) in the first year of studies and 0.24 g S · kg^{-1} (49 %) in the second year. In the third year there were unfavorable atmospheric conditions. Despite a drop in the Miscanthus biomass, the observed increase of the sulfur content in the stems fertilized with potassium was smaller but still significant, measured at 0.1 g S · kg^{-1} or 14 %.

Fertilizing with potassium modified the sulfur content in the Miscanthus stems even more than in the leaves (Table 2). On the average during the three years of studies an increase in the sulfur content in the stems from fertilizing with high doses of potassium was found to be over 35 %. An especially significant concentration of sulfur in the

Table 2

Sulfur content [g · kg^{-1}] in the stems of *Miscanthus x giganteus* during vegetation

Month	Dose of K	Dose of N	Years			Mean 2007–2009
			2007	2008	2009	
June	83	100	0.73	0.72	1.05	0.83
		150	0.61	0.69	1.03	0.78
		200	0.62	0.77	1.34	0.91
	124	100	0.87	0.86	1.40	1.04
		150	0.85	1.12	1.27	1.08
		200	0.92	1.09	1.29	1.10
July	83	100	0.60	0.59	0.92	0.70
		150	0.48	0.57	0.91	0.65
		200	0.47	0.62	1.19	0.76
	124	100	0.73	0.72	1.26	0.90
		150	0.72	0.99	1.14	0.95
		200	0.76	0.93	1.13	0.94
August	83	100	0.35	0.36	0.58	0.43
		150	0.29	0.40	0.71	0.46
		200	0.27	0.43	0.70	0.47
	124	100	0.50	0.71	0.57	0.59
		150	0.61	0.61	0.55	0.59
		200	0.54	0.61	0.53	0.56
September	83	100	0.22	0.47	0.42	0.37
		150	0.26	0.35	0.42	0.34
		200	0.24	0.39	0.40	0.34
	124	100	0.50	0.61	0.58	0.56
		150	0.55	0.53	0.72	0.60
		200	0.47	0.53	0.44	0.48
October	83	100	0.19	0.32	0.40	0.30
		150	0.19	0.24	0.39	0.27
		200	0.26	0.40	0.23	0.30
	124	100	0.42	0.54	0.51	0.49
		150	0.55	0.62	0.47	0.54
		200	0.52	0.51	0.34	0.45
Mean values						
June			0.77	0.87	1.23	0.96
July			0.63	0.73	1.09	0.82
August			0.42	0.52	0.60	0.51
September			0.37	0.48	0.49	0.45
October			0.35	0.44	0.39	0.39
LSD $\alpha_{0.05}$			**0.05**	**0.10**	**0.10**	**0.05**

Table 2 contd.

Month	Dose of K	Dose of N	Years			Mean 2007–2009
			2007	2008	2009	
	83		0.38	0.49	0.71	0.53
	124		0.63	0.73	0.81	0.72
LSD $\alpha_{0.05}$			**0.03**	**0.06**	**0.06**	**0.04**
		100	0.51	0.59	0.77	0.62
		150	0.51	0.61	0.76	0.63
		200	0.50	0.63	0.76	0.63
LSD $\alpha_{0.05}$			**n.s.**	**n.s.**	**n.s.**	**n.s.**
Years			0.51	0.61	0.76	0.63
LSD $\alpha_{0.05}$			**0.04**			—

n.s. – not significant difference.

stems was observed during the first two years of studies in which higher yields of Miscanthus were obtained (Fig. 1).

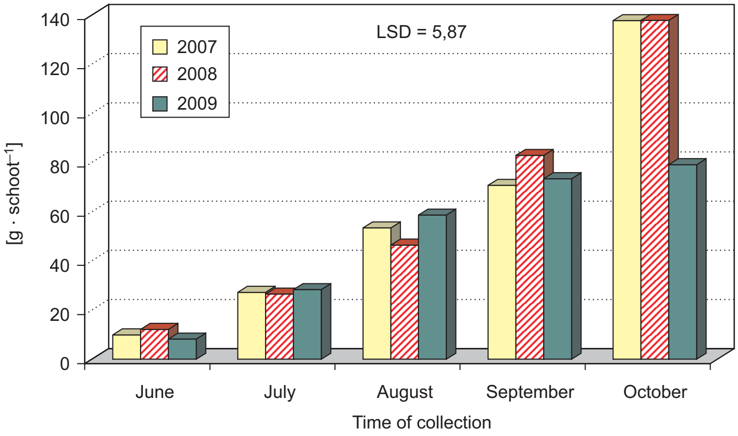

Fig. 1. Dynamics of the accumulation of dry mass in the Miscanthus shoots during the vegetation period

On the basis of the average proportion of the leaf mass and the stem mass in the final yield, the magnitude and the sulfur content of the yield was determined for the Miscanthus biomass collected in the fall in October (Fig. 2). The share of the leaves in the crop mass was an average of 26.6 % at the time of studies; however, the amount of accumulated sulfur in the leaves was over half of the total amount of sulfur in the biomass.

The concentration of sulfur in both the Miscanthus leaves and stems turned out to be significantly dependent on the year of planting (Table 1 and 2). This may be the result of stronger development and an increase in the range of the root system at the older

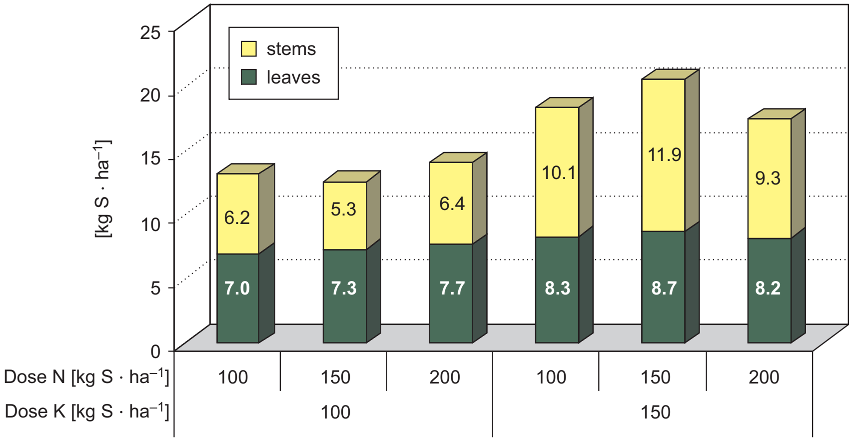

Fig. 2. Accumulation of sulfur in the Miscanthus leaves and stems towards the end of the vegetation period

plantations or varying weather conditions, since both of these could have impacted the access to nutritional elements and plant yields in the particular years of study (Fig. 1).

Other studies have found an increase in the sulfur content as well as other elements with the increasing age of an energy plant plantation [7]. This phenomenon is related to the withdrawal of elements from the aboveground parts to the rhizomes towards the end of the vegetation period and the successive reoccurrence in the spring in subsequent years. Because of this phenomenon an increase in the reserve base of nutritional elements is made possible and is available to be used by plants [13, 20].

In comparison with the amount of sulfur in mineral form transported with the transpiration current, the movement of this element from the leaves to the rhizomes is theoretically minimal, because movement in the phloem can only occur in the form of organic compounds. Thus, it seems that the increase in sulfur with the increased age of a plantation in the absence of sulfur fertilization is caused by the expansion of the rapidly growing root system and to a lesser extent to the withdrawal of sulfur from the aboveground parts to the rhizomes. The source of nutrition could also be fallen Miscanthus leaves; however, in the case of sulfur, the uptake of this element is connected with the earlier mineralization of the organic remains.

Conclusions

1. The sulfur content in the aboveground parts of Miscanthus was mostly dependent on the vegetation period and decreased with the age of the plant.

2. At all times of taking samples more sulfur was found in the leaves than in the stems of Miscanthus. During the vegetation period the drop in the sulfur content in the stems was higher than in the sulfur content in the leaves.

3. Applying intensive nitrogen fertilizing did not modify the sulfur content in the aboveground parts of Miscanthus.

4. Fertilizing with high doses of potassium caused a significant increase in the sulfur content in the Miscanthus leaves and stems.
5. With the age of a plantation the sulfur content in Miscanthus increased.

References

[1] Scherer HW. Europ J of Agron. 2001;14:81-111.
[2] Kaczor A, Zuzańska. J Chem Dydakt Ekol Metrol. 2009;14(1-2):69-78.
[3] Lewandowski I, Kicherer A. Europ J Agron. 1997;6:163-177.
[4] Borkowska H, Lipiński W. Acta Agrophys. 2007;10(2):287-292.
[5] Kalembasa S, Wiśniewska B. Acta Agrophys. 2008;11(3):667-675.
[6] Monti A, Di Virgilio N, Venturi G. Biomass and Bioenergy. 2008;32:216-223.
[7] Stolarski M. J Elementol. 2008;13(4):655-663.
[8] Kalembasa D, Malinowska E. Łąkarstwo w Polsce. 2007;10:99-110.
[9] Stolarski M. J Elementol. 2008;13(4):655-663.
[10] Kalembasa D, Malinowska E. Nawozy i Nawożenie. 2005;3(24):359-364.
[11] Malinowska E, Kalembasa D, Jeżowski S. Zesz Probl Post Nauk Roln. 2006;512:403-409.
[12] Ciubak J, Krzywy E, Krzywy-Gawrońska E., Styrczula P. Zesz Probl Post Nauk Roln. 2009;538:15-21.
[13] Kahle P, Beuch S, Boelcke B, Leinweber P, Schulten H-R. Europ J Agron. 2001;15:171-184.
[14] Szczukowski S, Kościk B, Kowalczyk-Juśko A, Tworkowski J. Fragm Agron. 2006;3(91):300-315.
[15] Kotecki A. Uprawa Miskanta Olbrzymiego. Energetyczne i pozaenergetyczne możliwości wykorzystania słomy. Wrocław: Wyd. UP Wrocław; 2010.
[16] Cichy W. Czysta Energia. 2005;6(44):39-40.
[17] Celińska A. Polityka Energetyczna, 2009;12(2/1):59-72.
[18] Kalembasa D, Janinhoff A, Malinowska E, Jaremko D, Jeżowski S. J Elementol. 2005;10(2):309-314.
[19] Kalembasa D, Wiśniewska B. Zesz Probl Post Nauk Roln. 2008;526:185-190.
[20] Himken M, Lammel J, Neukirchen D, Czypionka-Krause U, Olfs H. Plant and Soil. 1997;189:117-126.

ZMIANY ZAWARTOŚCI SIARKI W MISKANCIE OLBRZYMIM POD WPŁYWEM ZRÓŻNICOWANEGO NAWOŻENIA AZOTEM I POTASEM

Katedra Żywienia Roślin
Uniwersytet Przyrodniczy we Wrocławiu

Abstrakt: Celem prezentowanej pracy była ocena zmian zawartości siarki w częściach nadziemnych miskanta olbrzymiego pod wpływem stosowania wzrastających dawek azotu oraz potasu. Ścisłe doświadczenie polowe założono metodą split-plot, na glebie piaszczystej lekkiej. Zróżnicowane dawki nawozów azotowych wynosiły: 100 kg N; 150 kg N oraz 200 kg N $\cdot$ ha^{-1}, natomiast potasu 83 kg K i 124 kg K (100 kg i 150 kg K_2O). Próbki roślinne pobierano w latach 2007–2009, w okresie od czerwca do października. Podczas całego okresu wegetacji miskanta stwierdzano prawie o połowę mniejsze zawartości siarki w łodygach niż w liściach. Najwięcej tego składnika zawierały rośliny młode. W miarę upływu okresu wegetacji zawartość siarki zmniejszała się w liściach o około 30 %, natomiast w łodygach o 60 %. W żadnym z lat badań nawożenie azotem nie modyfikowało istotnie zawartości S w miskancie. Stwierdzono natomiast istotny wzrost zawartości siarki w liściach i łodygach miskanta nawożonego większą dawką potasu. W kolejnych latach badań na wszystkich obiektach obserwowano w nadziemnych częściach miskanta coraz wyższą zawartość siarki.

Słowa kluczowe: miskant olbrzymi, nawożenie N i K, zawartość siarki, okres wegetacji

DOI: 10.2428/ecea.2012.19(03)023 ECOL CHEM ENG A. 2012;19(3):223-229

Adam RADKOWSKI[1] and Iwona RADKOWSKA[2]

ESTIMATION OF NUTRITIONAL VALUE OF THE SILAGES FROM SELECTED GRASS-LEGUME MIXTURES UTILIZED IN ECOLOGICAL AGRICULTURE

OCENA WARTOŚCI POKARMOWEJ KISZONEK Z WYBRANYCH MIESZANEK TRAWIASTO-MOTYLKOWYCH STOSOWANYCH W ROLNICTWIE EKOLOGICZNYM

Abstract: This work presents the evaluation of the chemical composition and nutritive value of silages prepared from the selected grass-legume mixtures, which are recommended for the meadow sowing in ecological farms. Prepared plant material was subjected to the analysis of the basic chemical composition by the standard method, the analysis of NDF, ADF and ADL content using ANKOM Fiber Analyser according to the method of Goering and Van Soest. The nutritive value was evaluated in the INRA 1988 units using Winwar 1.6 software (DJG). The estimation was done on the basis of tabular coefficients of forage distribution in the rumen and intestines. The content of organic components was very diversified, what can be the result of the differentiated share of grasses and legumes in the examined silages.

The samples of silages collected from the examined mixtures were characterized with significantly higher mean contents of the investigated organic components than did the silage from the control object *ie* perennial sward. In the examined silages from the grass-legume mixtures the growth of the content of raw ash – by 53 %, total protein – by 46 % and raw fat – by 69 % (mean values for all mixtures) in comparison with the respective values of the control object was noticed. On the other hand, these silages were characterized with 14 % lower crude fiber content, 8, 21 and 25 % lower level of ADF (acid detergent fiber), ADL (acid detergent lignin) and NDF (neutral detergent fiber), respectively. It indicates that grass-legume plants of new highly productive cultivars are very important factor affecting the forage quality. High concentration of nutrients in silages derived from the examined mixtures in comparison with the silage from the perennial sward suggests that they are excellent material for the perennial meadow undersowing.

Keywords: silages, organic components, nutritive value

In recent years there is an increasing consumer concern about ecological food, what is strictly connected with the requirement for the proper method of its production. In

---

[1] Department of Grassland Management, Institute of Plant Production, Agricultural University of Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 43 61, fax: +48 12 633 62 45, email: rrradkow@cyf-kr.edu.pl

[2] National Research Institute of Animal Production Balice n. Krakow, ul. Krakowska 1, 32–083 Balice, Poland, phone: +48 66 608 12 49, email: iradkowska@izoo.krakow.pl

ecological farms with animal production the forages from ecological cultivations need to be utilized. The bulk fodders derived from grasslands are of a great importance, especially in the cattle feeding. As green forages, silages and hay constitute the basis of ruminant feeding they should be characterized with high quality and high nutritive value, should be microbiologically clean and free of microtoxins [1, 2].

The meadow and pasture sward in ecological farms is characterized with high diversity, the herbs contribute in the improvement of the forage palatability and influence the digestive tract activity of animals. Moreover, due to the content of legumes which are able to fix the nitrogen from the atmosphere, they are valuable source of this element for the other plants, what allows to significantly limit the level of mineral, nitrogen fertilization. The production efficiency in the eco-farms is highly affected by the environmental conditions, feeding technology used, kind of forages as well as breeding material. Good forage resource, the utilization of the proper preservation technique and the quality of the grass sward in the meadows and pastures are fundamental for the high yields of the dairy cows [3]. Reference data indicate that the silages from the meadow grasses in the ecological farms are poor in calcium, phosphorus, magnesium, manganese, zinc and beta carotene in the context of the requirements of ruminants. As a result this deficiency need to be supplemented with the vitamin preparations. The conditions of the grasslands and the quality of bulky fodder directly influence the conditions of the animals, their well-being, yielding and the quality of animal production, what is of great importance in the ecological agriculture more interested in the quality than in the maximization of the production [4].

The aim and scope of the study

The aim of the conducted study was to compare the chemical composition and the forage value of the silages derived from the grass-legume mixtures and from the perennial meadow sward. The following variants were taken into account:

Variant 1 – control object, silages derived from the perennial sward of the meadows typical for the farms of the investigated area. It constituted the initial material for the comparison with the other variants.

Variant 2 – silage derived from the mixture of the following composition: late perennial ryegrass (t) 45 %, late perennial ryegrass (d) 30 %, timothy grass 10 %, intermediate perennial ryegrass (d) 10 %, white clover 5 %.

Variant 3 – silage derived from the mixture of the following composition: meadow fescue 25 %, early perennial ryegrass 10 %, intermediate perennial ryegrass 10 %, late perennial ryegrass 10 %, smooth-stalked meadowgrass 10 %, red fescue 10 %, white clover 10 %, red clover 10 %, timothy grass 5 %.

Variant 4 – silage prepared from the mixture of the following composition: red clover 30 %, meadow fescue 30 %, early-intermediate perennial ryegrass 20 %, timothy grass 20 %.

Variant 5 – silage derived from the mixture of the following composition: lucerne 80 %, meadow fescue 15 %, timothy grass 5 %.

Variant 6 – silage derived from the mixture of the following composition: westerwold (dutch) ryegrass 80 %, persian clover 20 %.

Variant 7 – silage derived from the mixture of the following composition: intermediate perennial ryegrass (t) 40 %, late perennial ryegrass (t) 35 %, red clover 20 %, white clover 5 %.

The scope of the study included the estimation of the mixture kind influence on the content of nutrients in the silage.

Materials and methods

The field experiment was conducted in the years 2006–2008 in the private, individual farms in Silesia province, located at the altitude of 300–320 m.

The experiment of a sward character, was located in the brown, acidic soil (pH_{KCl} = 5.0–5.2) of a V soil quality class. The soil was characterized with a medium content of the assimilable potassium, manganese and zinc and low level of the assimilable phosphorus and copper.

During the vegetation (April–September) the average rainfall amounted to 338.1; 375.4 and 320.3 mm, respectively in the year 2006, 2007 and 2008, whereas average air temperatures reached the values of 15.2 ^{o}C; 14.3 ^{o}C and 14.9 ^{o}C, respectively.

The meadows were fertilized with a cattle manure in a single dose of 25 Mg (ton) $\cdot$ ha^{-1} used ones in the early spring. The content of chemical components of the manure was as follows: dry matter – 24.2 %; total N – 0.52 %; P – 0.15 %; K – 0.57 %; Ca – 0.28 %; Mg – 0.08 %; Na – 0.07 %.

25 Mg dose of manure provided: 130 kg of total nitrogen, 38 kg of phosphorus, 143 kg of potassium, 70 kg of calcium, 20 kg of magnesium and 18 kg of sodium.

The area of each field amounted to 500 m^2. The silages were derived from the first swath of the meadow flora. The plant material was collected in two stages, the first included mowing of the plants at the turn of earing and flowering stage of grasses with a rotary mower, than the green fodder was slightly dried by one-time turning it over. The fodder was raked 30 minutes before picking up. The material was collected using constant-chamber baler, then it was transported to the storage place and wrapped using bale wrapper. The average time from the bale forming to its wrapping with foil did not exceed 4 hours.

Before grazing the samples of silages were collected for the chemical analysis, which comprise the determination of the fundamental components by the Weenden method [5], pH using pH-meter, the ammonium level by the Conway method [6]. The content of organic acids was evaluated using the Varian 3400 type gas chromatograph. The nutritive value was evaluated in the INRA 1988 units using Winwar 1.6 software (DJG). The estimation was done on the basis of tabular coefficients of forage distribution in the rumen and intestines. The obtained results were subjected to the analysis of variance, and the significance of differences was estimated on the basis of Duncan test at the significance level of α = 0.05.

Results and discussion

As it is well known, **s**ilages and hay silage are first of all the source of protein in the feeding dose for dairy cows and cows during drying off as well as for young cattle [7]. The total protein content in the selected silages was diversified and fluctuated in the range of 96.2 – 251.6 g · kg^{-1} d.m. (Table 1). The highest value was determined in the silage derived from the mixture of the following composition: lucerne 80 %, meadow fescue 15 %, timothy grass 5 %. The data found in literature suggest that forage of good quality should contain 140–160 g of protein in kg of dry matter [8]. In that light it can be concluded that the level of protein in the absolutely dry matter of the control object was insufficient. On the other hand, all silages derived from the objects seven with new grass-legume mixtures were characterized with the values exceeding the optimal level of this component in the absolutely dry matter. The evaluation of the raw fat content in the dry matter provided information that its level was on average 66 % higher in the silages derived from the investigated mixtures than in the control material. Average raw fat content in the silages of high quality is estimated to be 40.0 g · kg^{-1} d.m. [5]. The content of this component in the examined silages was variable and fluctuated in the range of 33.7–62.2 g · kg^{-1} d.m., with the mean value of 53.5 g · kg^{-1} d.m. determined for the silages derived from the grass-legume mixtures. Fat in a feeding dose not only increases the energy concentration but also improves its palatability and utilization, constitutes the source of fat-soluble vitamins (A, D and E) and *polyunsaturated fatty acids* (PUFA). This component is of great importance as highly effective animals require high energy food.

The silages derived from the grass-legume mixtures were characterized with significantly lower level of raw fiber, which was on average 14 % lower than the value observed for the control object. The level of ADF (*acid detergent fiber*), ADL (*acid detergent lignin*) and NDF (*neutral detergent fiber*) fiber fractions were also higher in the control material and were not utilized by ruminants. It is assumed that 230–260 g g · kg^{-1} d.m. is the optimal raw fiber content in the dry matter of a food dose destined for the lactating cows. The respective optimal values for NDF, ADF and ADL fractions are: 420–500 g · kg^{-1} d.m., 240–290 g · kg^{-1} d.m. and 20–30 g · kg^{-1} d.m. [9]. It is worth to emphasize that components of the cell membranes after their bacterial decomposition are substantial energy source for ruminants. NDF content in a food ration gives the information about the forage utilization ability (the higher value the lower intake), whereas the content of *acid detergent fiber* (ADF) determines its digestability (the higher ADF content the lower digestability).

The content of organic acids is an important indicator of the quality and feeding value of silages [10].

Silage of good quality should be characterized with 4.2 pH reaction. The lower pH value (and the higher acidity), the higher content of lactic acid is stated in the silage. On the other hand, pH level equal or exceeding 5.0 is linked with the presence of butyric acid [11]. However, our research did not confirm this relationship. The high quality silage should contain lactic and acetic acid, the latter being present in the smaller

Table 1

Chemical composition of silages (means for three years of investigations)

Specification	Variant							SD	V [%]
	1	2	3	4	5	6	7		
	[g · kg^{-1} d.m.]								
pH	4.78 b*	4.41 a	4.51 a	4.60 a	4.78 b	4.79 b	4.57 a	0.15	3.27
Raw ash	84.2 a	159.3 b	153.7 b	151.7 b	177.0 c	150.1 b	159.8 b	148.0	29.5
Total protein	96.2 a	190.3 b	191.1 b	221.4 bc	251.6 c	210.4 bc	213.0 bc	196.3	48.7
Crude fiber	338.6 c	294.8 b	290.4 b	288.8 ab	281.6 a	293.3 b	296.2 b	297.7	18.7
Raw fat	35.2 a	55.9 b	61.8 c	62.2 c	33.7 a	56.2 b	50.9 b	50.8	11.8
Non-nitrogen extract	445.9 c	299.7 b	302.9 b	276.0 a	256.0 a	289.9 ab	280.1 ab	307.2	63.2
ADF	398.6 b	366.0 a	375.4 b	360.8 a	365.8 a	352.5 a	370.4 ab	369.9	14.6
ADL	72.8 b	51.1 a	53.7 a	50.6 a	84.1 c	47.5 a	58.0 ab	59.7	13.6
NDF	633.3 c	495.4 ab	499.1 ab	467.8 ab	395.0 a	513.0 b	490.1 ab	499.1	70.8
Lactic acid	10.12 b	10.04 b	8.23 ab	7.14 a	11.16 c	8.39 ab	11.86 c	9.56	1.70
Acetic acid	7.25 d	4.62 c	1.71 a	3.18 b	4.27 c	4.33 c	4.24 c	4.23	1,67
Butyric acid	—	—	—	0.29 a	0.33 a	—	—	0.31	0.03
Points in Flieg-Zimmer scale	74	86	98	66	72	80	92	—	—
Total note	good	very good	very good	good	good	good	very good	—	—
Content of N-NH$_3$ in total N [%]	4.70 a	6.32 b	8.31 c	8.54 c	10.52 d	7.94 bc	6.76 b	1.86	24.54
UFL [kg^{-1} d.m.]	0.705 a	0.771 b	0.783 b	0.786 b	0.789 b	0.776 b	0.761 b	0.03	3.79
UVF [kg^{-1} d.m.]	0.610 a	0.696 a	0.709 ab	0.711 ab	0.720 b	0.700 a	0.685 a	0.04	5.38
PDIN [g · kg^{-1} d.m.]	55 a	109 ab	112 ab	127 ab	145 b	121 ab	125 ab	28.31	24.96
PDIE [g · kg^{-1} d.m.]	55 a	60 ab	62 ab	69 ab	72 b	63 ab	65 ab	5.65	8.87

* Means marked with the same letter are not statistically different following verification with the Duncan test ($P = 0.05$). ADF (*acid detergent fiber*), ADL (*acid detergent lignin*) and NDF (*neutral detergent fiber*), UFL – *Feed Unit for Lactation* (1700 kcal EN), UVF – *Meat production Unit* (1820 kcal EN), PDIE – *protein digested* in the small intestine supplied by rumen-undegraded dietary protein plus protein digested in the small intestine supplied by microbial protein from rumen-fermented organic matter, PDIN – *protein digested* in the small intestine supplied by rumen-undegraded dietary protein plus protein digested in the small intestine supplied by microbial protein from rumen-degraded protein.

quantities. The presence of butyric acid indicates the low quality and the level of 10 g · kg^{-1} d.m. very low quality of silage.

The lactic acid content, the most desirable in silages, ranged from 7.14 to 11.86 g · kg^{-1} d.m. The acetic acid level was changeable and fluctuated in the range of 1.71–7.25 g · kg^{-1} d.m. Butyric acid was present in two variants in quantities of

0.29–0.33 $g \cdot kg^{-1}$ d.m., what in a consequence substantially determined lower total note of these silages. It possibly could be linked with too high share of legumes in the silages.

The content of ammonia nitrogen is an indicator of the depth of proteolysis and protein degradation processes. The content of $N\text{-}NH_3$ expressed in relation to the total nitrogen level determines the silage quality. The investigated silages were characterized with diversified level of this component which was equal to 4.70–10.52 %. These values were consistent with the required values and indicate high quality of silages as the $N\text{-}NH_3$ to total N ratio is taken into account.

As the energy value is taken into consideration the examined silages were not very diversified: UFL (V = 3.8 %) and UVF (V = 5.4 %). Higher level of diversification was stated in the case of the protein value which amounted to 55–145 for PDIN (V = 25.0 %) and 55–72 $g \cdot kg^{-1}$ d.m. for PDIE (V = 8.9 %). The mean value of this parameter in the silages from the investigated mixtures was higher when compared with the control object by 55 % for PDIN and 16 % for PDIE.

Analyzing the content of organic components it can be observed that lower values were determined for the silages from the perennial sward. The silages prepared from the grass-legume mixtures are characterized with very high quality what is reflected in their relatively high nutritive value. The kind of green fodders utilized for the preparation of silages has also some influence on the silage consumption. Zielinska et al [12] in their research reported higher degree of dry matter consumption and higher milk production for cows fed silages from legumes and their mixtures with grasses when compared with the silages prepared from grasses alone.

Conclusions

1. It was stated, that utilized grass-legume mixtures were characterized with significantly higher content of organic and mineral components when compared with the control object.
2. The content of total protein and raw fat in silages derived from the grass-legume mixtures was respectively 46 and 69 % higher than the respective values noticed for the control object.
3. UFL and UVF values of silages from the examined mixtures were respectively 9 and 13 % higher when compared to the control object. The PDIN and PDIE values were higher by 55 and 16 %, respectively.

References

[1] Baranowski A, Richter W. Występowanie mikotoksyn w kiszonkach. Przegl Hodowl. 2002;4:21-22.
[2] Zielińska K, Zastwany J, Wróbel B. Materiały z XIIIth International Silage Conference. Auchincruive. Scotland; 2002;204-206.
[3] Ziggers D. Managing your pasture pays off. “Dairy and beef”, Red Business Information (NL), 2003;2(5).
[4] Nazaruk M, Jankowska-Huflejt H, Wróbel B. Ocena wartości pokarmowej pasz z trwałych użytków zielonych pochodzących z gospodarstw ekologicznych. Woda – Środowisko – Obszary Wiejskie. 2009;1(25):61-76.

[5] Kamiński J, Borowiec F, Furgał K, Barteczko J, Kowalski Z, Pyś JB, Siuta A, Pisulewski P, Lehman B. Ćwiczenia z żywienia zwierząt i paszoznawstwa. Skrypt, Kraków: AR w Krakowie; 1995.
[6] Conway EJ. Microdiffusions Analysis and Volumetric Error. London: Crosby Locwood; 1962.
[7] Han KJ, Collins M, Vanzant ES, Dougherty CT. Characteristics of baled silage made from first and second harvests of wilted and severely wilted forages. Grass Forage Sci. 2006;61:22-31.
[8] Falkowski M, Kukułka I, Kozłowski S. Właściwości chemiczne roślin łąkowych. Poznań: Wyd. AR Poznań; 2000.
[9] Gudmundsson B. The influence of number of layers in big bales on silage quality when stored outdoors on shady and sanny sides of buildings. Proc. 326th The Nordic Association of Agr. Scientists (NJF) Production and utilization of silage. Lillehammer, Norway; 2001,125-127.
[10] Podkówka W, Potkański A. Wpływ czynników chemicznych i fizycznych na przydatność pasz do zakiszania. Post Nauk Rol. 1993;40:29-42.
[11] Nowak J, Šařec P. Wybrane czynniki decydujące o jakości kiszonek w belach cylindrycznych. Post Nauk Rol. 2001;5:95-110.
[12] Zielińska KJ, Stecka KM, Suterska AM, Miecznikowski AH. Ekologiczna metoda kiszenia pasz objętościowych. J Res Applicat Agricult Eng. 2006;51:219-223.

OCENA WARTOŚCI POKARMOWEJ KISZONEK Z WYBRANYCH MIESZANEK TRAWIASTO-MOTYLKOWYCH STOSOWANYCH W ROLNICTWIE EKOLOGICZNYM

[1] Zakład Łąkarstwa, Instytut Produkcji Roślinnej
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
[2] Instytut Zootechniki – Państwowy Instytut Badawczy w Balicach koło Krakowa

Abstrakt: W pracy przedstawiono ocenę składu chemicznego i wartości paszowej kiszonek z wybranych mieszanek trawiasto-motylkowych, które zalecane są do obsiewu łąk w rolnictwie ekologicznym. W przygotowanym materiale roślinnym oznaczono podstawowy skład chemiczny metodą standardową, analizy na zawartość NDF, ADF i ADL przeprowadzono za pomocą aparatu ANKOM Fiber Analyser, według metody zaproponowanej przez Goeringa i Van Soesta. Wartość pokarmową wyznaczono w jednostkach systemu INRA 1988 za pomocą programu komputerowego Winwar, wersja 1.6. firmy DJG. Do wyceny posłużono się tabelarycznymi współczynnikami rozkładu pasz w żwaczu oraz jelitach. Zawartość składników organicznych i mineralnych wykazywała dużą zmienność, co może wynikać ze zróżnicowanego udziału traw i roślin motylkowatych w badanych kiszonkach.

Próbki kiszonek pobrane z badanych mieszanek odznaczały się znacznym wzrostem średniej zawartości badanych składników organicznych w porównaniu z obiektem kontrolnym, jakim była kiszonka z wieloletniej runi. W badanych kiszonkach pochodzących z mieszanek trawiasto-motylkowych wykazano wzrost zawartości (uśredniono zawartości wszystkich mieszanek): popiołu surowego o 53 %, białka ogólnego o 46 %, tłuszczu surowego o 69 % w porównaniu z obiektem kontrolnym. Z kolei przy zawartości włókna surowego odnotowano spadek o 14 %, a frakcji włókna ADF (kwaśne włókno detergentowe), ADL (kwaśna lignina), NDF (neutralne włókno detergentowe) odpowiednio o 8 %; 21 % i 25 % w stosunku do obiektu kontrolnego. Wskazuje to, że bardzo ważnym czynnikiem wpływającym na jakość kiszonek są rośliny trawiasto-motylkowe, składające się z nowych odmian wysoko wydajnych. Duża koncentracja składników pokarmowych zawarta w kiszonkach pochodzących z badanych mieszanek w porównaniu z kiszonką z runi wieloletniej przemawia za faktem wykonania podsiewu tymi mieszankami łąk wieloletnich.

Słowa kluczowe: kiszonki, składniki organiczne, wartość pokarmowa

DOI: 10.2428/ecea.2012.19(03)024 ECOL CHEM ENG A. 2012;19(3):231-237

Jolanta RACZUK[1] and Janusz DESKA[2]

BUFFER PROPERTIES OF FOREST SOILS IN SELECTED PROTECTED AREAS

WŁAŚCIWOŚCI BUFOROWE GLEB LEŚNYCH WYBRANYCH OBSZARÓW CHRONIONYCH

Abstract: The paper presents the results of research on forest soil buffer capacity in selected protected areas. The soil buffer capacity was determined for each soil horizon using modified Arrhenius method. Buffer curves were sketched and soil buffer areas were measured with computer techniques. The date obtained were compared with some soil physical and chemical properties using statistical method. Organic (O) and humus (A) horizons of forest soils demonstrate a greater abilities of buffering alkalines than acids whereas buffer capacity of deeper-lying horizons changes as their physical and chemical properties change. Of the examined types of forest soils, the greatest buffer capacity was found for river alluvial soils. The results of the study indicate that the buffer areas are significantly correlated with pH, hydrolytic acidity, content of organic C, clay fractions, sum of exchangeable bases and cation exchangeable capacity.

Keywords: forest soil, buffer capacity, soil physical and chemical properties

Soils are capable of resisting factors due to a set of physical, chemical and biological properties [1]. The extent of changes in the soil environment depends not only on toxicity of substances introduced to the soil but also soil buffer properties.

The pH value is an indicator of nutrient availability in soils, nitrification properties, an occurrence of aluminium ions and heavy metals, as well as conditions of soil life [2]. Also soil buffer properties are associated with soil pH.

Soil buffer properties are understood and defined as the ability of a soil to maintain a relatively stable pH in spite of an effect of acidifying or alkalising factors [3]. Various buffering processes may take place in one and the same soil depending on the parent rock geological origins and mineralogical composition as well as weathering intensity.

The soil is a mixture of buffer systems. It contains components some of which are able to neutralize acids by means of H^+ ion fixation whereas others neutralize alkalis as

---

[1] Department of Ecology and Environment Protection, Institute of Biology, University of Natural Science and Humanities, ul. B. Prusa 12, 08–110 Siedlce, Poland, phone: +48 25 643 12 15, email: jraczuk@uph.edu.pl

[2] Department of Experimentation, Breeding and Seed Production of Agricultural Plants, University of Natural Science, ul. B. Prusa 14, 08–110 Siedlce, Poland, phone: +48 25 643 13 09, email: kaktus1@uph.edu.pl

a result of releasing H^+ ions [3–5]. Numerous studies [6–17] have shown that soil buffer capacity depends on physical, chemical and biological properties of the soil, soil-forming processes, habitat type and nature, climatic conditions as well as an effect of anthropogenic factors. Examination of soil buffer properties enables an assessment of an influence of degrading factors and makes it possible to predict the extent of degradation of the soil environment over time [3,13].

The purpose of the study was to estimate the buffer abilities of forest soils in selected protected areas, with regard to their typology and physical and chemical properties.

Material and methods

Studies were conducted on 7 profiles of forest soils which were different typologically and located in protected areas:

– profile 1 – brown river alluvial soil (acc. to "Klasyfikacja gleb leśnych Polski" [18]), (*Fluvisols* acc. to WRB [19]), developed from alluvial sands, covered by the *Ficario-Ulmetum* plants association, the reserve "Leg Debowy", the landscape park "Podlaski Przelom Bugu", the Lublin province;

– profile 2 – humus river alluvial soil (*Fluvisols*), developed from alluvial silts, covered by the *Fraxino-Alnetum* plant association the reserve "Leg Dębowy", the landscape park "Podlaski Przelom Bugu";

– profile 3 – glossic soil lessive (*Albeluvisols*), developed from fluvioglacial silts, covered by the *Tilio-Carpinetum* plants association, the reserve "Stary Las", the landscape park "Podlaski Przelom Bugu";

– profile 4 – brownish rusty soil (*Arenosols*), developed from moraine sands, covered by the *Carpinion-betuli* plants association, the reserve "Stary Las", the landscape park "Podlaski Przelom Bugu";

– profile 5 – proper rusty soil (*Arenosols*), developed from fluvioglacial sands, covered by the *Pino-Quercetum* plants association, "Uroczysko Ceranow", "Nadburzanski Park Krajobrazowy", the Mazovia province;

– profile 6 – proper rusty soil (*Arenosols*), developed from fluvioglacial sands, covered by the *Pino-Quercetum* plants association, the reserve "Pionki", "Kozienicki Park Karjobrazowy", the Mazovia province;

– profile 7 – proper podzol soil (*Podzols*) developed from fluvioglacial sands, covered by the *Pino-Quercetum* plants association, the reserve "Stawy Broszkowskie", the county of Siedlce, the Mazovia province.

Soil samples were taken from selected horizons of the soil profiles researched and dried under laboratory conditions. Sample from raw horizons were ground while samples from the mineral horizons were crushed in a mortar and sieved through 2 mm sieve.

Buffer curves were drawn according to Arrhenius with Brenner and Kappen modification [20] adding increasing amounts of 0.1 mol HCl $\cdot$ dm^{-3} and 0.1 mol NaOH $\cdot$ dm^{-3} to soil, with after 24 h was followed by solution pH measurement. Areas between standard curve and buffer curve drawn for particular genetic horizons were measured with the computer techniques. Similarly there were defined the granulometric composition according to Bouyoucos method with Cassagrande and Proszynski modifications, carbon in organic compounds – the Tiurin method, H (*hydrolytic acidity*) and TEB (*sum of exchangeable bases*) with the Kappen method, pH in H_2O and 1 mol KCl · dm^{-3} with the potentiometric method (the soil : solution ratio 1 : 2.5). CEC (*cation exchange capacity*) was calculated using to the following equation: CEC = = TEB + H_h, BS (*the degree of base saturation of soil sorption complex*) was definite with % BS = TEB · 100/CEC.

Using the statistical package Statistica 9.0 PL Pearson correlation coefficients (r) were calculated between the buffer area in acidic and alkaline range and physical and chemical properties of soil mineral horizons.

Results and discussion

The forest soils examined were characterised by varying physical and chemical properties. The parent rocks of the examined soils differed as to the geological origins and granulometric composition The content of clay fractions (< 0.002 mm) in the soils ranged between 0 and 13 % (Table 1). The pH of the soils ranged from very acid to neutral (pH in 1 mol KCl · dm^{-3} from 3.1 to 7.0). A very acid pH (3.1–4.4) was determined in the organic horizons – O and humus horizons – A of the examined soils (Table 2). The neutral pH was measured in the deeper horizons of river alluvial soils. Calcium carbonate was not detected in the studied soils.

Table 1

Some physical and chemical properties of the soil investigated

Taxonomic Unit	Fraction < 0.002 mm [%]	$C_{org.}$ [g · kg^{-1}]	H	TEB	CEC	BS [%]
			[mmol(+) · kg^{-1}]			
Ranges of values						
River alluvial soil (*Fluvisols*) profile 1, 2	0–13	1.0–337.2	10–712	90–3240	100–3911	65.3–96.7
Soil lessive (*Albeluvisols*), profile 3	1–12	0.4–358.5	202–1168	30–340	276–1498	8.6–60.2
Rusty soils (*Arenosols*), profile 4, 5, 6	1–5	0.4–438.4	120–975	10–521	132–1204	5.9–43.3
Podzolic soil (*Podzols*), profile 7	1–3	0.8–473.2	259–846	13–41	272–887	3.0–6.3

H – hydrolytic acidity, TEB – sum of exchangeable bases, CEC – cation exchange capacity, BS – base saturation.

Organic carbon content in the humus layers of the analysed soils ranged between 20.2 and 57.2 g · kg^{-1}. Most organic C – from 287.4 to 473.2 g · kg^{-1} – was determined in organic horizons (Table 1). The content of organic carbon decreased with the depth of soil horizons.

The soils analysed had different exchangeable capacity. The greatest cation exchangeable capacity (887–3911mmol(+) $\cdot$ kg^{-1}) was found in the humus horizons of the examined soils. The saturation degree of the sorption complex with exchangeable bases ranged from BS = 3.0 % in the eluvial horizon Ees of the proper podzol soil to BS = 96.7 % in the horizon Gor of the humus river alluvial soil (Table 1).

The analyses showed that the very acid and acid reaction of the soils is the result of natural soil-forming processes, such as relations between parent rock and plant cover (low pH, high value of hydrolytic acidity and low degree of saturation of the sorption complex with bases).

The buffer capacity of the soil profiles analysed was assessed based on the area of buffer surfaces in acidic (P_{HCl}) and alkaline (P_{NaOH}) range, drawn for soil samples taken from individual genetic horizons. Data presented in Table 1 demonstrate that the buffer capacities of genetic horizons of the examined forest soils were clearly different. The buffer area in acidic range was between 0.25 and 11.95 cm^2 and, in alkaline range, between 0.77 and 37.90 cm^2 (Table 2).

There was determined greater capacity of buffering alkalines than acids in the genetic horizons of the brownish rusty soil (profile 4), proper rusty soils (profile 5, 6), proper podzol soil (profile 7) and in almost all horizons of the glossic soil lessive (profile 3), as indicated by the ratio of $P_{NaOH} : P_{HCl}$ buffer areas which ranged between 1.30 to 64.64 (Table 2). In the profile of the glossic soil lessive, the horizons IIBt and IIIC2 were an exception as they revealed a greater capacity of buffering acids than alkalines.

Of the examined forest soils, river alluvial soils had the greatest buffer capacity. The surface horizons of these soils display a greater capacity of buffering alkalines than acids although their buffer area in the acid range is greater compared with the remaining soils. The buffering capacity of samples rich in organic matter are higher than soils with low humus, which are usually poor-buffered. In the horizons of deeper-lying river alluvial soils, there was found a greater capacity of buffering acids than alkalines, which is associated with their pH. The buffer capacity of river alluvial soils is also associated with their granulometric composition (loamy sand, loamy silt, clayey silt), and the content of mineral and organic colloids. The buffer curve of the eluvial horizon Ees of the podzol soil the closest resembled the standard curve in acidic range, which was affected by the process of podzolization by limiting the buffer area P_{HCl} for this horizon to 0.25 cm^2 (Table 2).

In order to find an association between the buffer area in acidic (P_{HCl}) and alkaline (P_{NaOH}) range and physical and chemical properties of the soils, coefficients of Pearson linear correlation (r) were calculated. Statistical analysis showed that the buffer area in the acid range was significantly positively correlated with pH in H_2O (r = 0.610***) and in 1 mol KCl $\cdot$ dm^{-3} (r = 0.560***), clay fraction (r = 0.746***) and organic C content (r = 0.373*). The strongest correlation (r = 0.859***) was found between P_{HCl} and cation exchangeable bases (TEB) in the mineral horizons of the examined soils. However, there was found no significant effect of cation exchangeable capacity and hydrolytic acidity (H) on the buffer area in acidic range.

Table 2

Buffering areas in acid range and alkaline range of soil investigated

Profile No.	Genetic horizon	Depth [cm]	pH		P_{NaOH} [cm^2]	P_{HCl} [cm^2]	P_{NaOH}/P_{HCl}	P_{HCl}/P_{NaOH}
			H_2O	KCl				
1	O	0–2	5.3	4.7	21.45	7.91	2.71	0.37
	A1	2–9	5.2	4.5	24.31	7.32	3.32	0.30
	A2	9–16	5.6	4.8	18.01	8.95	2.01	0.50
	Bbr1	16–41	6.0	5.8	9.92	7.35	1.35	0.74
	Bbr2	41–84	6.9	6.7	5.57	7.53	0.74	1.35
	Cgg	84–180	7.6	6.9	1.10	4.28	0.25	3.89
2	O	0–1	5.7	5.2	24.32	10.82	2.24	0.44
	A	1–20	5.6	4.9	26.11	10.24	2.55	0.39
	ACgg	20–46	6.4	6.0	11.06	11.95	0.93	1.08
	Gor	46–81	7.2	7.0	1.86	6.63	0.28	3.56
	Gr	81–112	7.1	6.9	5.09	9.93	0.51	1.95
	DG	112–180	7.2	7.0	0.77	1.33	0.58	1.73
3	O	0–3	4.1	3.3	15.97	4.03	3.96	0.25
	A	3–7	4.0	3.1	12.36	3.73	3.31	0.30
	AEet	7–22	4.2	3.5	9.23	3.92	2.35	0.42
	Eet	22–43	4.5	3.9	6.28	4.22	1.49	0.67
	Eet/Bt	43–64	4.7	4.0	3.81	2.27	1.67	0.60
	IIBt	64–98	5.3	4.2	3.02	4.52	0.67	1.50
	IIIC1	104–126	4.3	3.7	3.50	2.55	1.37	0.73
	IIIC2	126–150	4.6	3.8	4.60	5.20	0.88	1.13
4	O	0–2	5.6	4.9	16.42	5.34	3.07	0.33
	A	2–7.5	5.4	4.7	11.54	5.16	2.24	0.45
	ABbr	7.5–28	4.7	4.1	10.72	4.30	2.49	0.40
	BbrBv	28–54	4.9	4.3	8.38	3.82	2.19	0.46
	C1	54–89	5.0	4.2	6.08	2.20	2.76	0.36
	C2	89–150	5.1	4.4	2.60	2.00	1.30	0.77
5	O	0–3	3.8	3.4	24.93	1.09	22.87	0.04
	A	3–11	3.6	3.1	22.66	0.88	25.73	0.04
	Bv	11–42	4.5	4.3	8.98	2.92	3.08	0.33
	C	> 42	4.9	4.5	3.30	2.33	1.42	0.71
6	O	0–5	3.7	3.3	25.75	1.15	22.39	0.04
	A	5–15	3.6	3.1	24.02	0.99	24.26	0.04
	Bv1	15–35	4.5	4.3	11.07	4.96	2.23	0.45
	Bv2	35–50	4.6	4.4	10.73	5.09	2.10	0.47
	C	> 50	4.8	4.6	3.59	2.73	1.31	0.76
7	O	0–10	3.4	3.0	37.90	0.89	49.22	0.02
	A	10–19	3.8	3.2	24.69	0.67	36.85	0.03
	Ees	19–30	4.1	3.5	16.16	0.25	64.64	0.02
	Bhfe	30–40	4.5	4.3	17.17	2.50	6.87	0.19
	Bfe1	40–46	4.7	4.5	13.63	2.11	6.46	0.22
	Bfe2	46–60	4.5	4.4	12.45	2.05	6.07	0.24
	C	> 60	4.8	4.8	5.35	1.95	2.74	0.43

High values of correlation coefficients indicate a strong association between the buffer area in alkaline range and hydrolytic acidity (r = 0.763***) and organic C content (r = 0.738***). A weaker yet significant association was found between P_{NaOH} and cation exchangeable capacity (CEC) (r = 0.396*). The buffer area in alkaline range was significantly and negatively associated with pH in H_2O (r = –0.478***) and pH in 1 mol $KCl \cdot dm^{-3}$ (r = –0.473***). There was found no significant association between the buffer area P_{NaOH} and the clay fraction content.

Of the examined forest soil horizons, the organic horizons O and humus horizons A were most resistant to alkalisation, which has also been reported by other authors [2, 5, 6, 8, 10, 11, 17] who examined forest soils. Statistical analysis demonstrated a stronger association between organic C content and the buffer area in alkaline range (P_{NaOH}) compared with the buffer area in acidic range (P_{HCl}). It is most probable the result of unsaturated character of forest litter where acid cations predominate. Hydrogen bound with acid groups and exchangeable aluminium in forest soils may neutralize marked amounts of bases. Many works [2, 5, 6, 9, 10, 13–16] stress the important role of humus and clay minerals as buffer systems. When soil pH is alkaline, hydrogen ions are bound by negatively charges of clay minerals and humus. The H^+ ions is weakly adsorbed onto clay (ionic bonding) and it is strongly adsorbed to carboxyl groups of humus (covalent bonding) [21]. When pH changes into neutral or alkaline, the hydrogen bound is deionised and becomes changeable.

The forest soil types analysed and their genetic horizons varied in their buffer capacity between one another which, as indicated by literature [3, 10–14, 21], is associated with distribution of various buffer systems in soil profiles (silicate, aluminium, iron, ion exchange, carbonate buffers). Urlich [22] defined pH ranges in which individual buffers play the main role. The effectiveness of individual buffer systems depends on physical, chemical and biological soil properties.

Conclusions

1. Organic and humus horizons of forest soils demonstrate a greater abilities of buffering alkalines than acids whereas buffer capacity of deeper-lying horizons changes as their physical and chemical properties change.

2. Of the examined types of forest soils, the greatest buffer capacity was found for river alluvial soils.

3. The statistical analysis showed that the soil resistance to acid activity defined with correlation coefficient was significantly correlated with colloidal clay fractions content, sum of exchangeable basis (TEB), pH value in H_2O and 1 mol $KCl \cdot dm^{-3}$ and with organic C content.

4. The resistance of the forest soils to alkaline activity was significantly correlated with hydrolytic acidity (H), organic C content, pH value in H_2O and in 1 mol $KCl \cdot dm^{-3}$ and with cation exchangeable capacity (CEC).

References

[1] Baran S, Turski R. Degradacja, ochrona i rekultywacja gleb. Lublin: Wyd. AR w Lublinie; 1996.
[2] Kowalkowski A. Regional Monitor Środ Przyrodn. 2002;3:31-43.
[3] Bednarek R, Dziadowiec H, Pokojska U, Prusinkiewicz Z. Badania ekologiczno-gleboznawcze. Warszawa: PWN; 2004.
[4] Hornbeck JW, Federer CAJ. Forestry. 1985;83:690-691.
[5] Pokojska U. Rocz Glebozn. 1986;37(2–3):249-263.
[6] Adamczyk B, Oleksynowa K, Niemyska-Łukaszuk J, Drożdż-Hara M, Miechówka A, Kozłowska E, Fajto A. Rocz Glebozn. 1983;34(4):81-92.
[7] Natscher L, Schwertmann U. Geoderma. 1991;48(1/2):93-106. DOI: 10.1016/0016-7061(91)90091-7.
[8] Porębska G, Ostrowska A. Ochr Środ i Zasob Natur. 1992;4:63-66.
[9] Miechówka A, Mazurek R, Ciarkowska K. Zesz Probl Post Nauk Roln. 1995;421a:285-294.
[10] Malczyk P. Zesz Probl Post Nauk Roln. 1998;456:469-475.
[11] Raczuk J. EJPAU. 2001;4(1), http://www.ejpau.media.pl.
[12] Chodorowski J, Dębicki R, Smalej M. Acta Agrophys. 2001;50:59-70.
[13] Jaworska H, Dąbkowska-Naskręt H, Malczyk P. Ecol Chem Eng. 2005;12(3):231-239.
[14] Malczyk P, Kobierski M, Jaworska H, Dąbkowska-Naskręt H. Rocz Glebozn. 2008;59(3/4):149-154.
[15] Niewiadomski A, Tołoczko W, Trawczyńska A. Rocz Glebozn. 2009;60(1):85-91.
[16] Walenczak K, Licznar SE, Licznar M. Rocz Glebozn. 2009;60(2):102-107.
[17] Bartmański P, Plak A, Dębicki R. Proc ECOpole. 2010;4(1):99-102.
[18] Klasyfikacja Gleb Leśnych Polski. Warszawa: CILP; 2000.
[19] Word Reference Base for Soil Resources (WRB). Word Soil Resources Reports No 103, FAO. Rome; 2006.
[20] Ostrowska A, Gawliński S, Szczubiałka Z. Metody analizy i oceny właściwości gleb i roślin. Katalog IOŚ. Warszawa; 1991.
[21] Bolt GH, Brugenwert MG. Soil Chemistry. New York: Elsevier; 1978.
[22] Urlich B. Zeitschr Pflanzenern Bodenk. 1981;144(3):289-305.

WŁAŚCIWOŚCI BUFOROWE GLEB LEŚNYCH WYBRANYCH OBSZARÓW CHRONIONYCH

[1] Katedra Ekologii i Ochrony Środowiska, Instytut Biologii
[2] Katedra Doświadczalnictwa, Hodowli i Nasiennictwa Roślin Rolniczych
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach

Abstrakt: W pracy przedstawiono wyniki badań nad zdolnościami buforowymi gleb wybranych obszarów chronionych. Analizie poddano 7 profili gleb leśnych reprezentujących następujące podtypy gleb: mady rzeczne brunatne, mady rzeczne próchnicze, gleby płowe zaciekowe, gleby rdzawe brunatne, gleby rdzawe właściwe oraz gleby bielicowe właściwe. Wykreślono dla nich krzywe buforowe zmodyfikowaną metodą Arrheniusa i zmierzono techniką komputerową powierzchnie buforowe gleb. Uzyskane dane porównano statystycznie z niektórymi właściwościami fizyczno-chemicznymi gleb. Poziomy organiczne (O) i próchniczne (A) gleb leśnych wykazują większe zdolności buforowania zasad niż kwasów, natomiast zdolności buforowe poziomów głębiej zalegających zmieniają się wraz z ich właściwościami fizyczno-chemicznymi. Wśród badanych typów gleb leśnych największe zdolności buforowe wykazują mady rzeczne. Wyniki badań wskazują, że powierzchnie buforowe badanych gleb są istotnie skorelowane z pH, kwasowością hydrolityczną, zawartością węgla związków organicznych, frakcja iłu, sumą zasad wymiennych oraz z kationową pojemnością sorpcyjną.

Słowa kluczowe: gleby leśne, buforowość gleb, właściwości fizyczno-chemiczne gleb

DOI: 10.2428/ecea.2012.19(03)025 ECOL CHEM ENG A. 2012;19(3):239-248

Magdalena GRZEGORZAK[1*], Ewa SZPYRKA[1],
Magdalena SŁOWIK-BOROWIEC[1], Anna KURDZIEL[1],
Aneta MATYASZEK[1] and Julian RUPAR[1]

POTENTIAL RISK TO CONSUMERS RELATED WITH OCCURRENCE OF PESTICIDE RESIDUES IN EARLY VEGETABLES

POZOSTAŁOŚCI ŚRODKÓW OCHRONY ROŚLIN W NOWALIJKACH JAKO POTENCJALNE RYZYKO NARAŻENIA KONSUMENTÓW

Abstract: In years 2009–2011 Laboratory of Pesticide Residue Analysis from March to June carried out research on 96 samples of early vegetables. In 35 tested samples were detected pesticide residues. The aim of the study was to estimate long-term and short-term intake of pesticide residues in early vegetables in years 2009–2011 for toddlers and adults. The estimation of dietary exposure was based on pesticide residue data from research carried out by Laboratory of Pesticide Residue Analysis and was calculated using Pesticide Safety Directorate model. The highest long-term intake was for parsley leaves but did not exceed for adult 26.6 % ADI and for toddlers 17.3 % of the ADI. In case of consumption other early vegetables, long-term exposure in both age groups did not exceed 2.5 % ADI. The highest estimated values for short-term exposure were obtained for tomato, and in the group of toddlers it was 41.4 % ARfD, and in the adult group it was 10.4 % ARfD. In the remaining samples the short-term exposure did not exceed 5 % ARfD.

Keywords: pesticide residues, dietary exposure, early vegetables

Introduction

Early vegetables are young spring vegetables available for the first time in the season. Early vegetables include radishes, tomatoes, lettuce, cucumbers, dill and chives. In Poland the seasons for early vegetables falls in the period from March to June. It is the time when the human body is "tired" with winter weather, "hungry" and even demanding stocking up of vitamins and minerals warehouse depleted during the winter.

---

[1] Plant Protection Institute – National Research Institute, Regional Experimental Station, Laboratory of Pesticide Residue Analysis, ul. Langiewicza 28, 35–101 Rzeszów, Poland, phone: +48 17 854 38 62, fax: +48 17 854 38 62, email: adres@email.pl

[*] Corresponding author: m.grzegorzak@iorpib.poznan.pl

Young, fresh looking vegetables doubtlessly bring some variation into the spring diet and are a good looking addition to a menu.

Vegetables are grown in 2 main ways: in a field or in a greenhouse. Early vegetables are grown in the second way, as in our climate cold days with small amount of sunshine and significant temperature variations prevail in the early spring. These are not conditions favourable for plant growth. Therefore, it is necessary to create an appropriate artificial environment for them. Cultivation of early vegetables is carried out in specially adapted greenhouses, ensuring temperature conditions, humidity and sunshine conditions favourable for plant growth.

To facilitate plant growth a large number of *plant protection products* (p.p.p) is used, as in unfavourable weather conditions they require more growth stimulators. Chemical substances also ensure protection against diseases and pests.

Besides bringing advantages to producers, p.p.p are also encumbered with undeniable negative effects on human health, as, obviously, food should have appropriate nutrition value, and contain as low as possible content of pesticide residues, being exceptionally toxic substances amongst those to which humans are exposed. Therefore, does the real nutrition value of early vegetables match their healthiness as expected by customers?

Tests of food are particularly important for assessment of human exposure to pesticide residues in food, as they allow determining those pesticides present in the largest amounts in food and those foods that most often contain their residues. A need to monitor residues of chemical p.p.p in food also results from their toxic effect and common use in agriculture [1].

The food safety strategy in force in the European Union requires the Member States to maintain monitoring programmes and official food monitoring for contamination, including pesticides, against *the maximum residue levels* (MRLs). Their objective is to evaluate the actual pesticide intake and, on that basis, assess the risk for consumers related to short-term (acute) and prolonged exposure. It should be emphasised here, however, that established and valid MRL values are not the safety limit. The safe level of pesticide residues can be established using the *Acceptable Daily Intake* (ADI) or the *Acute Reference Dose* (ARfD) for prolonged and acute exposure, respectively [2].

The evaluation of dietary exposure was based on data concerning residues found during official testing of Polish crops carried out at the Laboratory of Pesticide Residue Analysis in Rzeszow, and on food consumption in Great Britain, due to lack of Polish data.

The aim of this work was to assess the actual pesticide intake with early vegetables eaten in years 2009–2011 and to evaluate on that basis the risk for consumers related to short-term and long-term exposure.

Materials and methods

The studied material contained samples of greenhouse cucumbers (g), tomatoes (g), lettuce (g), parsley leaves, dill, chives, radishes and peppers. Analyses were conducted as a part of official monitoring ordered by the Ministry of Agriculture and Rural Development, and to orders of producers and companies processing, purchasing and exporting fruit and vegetables. Data concerning residues for risk estimations were obtained in tests carried out in years 2009–2011 from March to June at the Laboratory of Pesticide Residue Analysis of the Plant Protection Institute at the Regional Experimental Station in Rzeszow. In total, 96 samples of early vegetables from the south-east Poland were analysed at the laboratory.

During the studies, from 126 in 2009 to 147 in 2011 of p.p.p substances, together with their metabolites and decomposition products, were determined. Samples were tested using chromatographic and spectrophotometric methods accredited in accordance with ISO/IEC 17025. Residues of pesticides were determined using the analytical method required by the European Commission [3].

Obtained results were compared against the maximum residue levels (MRLs) in force in Poland [4].

Each year the laboratory participates in the international proficiency tests organised by the European Union (University of Almeria, Spain), and also in interlaboratory comparative research, in which it achieves correct results. This proves that the system for monitoring of residue levels is correct. This way the laboratory confirms its competences for carried out tests, ensuring obtaining correct results.

Gas chromatography with an *electron capture detector* and ion monitoring (GC/ECD/NPD) for simultaneous detection of many compounds and the spectrophotometric method were used in analyses.

Determination of plant protection products residues using the gas chromatography method

100 g of the analytic sample was homogenised with acetone and filtered in vacuum through a filter placed in a Büchner funnel. A homogeniser container was rinsed with acetone and the washings were used to rinse the filter. For further analysis, 1/5 of obtained filtrate was sampled (sample of 20 g) and placed in a separator containing 2.5 % sodium sulphate(VI) Pesticide residues were extracted three times with dichlorometane. The combined extracts were evaporated until dryness using a rotary evaporator Rotavapor-R from the company Büchi in a temperature below 40 ^{o}C, and then transferred with petroleum ether into a measuring flask, of 10 cm^3 capacity. The obtained extracts were purified in the florisil column. Pesticides were eluated with a mixture of diethylene ether : petroleum ether, 3:7 (v/v) and then with a mixture of petroleum ether : acetone 7:3 (v/v). Eluates were vaporated until dryness in the rotary evaporator and the residues transferred quantitatively to measuring flasks and made up to volume with petroleum ether [5–8].

Purified extracts were analysed in a gas chromatograph Agilent 6890 equipped with EC and NP detectors (DB-1701 column; sequence of temperatures: initial temperature 100 ^{o}C → 20 ^{o}C/min → 180 ^{o}C – 4 min → 20 ^{o}C/min → 220 ^{o}C – 5 min → 20 ^{o}C/min → 260 ^{o}C – 43 min; the total analysis time of 60 minutes). The results were confirmed in a chromatograph Hewlett Packard 7890 equipped with EC and NP detectors (HP-5MS column; sequence of temperatures: initial temperature 100 ^{o}C → 10 ^{o}C/min

→ 180 °C – 4 min → 3 °C/min → 220 °C – 15 min → 10 °C/min → 260 °C – 11 min; the total analysis time of 55 minutes) for linearity of their determinations.

Determination of plant protection products residues using the spectrophotometric method

Determination of dithiocarbamates residues were by spectrophotometric method through decomposition to CS_2 in the acid environment in a presence of tin(II) chloride and transfer to the methylene blue analysed at the spectrophotometer Unicam Helios at the wavelength $\lambda = 662$ nm [9].

Intake estimation

For consumer residues intake estimation, new models from Pesticides Safety Directorate of the Department for Environment, Food and Rural Affairs (PSD-Defra, UK) were applied. Calculations were performed using a Chronic_and Acute_Consumer_ver1.1 software with built-in consumption database for 10 groups of people [10].

Long-term risk was calculated as follows:

$$\text{NEDI} = \Sigma\ [(F_i \times RL_i \times P_i)/\text{mean body weight}]$$

where: NEDI – *National Estimated Daily Intake*,
F_i – food consumption data for given food commodity,
RL_i – appropriate residue level corresponding to that commodity,
P_i – correction value that takes into account the reduction or increase in residue which might occur on storage and/or processing.

Short-term risk was calculated according to the following formula:

$$\text{NESTI} = \Sigma\ [(F \times HR)/\text{mean body weight}]$$

where: NESTI – *National Estimates of Short-Term Intake*,
F – full portion consumption data for the commodity unit,
HR – *the highest residue level* detected incorporating processing or edible portion factor.

Values of ADI and ARfD are elaborated by the European Commission, European Food Safety Authority (EFSA), Standing Committee on the Food Chain and Animal Health (SCoFCAH) and the values are derived from pesticide database [11].

Results and their discussion

96 samples in total were analysed at the laboratory. Residues of p.p.p were found in 35 of tested samples, corresponding to 36.5 % of all samples. The largest amounts of pesticide residues were found in chives 100 %, pepper (g) 71 %, parsley leaves and dill,

50 %, and tomatoes (g) 47 %, of tested crops. The most often found substances were fungicide residues: chlorothalonil – 14 samples, azoxystrobin – 9 samples, dithiocarbamates – 7 samples, cyprodinil and fludioxonil – in 5 samples, and of insecticides: bifenthrin – 7 samples. In 3 samples determined levels of pesticide residues exceeded MRLs. Presence of multiple residues was also found. In the tomato samples residues of 4 active substances were found, with one substance exceeding MRL. In the green parsley sample residues of 4 active substances were found, with 3 substances exceeding MRLs, and in the dill sample 3 residues were found, all of which exceeded MRLs. The details are shown in Table 1.

Table 1

Occurrence of pesticide residues in early vegetables in 2009–2011

Crop	Number of analyzed samples	Active substance	Samples with residues		Range of found residues		MRL [mg/kg]
			number	[%]	min [mg/kg]	max [mg/kg]	
Greenhouse cucumber	27	Azoxystrobin	3	11	0.09	0.23	1
		Bifenthrin	3	11	0.02	0.02	0.1
		Chlorothalonil	3	11	0.02	0.35	1
Greenhouse tomato	45	Azoxystrobin	2	4.4	0.007	0.05	3
		Bifenthrin	3	6.6	0.02	0.3	0.2
		Boscalid	3	6.6	0.05	0.14	1
		Chlorothalonil	8	17.8	0.01	0.09	2
		Cyprodinil	5	8.9	0.01	0.07	1
		Ditiocarbamates	6	13.3	0.03	0.95	3
		Fludioxonil	5	8.9	0.02	0.08	1
		Iprodione	2	4.4	0.08	0.19	5
Greenhouse lettuce	5	Ditiocarbamates	1	20	0.03	—	5
Parsley leaves	6	Azoxystrobin	1	16.7	0.24	—	70
		Chlorpyrifos	1	16.7	1.45	—	0.05
		Chlorothalonil	2	33.3	1.18	45.9	5
		Cypermethrin	1	16.7	0.25	—	2
		Propiconazole	1	16.7	0.65	—	0.05
		Tetraconazole	1	16.7	0.3	—	0.02
Dill	4	Chlorpyrifos	1	25	0.12	—	0.05
		Diazynon	1	25	0.08	—	0.01
		Captan	1	25	0.05	—	0.02
		Pendimethalin	1	25	0.6	—	0.05
Chive	1	Azoxystrobin	1	100	0.28	—	3
Radish	1	—	—	—	—	—	—
Greenhouse peppers	7	Azoxystrobin	2	28.5	0.02	—	3
		Bifenthrin	1	14.3	0.03	—	0.2
		Chloropyrifos	1	14.3	0.09	—	0.5
		Chlorthalonil	1	14.3	0.08	—	2
		Cypermethrin	1	14.3	0.02	—	0.5
		Iprodione	1	14.3	0.02	—	5

Table 2

Estimation of long-term dietary exposure to pesticide residues for early vegetables in years 2009–2011

Crop	Number of analyzed samples	Active substance	Average residue level [mg/kg]	ADI [mg/kg body weight]	High level (97.5 percentile) of long term consumption [kg/person/day for]		Intake			
							adults [76 kg]		toddlers [14.5 kg]	
					adults	toddlers	mg/kg body weight	% ADI	mg/kg body weight	% ADI
Greenhouse cucumber	27	Azoxystrobin	0.15	0.2	0.0309	0.0351	0.00036	0.180	0.00006	0.030
		Bifenthrin	0.02	0.15	0.0309	0.0351	0.00005	0.033	0.00001	0.006
		Chlorothalonil	0.14	0.015	0.0309	0.0351	0.00034	2.267	0.00006	0.400
Greenhouse tomato	45	Azoxystrobin	0.03	0.2	0.1047	0.0382	0.00008	0.040	0.00004	0.020
		Bifenthrin	0.12	0.15	0.1047	0.0382	0.00032	0.213	0.00017	0.113
		Boscalid	0.11	0.04	0.1047	0.0382	0.00029	0.725	0.00015	0.375
		Chlorothalonil	0.04	0.015	0.1047	0.0382	0.00011	0.733	0.00006	0.400
		Cyprodinil	0.04	0.03	0.1047	0.0382	0.00011	0.367	0.00006	0.200
		Ditiocarbamates	0.23	0.03	0.1047	0.0382	0.00061	2.033	0.00032	1.067
		Fludioxonil	0.05	0.37	0.1047	0.0382	0.00013	0.035	0.00007	0.019
		Iprodione	0.135	0.06	0.1047	0.0382	0.00036	0.600	0.00019	0.317
Greenhouse lettuce	5	Ditiocarbamates	0.03	0.03	0.0471	0.0124	0.00003	0.100	0.00002	0.067

Table 2 contd.

Crop	Number of analyzed samples	Active substance	Average residue level [mg/kg]	ADI [mg/kg body weight]	High level (97.5 percentile) of long term consumption [kg/person/day for]		Intake			
							adults [76 kg]		toddlers [14.5 kg]	
					adults	toddlers	mg/kg body weight	% ADI	mg/kg body weight	% ADI
Parsley leaves	6	Azoxystrobin	0.24	0.2	0.0129	0.0016	0.00003	0.015	0.00004	0.020
		Chloropyrifos	1.45	0.01	0.0129	0.0016	0.00016	1.600	0.00025	2.500
		Chlorothalonil	23.5	0.015	0.0129	0.0016	0.00259	17.267	0.00399	26.600
		Cypermethrin	0.25	0.05	0.0129	0.0016	0.00003	0.060	0.00004	0.080
		Propiconazole	0.65	0.04	0.0129	0.0016	0.00007	0.175	0.00011	0.275
		Tetraconazole	0.3	0.004	0.0129	0.0016	0.00003	0.750	0.00005	1.250
Dill	4	Chlorpyrifos	0.12	0.01	0.0291	L/C	L/C	—	0.00005	0.500
		Diazynon	0.08	0.0002	0.0291	L/C	L/C	—	0.00003	15.00
		Captan	0.05	0.1	0.0291	L/C	L/C	—	0.00002	0.020
		Pendimethalin	0.6	0.125	0.0291	L/C	L/C	—	0.00023	0.184
Chive	1	Azoxytstrobin	0.28	0.2	0.0190	0.0023	0.00004	0.020	0.00007	0.035
Radish	1	—	—	—	—	—	—	—	—	—
Greenhouse peppers	7	Azoxytstrobin	0.02	0.2	0.0281	0.0117	0.00002	0.010	0.00001	0.005
		Bifenthrin	0.03	0.15	0.0281	0.0117	0.00002	0.013	0.00001	0.007
		Chlorpyrifos	0.09	0.01	0.0281	0.0117	0.00007	0.700	0.00003	0.300
		Chlorothalonil	0.08	0.015	0.0281	0.0117	0.00006	0.400	0.00003	0.200
		Cypermethrin	0.02	0.05	0.0281	0.0117	0.00002	0.040	0.00001	0.020
		Iprodione	0.02	0.06	0.0281	0.0117	0.00002	0.030	0.00001	0.017

Table 3

Estimation of short-term exposure to pesticide residues for early vegetables in years 2009–2011

Crop	Active substance	HR [mg/kg]	ARfD [mg/kg body weight]	Full portion consumption data (97.5 percentile) [kg]		Intake			
				toddlers	adults	toddlers [14.5 kg]	% ARfD	adults [76 kg]	% ARfD
Greenhouse tomato	Bifenthrin	0.3	0.03	0.2830	0.0905	0.01242	41.41	0.00313	10.43
Parsley leaves	Chlorpyrifos	1.45	0.1	0.0497	0.0045	0.00045	0.45	0.00095	0.95
	Chlorothalonil	45.9	0.6	0.0497	0.0450	0.014245	2.37	0.03016	5.03
	Propiconazole	0.65	0.3	0.0497	0.0450	0.000202	0.07	0.00042	0.14
Dill	Chlorpyrifos	0.12	0.1	0.1558	—	—	—	0.00172	1.72
	Diazynone	0.08	0.025	0.1558	—	—	—	0.00115	4.60
	Pendimethalin	0.6	no applicable	—	—	—	—	—	—

Tests for p.p.p residues showed presence of active substances not recommended for protection of a given crop [12]. The largest amount of not recommended substances was found in dill, and they were: chlorpyrifos, diazinon and captan, and in parsley leaves and pepper (g): propiconazole and chlorpyrifos, respectively.

Long-term and short-term exposure was estimated for 2 age groups: toddlers and adults. As acceptable and not health-threatening were considered values of evaluated consumer exposure not exceeding 100 % of ADI or ARfD value.

Table 2 shows evaluated long-term exposure of humans following consumption of all determined p.p.p. The highest long-term consumer exposure was found for both groups, of adults and of toddlers, in case of consumption of products such as parsley leaves (17.3 % ADI – toddlers, 26.6 %ADI – adults) and dill (15 % ADI – adults). In case of consumption of other early vegetables, long-term exposure in both age groups did not exceed 2.5 % ADI. The intake values for toddlers marked as L/C mean the consumption (very low) was determined as being below 0.1 g/day.

Short-term exposure is shown in Table 3. It is estimated by comparing a single intake of pesticide residues to the amount of its acute reference dose (ARfD). It was calculated for compounds exceeding MRLs. The highest values of short-term exposure were obtained for tomato, and for the group of toddlers it was 41.4 % ARfD, and for the adult group it was 10.4 % ARfD. In both cases these values did not exceed the acceptable 100 % threshold. In case of consumption of other products the short-term exposure did not exceed 5 % ARfD.

On a basis of the determined levels of pesticide residues in analysed crops, estimation of the risk to human health, both long- and short-term, it can be stated that intake of pesticide residues with Polish early vegetables did not pose a risk to consumers' health.

Conclusions

1. The substances most often found in early vegetables were residues of the fungicide group.
2. The highest estimated long-term consumer exposure was found for both groups of adults and toddlers in case of consumption of products such as parsley leaves (17.3 % ADI – toddlers, 26.6 % ADI – adults) and dill 15 % ADI – adults. In case of consumption of other early vegetables, long-term exposure in both age groups did not exceed 2.5 % ADI.
3. The highest estimated values of short-term exposure were obtained for tomato, and in the group of toddlers it was 41.4 % ARfD, and in the adult group it was 10.4 % ARfD. In the remaining samples the short term exposure did not exceed 5 % ARfD.

References

[1] Góralczyk K, Ludwicki JK, Czaja K, Struciński P. Monitoring pozostałości pestycydów w żywności w Polsce. Roczn PZH. 1998;49:331-339.

[2] Kostka G, Urbanek-Olejnik K, Liszewska M. Szacowanie ryzyka dla łącznego narażenia na pozostałości pestycydów w żywności. Roczn PZH. 2011;62(2):127-136.

[3] Document SANCO/12495/2011. Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed.

[4] Rozporządzenie (WE) nr 396/2005 Parlamentu Europejskiego i Rady z dnia 23 lutego 2005 r. zmieniające dyrektywę Rady 91/414/EWG w sprawie najwyższych dopuszczalnych poziomów pozostałości pestycydów w żywności i paszy pochodzenia roślinnego i zwierzęcego oraz na ich powierzchni (Dz Urz UE L 70/1 z dnia 16.03.2005 r. z późn. zm.).

[5] Ambrus A, Lantos J, Visi E, Csatlos I, Sarvari L. General method for determination of pesticide residues of plant origin, soil, and water. I. Extraction and cleanup. J Assoc Off Anal Chem. 1981; 64:733-768.

[6] Luke MA, Froberg JE, Masumoto HT. Extration and Cleanup of Organochlorine, Organophosphate, Organonitrogen, Hydrokarbon Pesticides in Produce for Determination by Gas-Liquid Chromatography. J Assoc Off Anal Chem. 1975;58,1020-1026.

[7] Luke MA, Froberg JE, Dosse GM, Masumoto HT. Improved Multiresidue Gas Chromatographic Determination of Organophosphorus, Organonitrogen and Organohalogen Pesticides in Produce, Using Flame Photometric and Electrolytic Conductivity Detectors. J Assoc Off Anal Chem. 1981;64:1187-1195.

[8] Sadło S. Partition coefficient – its determination and significance in estimation of pesticide residue losses in thebcourse of extraction procedure. J Plant Protect Res. 1998;38(2):179-184.

[9] Chmiel Z. Spektrofotometryczne oznaczanie śladowych pozostałości dwutiokarbaminianów w materiale roślinnym. Chem Anal. 1979;24:505-512,

[10] New intake calculation models for consumer intake assessments 2006. Pesticides Safety Directorate.http://www.pesticides.gov.uk/guidance/industries/pesticides/topics/pesticide-approvals/pesticides-registration/data-requirements-handbook/uk-consumer-intake-models

[11] EU PESTICIDES database http://ec.europa.eu/sanco_pesticides/public/index.cfm?event= activesubstance.selection&a=1

[12] Zalecenia Ochrony Roślin na lata 2008/2009 oraz 2010/2011 cz. III, Zakład upowszechniania Wydawnictw i Współpracy z Zagranicą Poznań 2008/2009, 2010/2011.

POZOSTAŁOŚCI ŚRODKÓW OCHRONY ROŚLIN W NOWALIJKACH JAKO POTENCJALNE RYZYKO NARAŻENIA KONSUMENTÓW

Laboratorium Badania Pozostałości Środków Ochrony Roślin
Instytutu Ochrony Roślin – Państwowego Instytutu Badawczego w Rzeszowie

Abstrakt: W latach 2009–2011 w Laboratorium Badania Pozostałości Środków Ochrony Roślin od marca do czerwca przeprowadzono badania 96 próbek nowalijek. W 35 próbkach stwierdzono obecność pozostałości pestycydów. Celem pracy była ocena długo- i krótkoterminowego spożycia pozostałości pestycydów w nowalijkach dla dwóch grup konsumentów: małych dzieci i dorosłych. Oszacowane wartości narażenia wyznaczono z danych dotyczących pozostałości środków ochrony roślin z badań przeprowadzonych w Laboratorium. Wartości te zostały oszacowane na podstawie modelu brytyjskiego Urzędu Bezpieczeństwa Pestycydów Ministerstwa ds. Środowiska, Żywności i Rolnictwa. Najwyższe pobranie oszacowano dla liści pietruszki, jednak nie przekraczało ono: dla dorosłych 26,6 % ADI, a dla małych dzieci 17,3 % ADI. W przypadku konsumpcji innych nowalijek długoterminowe narażenie nie przekraczało 2,5 % ADI. Największe szacowane wartości krótkotrwałego narażenia uzyskano dla pomidorów w grupie małych dzieci – 41,4 % ARfD i w grupie dorosłych – 10,4 % ARfD. W pozostałych próbkach krótkoterminowe narażenie nie przekraczało 5 % ARfD.

Słowa kluczowe: pozostałości środków ochrony roślin, narażenie, nowalijki

DOI: 10.2428/ecea.2012.19(03)026 ECOL CHEM ENG A. 2012;19(3):249-255

Krzysztof FRĄCZEK[1], Dariusz ROPEK[2],
Jacek GRZYB[1] and Helena BIS[1]

CHANGES IN NUMBER OF FUNGI UNDER CULTIVATION OF AGRICULTURAL PLANTS IN THE VICINITY OF THE MUNICIPAL LANDFILL SITE

ZMIANY LICZEBNOŚCI GRZYBÓW W GLEBACH POD UPRAWĄ ROŚLIN ROLNICZYCH WOKÓŁ SKŁADOWISKA ODPADÓW KOMUNALNYCH

Abstract: Research for the present paper was carried out in field experiment localized in the vicinity of the municipal landfill site in Tarnow. In designated areas 8 plots were established, in which horse bean 'Nadwislanski' var., spring wheat 'Zura' var. and potatoes 'Kuklik' var. were cultivated in 2006 and 2007 using the same agricultural technology. Additional plot was located in restored sector of the landfill.

On the basis of the obtained results it can be concluded that in soil under cultivation of various agricultural plants, microscopic fungi – *Micromycetes* occurred with frequency from 8440 to 179150 cfu $\cdot$ g^{-1} of soil dry mass. The abundance of fungi in soil on the plots with spring wheat was lower than under other agricultural plants (potatoes, horse bean). Based on the analyses it was also found that the occurrence of fungi in tested soils was influenced by plot location in relation to the landfill and type and period of growth of the cultivated plant.

Keywords: fungi, soil, agricultural plants, landfill site

Municipal landfills site pose a threat to the surrounding natural environment and negatively affect the conditions of agricultural production, reducing soil quality and value of crops. In many cases they are located nearby the fields, which often leads to significant deterioration of vegetation conditions of cultivated plants. Therefore, there is a growing need to improve technologies that lead to minimisation of their negative influence not only on atmosphere but also on adjacent soil [1–3].

There is a clear correlation between parameters of soil environment, its microorganisms and plants. Microorganisms increase efficiency of root exudates, and thus

---

[1] Department of Microbiology, University of Agriculture in Krakow, al. A. Mickiewicza. 24/28, 30–058 Kraków, Poland, phone: +48 12 662 41 81, email: rrfracze@cyf-kr.edu.pl

[2] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 02, email: rrropek@cyf-kr.edu.pl

they can facilitate the assimilation of minerals and stimulate plant growth. Fungi play a significant role in soil environment, they are common in natural biocenoses and are used for agrocenoses [1, 4].

Therefore, the objective of the research was to measure the changes in abundance of fungi in soils under cultivation of agricultural plants in the vicinity of a municipal landfill site.

Material and methods

The research for present paper was carried out in the vicinity of the municipal landfill site in Tarnow from March 2006 to September 2007. The analyses were carried out in field experiment. On each side of the landfill two zones were set to its borders – zone I from 50 to 250 m, whereas II zone between 250 and 500 m. 8 plots were established in the designed areas, in which horse bean ‘Nadwislanski’ var., spring wheat ‘Zura’ var. and potatoes ‘Kuklik’ var. were cultivated in 2006 and 2007 using the same agricultural technology. Additionally, the 9th plot was located in reclaimed sector of the landfill, which role was to indicate the influence of the deposited waste in this sector on mycological state of soils in the tested plot. Each plot was divided into microplots 20 m^2 each. Characteristics of the plots location are presented in Table 1. Soil samples for analyses were taken once in each season from the plots from 0–20 cm layer. Samples were transported to the laboratory of Department of Microbiology, University of Agriculture in Krakow, where the microbiological analyses of abundance of fungi in one gram of soil dry matter were performed. *Colony forming units* (cfu) of fungi on malt extract agar (Malt Extract Agar, MEA, Oxoid, Basingstoke, Great Britain) were determined using plating dilutions method.

Table 1

Plots situated in the vicinity of municipal landfill site in Tarnow in 2006–2007

Plot	Plot location – zone [m]	Soil
W I	50–250	light loam
W II	250–500	heavy loamy sand
N I	50–250	light loam
N II	250–500	light loam
E I	50–250	slightly loamy sand
E II	250–500	heavy loamy sand
S I	50–250	light loamy sand
S II	250–500	slightly loamy sand
Z	restored sector	light loam

Explanations: letters in plots’ marking mean cardinal points.

Results and discussion

On the basis of the results presented in Table 2 it can be concluded that in soil under cultivation of various agricultural plants, microscopic fungi *Micromycetes* occurred in the range between 8440 and 179150 cfu $\cdot$ g^{-1} soil d.m.

Table 2

Abundance of fungi in soils under cultivation of agricultural plants in the vicinity of municipal landfill site in Tarnow

No.	Plot/plant	Abundance [cfu per 1 g of soil d.m.]							
		III 2006	V 2006	VII 2006	IX 2006	III 2007	V 2007	VII 2007	IX 2007
1	W I								
	Spring wheat	10270	42700	113500	90534	27362	14634	33200	19700
	Horse bean	14273	42054	45567	58300	23367	33564	43267	16200
	Potatoes	18730	23360	42267	24300	13380	11634	23200	20300
2	W II								
	Spring wheat	23004	26816	31966	131633	68700	19466	39520	27050
	Horse bean	13804	86816	109500	48200	88700	69045	90526	17000
	Potatoes	10900	16800	68867	34700	26050	11950	58660	44720
3	N I								
	Spring wheat	24590	72560	83000	76540	18700	9667	61090	21300
	Horse bean	16598	112500	157500	98000	28700	139024	126190	10300
	Potatoes	23900	68930	92500	163500	42720	45900	92520	63450
4	N II								
	Spring wheat	43086	47890	37100	100866	34800	45330	59000	22800
	Horse bean	23029	57800	70823	37950	14866	32970	54810	12400
	Potatoes	14360	48400	123500	141000	35800	69434	84170	71240
5	E I								
	Spring wheat	17600	42646	55766	64720	23900	49300	78200	21740
	Horse bean	27628	22695	28900	64366	13966	27600	38000	41734
	Potatoes	29300	50130	84700	24900	67830	41734	82400	34460
6	E II								
	Spring wheat	13200	27440	32066	8440	19600	9500	29360	13200
	Horse bean	13240	17170	30000	63033	15100	24573	39361	17640
	Potatoes	16280	33290	62200	66534	9420	12500	42610	46540
7	S I								
	Spring wheat	21600	59220	90400	130500	17600	31600	46200	14500
	Horse bean	11698	79780	107533	26234	32650	82600	56780	10500
	Potatoes	23620	17600	44260	34567	20600	12600	26240	19500
8	S II								
	Spring wheat	54196	92260	113510	132500	82502	41967	54000	29900
	Horse bean	10196	102248	179150	120000	27567	122863	154562	69943
	Potatoes	44500	32680	64572	74567	17560	16400	66420	49900

Table 2 contd.

No.	Plot/plant	Abundance [cfu per 1 g of soil d.m.]							
		III 2006	V 2006	VII 2006	IX 2006	III 2007	V 2007	VII 2007	IX 2007
9	Z								
	Spring wheat	18600	23290	15350	35567	10200	17350	21560	16800
	Horse bean	14772	32795	31967	25000	10634	31793	43967	66300
	Potatoes	21684	22600	24600	32567	27020	23620	53640	33238

Explanations: location of plots as in Table 1.

The lowest value was recorded on the plot under wheat cultivation, which was located in the zone 250 to 500 m east of the landfill (E II plot), and the highest value was recorded on the plot under horse bean cultivation, which was located behind the trees strip in zone II, on the south side of the landfill (plot S II). Analysing changes in the number of fungi showed that their abundance in the tested soil varied depending on the plot location, type of the cultivated plant and sampling period. It needs to be stressed that soil is excellent substrate for growth and development of various group of microorganisms, because it is usually sufficient with organic and mineral nutrients and has adequate humidity, reaction and favourable aerobic conditions. There is also a strong correlation between soil environment parameters, its microorganisms and plants [2, 5–7].

Analytical data presented in Table 2 indicates that the plot under horse bean cultivation contained from 10196 to 179150 cfu of microscopic fungi in 1 g of soil dry matter. Both the maximum and minimum number was found in the plot situated south of the landfill, in a zone 250–500 m from its area (S II plot). Analysing changes of fungi abundance in soil on the plots under cultivation of each agricultural plant indicated that their number in spring wheat ranged from 8440 to 132500 cfu in 1 g of soil d.m. Their highest number occurred on the plot located south of the landfill (S II plot) whereas the minimal number on the east side of the landfill (E II plot). On the other hand, abundance of microscopic fungi in soil under cultivation of potatoes was from 9420 to 163500 cfu $\cdot$ g^{-1} soil d.m. The study showed that in soil under cultivation of potatoes the highest number of fungi occurred on a plot located on the north side of the landfill in the area distant from 50 to 250 m from its territory (plot N I) and the lowest number was found on the plot located on the east side of the landfill in the area 250–500 m (E II plot) distant. Obtained analytical data indicate that in 1 g of soil d.m. on the plots under horse bean there were from 10196 to 179150 cfu of microscopic fungi. Maximum as well as minimum value was found on the plot situated south of the landfill in the area distant from 250 to 500 m (S II plot). Similarly increase in abundance of fungi in soil layers (20–25 cm) was observed in the measuring point located further from the landfill in Leczyca (400 m) [3].

Comparing the average number of fungi under cultivation of all agricultural plants included in the experiment indicated that the highest average number (98316 cfu $\cdot$ g^{-1} soil d.m.) occurred in soil under cultivation of horse bean, on the plot located on the south side of the landfill (S II plot) which was over 30 % higher than the maximum average number of fungi in the soil on the control plot located in the zone II on the west side of the landfill, 250 to 500 m from its area (W II plot). On the other hand the lowest value (19101 cfu $\cdot$ g^{-1} d.m. of soil) was found in the soil under cultivation of spring wheat, on the plot situated on the east side of the landfill (E II plot) (Table 3) which was comparable with the minimum average number of fungi (19840 cfu $\cdot$ g^{-1} d.m. of soil) found in the plot located in the restored area. It is worth noting that on the plot located in the area of the landfill (Z plot) under cultivation of all agricultural plants much lower average numbers of fungi was recorded in comparison with other plots. Therefore, it is clear that waste deposited in this sector significantly impacted mycological state of the soils in the tested area.

Table 3

Average number of fungi in soil under cultivation of agricultural plants in the vicinity of municipal landfill site in Tarnow (years 2006–2007)

No.	Plot	Abundance [cfu in 1 g of soil d.m.]		
		Spring wheat ‘Zura’ variety	Horse bean ‘Nadwislanski’ variety	Potatoes ‘Kuklik’ variety
1	W I	43988	34574	22146
2	W II	46019	65449	34081
3	N I	45931	86102	74178
4	N II	48859	38081	73488
5	E I	44234	33111	51932
6	E II	19101	27515	36172
7	S I	51453	50972	24873
8	S II	75104	98316	45825
9	Z	19840	32154	29871

Explanations: location of plots as in Table 1.

Based on the obtained average numbers of fungi it was also shown that in the soil under cultivation of potatoes and beans a significant increase of fungi abundance compared with the control plot (W II) can be noticed on the plots located on the north side of the landfill, whereas under cultivation of spring wheat – on the plots located on the south side.

The above relationship may result from the fact that due to location the plots situated on the east side of the landfill should be the most vulnerable to impact of pollutants emission from the landfill. This results both from predominant winds in this area that blow in an easterly direction and from the fact that the active section is situated on the north-east side of the landfill. It also needs to be remembered that soil microflora is the fastest growing and reacting to changes of environmental parameters part of the biocenosis. This is due to typical of microorganisms variety of biochemical functions and abnormally high physiological activity [2, 5, 8].

The obtained results also showed that abundance of fungi on the plots under cultivation of spring wheat was slightly lower than under the rest of agricultural plants included in the experiment. The results may therefore indicate that different chemical composition of root exudates of each agricultural plant species in the experiment had modifying influence on the soil microorganisms. Microflora is affected by plant genus, plant species, plant variety as well as plant growth period. Plants by their root exudates can change soil dwelling microflora in various ways [4, 9]. Taking into account the terms of the analyses, significant increase of fungi abundance was noted in soil on the plots during active growth of plants which is in summer and which is probably caused by intensive mineralisation of soil organic matter due to usually preferable humidity and thermal conditions for growth of microflora in this period [4, 10].

Conclusions

1. The carried out research showed varied occurrence of fungi in soil environment on plots located in the vicinity of the municipal landfill site.
2. The abundance of fungi in soil on the plots under cultivation of spring wheat was lower than under other tested agricultural plants (potatoes, horse bean).
3. The plot location in relation to the landfill and type and period of growth of the cultivated plant influenced the abundance of fungi in the tested soils.

Acknowledgments

The research was financed from budgetary funds on science in 2005–2008 as a research project 2P06R 089 29.

References

[1] Ropek D, Frączek K. Acta Agr Silv. ser. Agr. 2006;XLIX:428-432.
[2] Frączek K. Woda – Środowisko – Obszary Wiejskie. 2010;2(30):61-71.
[3] Przybulewska K, Nowak A, Głąbowska D. Woda – Środowisko – Obszary Wiejskie. 2010;2(30):159-166.
[4] Wielgosz E, Szember A. Wpływ wybranych roślin na liczebność i aktywność drobnoustrojów glebowych. Ann UMCS Sec E. 2006;61:107-119.
[5] Paul EA, Clark FE. Mikrobiologia i biochemia gleby. Lublin: Wyd. UMCS; 2000.
[6] Prado AG, Airoldi C. Thermochim Acta. 1999;332:71-74.
[7] Kucharski J. Drobnoustroje w środowisku. Występowanie, aktywność i znaczenie. Kraków: Wyd. Akademii Rolniczej; 1997.
[8] Libudzisz Z, Kowal K. Mikrobiologia techniczna. Łódź: Wyd. Politechniki Łódzkiej; 2000.
[9] Wielgosz E, Szember A, Skwarek J. Ann. UMCS Sec E. 2004;59(4):1689-1696.
[10] Przybulewska K, Nowak A. Acta Agr Silv ser Agr. 2004;XLII:378-391.

ZMIANY LICZEBNOŚCI GRZYBÓW W GLEBACH POD UPRAWĄ ROŚLIN ROLNICZYCH WOKÓŁ SKŁADOWISKA ODPADÓW KOMUNALNYCH

Katedra Mikrobiologii
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Badania do niniejszej pracy zostały przeprowadzone w doświadczeniu polowym zlokalizowanym wokół składowiska odpadów komunalnych w Tarnowie. W wyznaczonych strefach założono 8 poletek doświadczalnych, na których uprawiano w latach 2006 i 2007 bobik odmiany ‘Nadwiślański’, pszenicę jarą odmiany ‘Żura’ oraz ziemniaki odmiany ‘Kuklik’ przy zastosowaniu tej samej agrotechniki. Dodatkowe poletko zlokalizowano w sektorze zrekultywowanego składowiska. Na podstawie otrzymanych wyników można stwierdzić, że w glebie na poletkach pod uprawą różnych roślin rolniczych grzyby mikroskopowe – *Micromycetes* występowały od 8440 do 179150 jtk · g^{-1} s.m. gleby. Stwierdzono, że liczebność grzybów w glebie na poletkach w uprawie pszenicy jarej była mniejsza niż pod pozostałymi ujętymi w doświadczeniu roślinami rolniczymi (ziemniaki, bobik). Na podstawie przeprowadzonej analizy stwierdzono także, że występowanie grzybów w badanych glebach było zależało od miejsca położenia poletka doświadczalnego względem składowiska oraz rodzaju i okresu wzrostu uprawianej rośliny rolniczej.

Słowa kluczowe: grzyby, gleba, rośliny uprawne, składowisko odpadów

DOI: 10.2428/ecea.2012.19(03)027 ECOL CHEM ENG A. 2012;19(3):257-265

Helena BIS[1], Krzysztof FRĄCZEK[1]
and Renata BROŻEK[1]

OCCURRENCE OF FUNGI AND INCLUDING TOXICOGENIC FUNGI IN STORED GRAIN SEEDS

WYSTĘPOWANIE GRZYBÓW W TYM TOKSYNOTWÓRCZYCH W MAGAZYNOWYM ZIARNIE ZBÓŻ

Abstract: Analyses for the present paper were carried out from October 2009 to February 2010. Grain seeds for microbiological analyses were sampled from various grain stores, both from granaries and from silos, in Brzeznica, Czernichow and Tomice communes in Malopolskie province, from individual farms and from agricultural schools.

Quantity of microscopic fungi – *Micromycetes* in the stored grain seeds was influenced by the following factors: seed moisture, store type, grain type and length of storage. 10 out of 22 isolated fungi belonged to potentially toxicogenic genera: *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium graminearum*, *Fusarium nivale*, *Fusarium oxysporum*, *Fusarium redolens*, *Penicillium citrinum*, *Trichoderma viride*. Metabolites of the following strains showed the strongest phytotoxicity: *Aspergillus flavus* – strain isolated from spring-wheat 'Bombona' cv., *Penicillium citrinum* – strain isolated from spring barley 'Poldek' cv. and *Fusarium graminearum* – strain isolated from winter-wheat 'Tonacja' cv.

Keywords: grain seeds, fungi, phytotoxicity

Grains are the major group of nutritional plants in the world. They are the most important food source for people all over the world due to favourable seed composition that contains almost all components necessary for the proper development of human organism [1, 2]. Grain seeds are also the most comprehensive concentrate for all types of livestock especially for poultry, pigs and horses, mainly due to high carbohydrates' concentration [3]. It needs to be stressed that every year as much as 4 % of the grain seeds production is loss caused by different factors, such as: improper storage, insufficient protection against pests and mould fungi [1, 4]. Danger to human and animal health, effects of mould fungi and mycotoxins depend mostly on grain seeds' storage. Therefore, the only action that allows for minimisation and reduction of this

---

[1] Department of Microbiology, University of Agriculture in Krakow, al. A. Mickiewicza 24/28, 30–058 Kraków, Poland, phone: +48 12 662 4181, email: rrfracze@cyf-kr.edu.pl

problem is proper agricultural engineering and proper conditions of seeds' storage as well as examination of material samples before its processing [5, 6].

Consequently, the objective of the research was to find the presence and to recognise the mould fungi – *Micromycetes,* with particular emphasis to potentially toxicogenic fungi in the stored grain seeds.

Material and methods

The research was carried out in the laboratory of the Department of Microbiology, University of Agriculture in Krakow, monthly from October 2009 to February 2010. Seeds for microbiological analyses were sampled from various grain stores, both from granaries and silos in the following communes: Brzeznica, Czernichow and Tomice in Malopolskie province from individual farms and from agricultural schools. The Table 1 shows the characteristics of the analysed objects.

Table 1

Characteristics of the analysed objects

Sample number	Form of grain	Cultivar	Sampling point (commune)	Owner of the store	Store type
1	Spring-wheat	Helia	Brzeznica	Individual farmers	Silo
2		Tybalt	Czernichow		Granary (pile)
3		Bombona	Brzeznica		
4	Winter-wheat	Rywalka			
5		Tonacja			
6		Turnia	Tomice	Agricultural school	Silo
7	Spring-barley	Stratus		Individual farmers	Granary (pile)
8		Poldek			
9		Sezam	Czernichow		
10	Oat	Dukat		Agricultural school	Silo
11		Hetman			
12		Krezus	Brzeznica	Individual farmer	

Grain seeds for the analyses were sampled with a cane probe, subject to principles of microbial purity.

During the experiment the samples were taken once in each month. Each grain sample was collected from the seed lot by random collection of small samples in various parts of the lot in different depths. Subsequently, they were integrated. Minimal number of primary samples was calculated as follows: square root was calculated from the number of tones of the loose statistical lot and then the result was divided into two and rounded up to the next integer. The average sample weighed about 1000 g mass and was placed in sterile box. Seed sampling was performed in accordance with the recommendations of Polish Standard PN-EN ISO 13690:2007. Moreover, each time grain seeds' moisture was measured. The analysis was performed within 24 hours from the seed sampling. Mycological analyses were carried out with the serial dilution method. Fungi were cultured on the wort agar at 28 $^{\circ}$C for 5 days and then pure cultures were isolated and inoculated on the diagnostic media accordingly to the standard requirements [7–10]. Fungi that are known to be potentially toxigenic were tested to check their ability to produce toxins. Consequently these isolates were inoculated on the liquid Eldrige's medium. They were cultured for 14 days at room temperature. The ability to produce toxins was evaluated in a biological test [11], using the testing plant – green peas seeds "Nike" ST93137(115)24G/C. Fungi strains were considered to be toxic when their metabolites inhibited 30 % germination energy of the tested plant in relation to the control plants.

Results and discussion

High quantitative differentiation of microscopic fungi – *Micromycetes* – was proved on the basis of mycological analyses of seeds of 12 grain cultivars. Table 2 presents the abundance of fungi in the tested seed samples.

Table 2

Abundance of microscopic fungi – *Micromycetes* in 1 g of seeds in the performed analyses

Grain cultivar	Number of cfu $\cdot$ g^{-1} of seeds				
	2.10.2009	4.11.2009	3.12.2009	6.01.2010	2.02.2010
Spring-wheat Helia	1200	167	100	100	26
Spring-wheat Tybalt	2000	4620	4237	3383	2413
Spring-wheat Bombona	1900	1047	693	793	580
Winter-wheat Rywalka	2400	1833	2800	1730	2357
Winter-wheat Tonacja	1163	757	617	657	503
Winter-wheat Turnia	650	300	117	150	43
Spring-barley Stratus	2717	1513	2807	1527	2630
Spring-barley Poldek	2440	1680	1547	1683	1024
Spring-barley Sezam	2543	2833	2033	1780	433
Oat Dukat	797	393	213	90	35
Oat Hetman	200	53	67	37	5
Oat Krezus	443	367	700	80	10

Quantity of mould fungi *Micromycetes* in the analysed samples ranged from 5 to 4620 cfu $\cdot$ g^{-1} of seeds, which proves that the admissible concentration of microscopic fungi was not exceeded in any of the tested seed samples. Evaluation of the obtained results was performed on the basis of the microbial contamination limit of the cereal grain proposed by ICC (*International Association of Cereal Science and Technology*). The general number of mould fungi on the seed surface according to the above standards should not exceed $3.0 \cdot 10^4$ cfu $\cdot$ g^{-1} of seeds. Figure 1 presents arithmetic

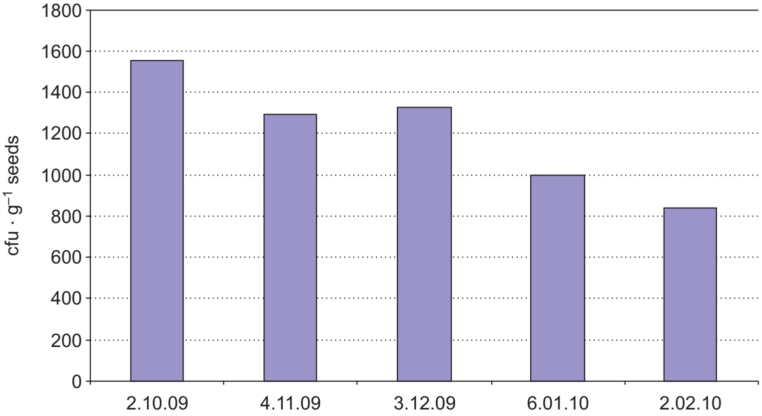

Fig. 1. Arithmetic mean of microscopic fungi – *Micromycetes* in different periods of analyses

mean of the *Micromycetes* fungi in each analysis. In October 2009 the abundance of fungi reached 1558 cfu · g^{-1} of seeds, which constituted 25.6 % of all fungi cultured during the whole test period (from October 2009 to February 2010). In November 1297 cfu · g^{-1} of seeds were found, which constitutes 21.6 % of all fungi from the test period. In December 1328 cfu · g^{-1} of seeds (22.1 %), in January 1001 cfu · g^{-1} of seeds (16.7 %), whereas in February 838 cfu · g^{-1} of seeds were cultured, which constituted 14 %. Based on the presented data it was ascertained that the abundance of fungi decreased with time.

Table 3 presents comparison of arithmetic mean of fungi abundance in 1 g of seeds with seeds' moisture.

Table 3

Comparison of arithmetic mean of fungi count in 1 g of seeds with seed moisture in each grain genus and cultivar

Sample number	Grain cultivar	Seed moisture [%]	Number of fungi in 1 g of seeds	Average
1	Spring-wheat Helia	9.3–13.1	319	1551
2	Spring-wheat Tybalt	19.5–21.4	3331	
3	Spring-wheat Bombona	15.3–16.7	1003	
4	Winter-wheat Rywalka	15.5–17.7	2224	1072
5	Winter-wheat Tonacja	11.5–13.4	739	
6	Winter-wheat Turnia	12.3–13.2	252	
7	Spring-barley Stratus	18.7–19.5	2239	1946
8	Spring-barley Poldek	16.1–17.9	1675	
9	Spring-barley Sezam	15.6–17.2	1924	
10	Oat Dukat	11.4–12.6	306	233
11	Oat Hetman	12.3–13.7	72	
12	Oat Krezus	12.8–13.4	320	

The highest arithmetic mean of fungi abundance in the whole research period – 3331 cfu $\cdot$ g^{-1} of seeds was found in 'Tybalt' spring-wheat sample. On the other hand the lowest abundance was found in oat sample – 'Hetman'. It reached 72 cfu $\cdot$ g^{-1} of seeds. The average abundance of fungi found in all analysed objects was 1200 cfu $\cdot$ g^{-1} of seeds. The results presented in the Table 3 indicate that the most contaminated grain was the spring-barley, and the average abundance of fungi in 1 gram of seeds was 1946 cfu. All cultivars of spring-barley sampled for microbiological analyses were taken from the pile in granaries from individual farmers. Oat was the least contaminated by microscopic fungi – *Micromycetes* – 233 cfu $\cdot$ g^{-1} of seeds. This amount definitely differs from the other amounts. All the oat seeds were stored in silos. In case of the spring-wheat fungi amount reached 1551 cfu $\cdot$ g^{-1} of seeds, whereas in winter-wheat it reached 1072 cfu $\cdot$ g^{-1} of seeds. About 66 % of seed samples from the analysed spring-wheat and winter-wheat were stored in the granaries (piles) that belonged only to individual farmers and the rest was stored in silos. As presented in the Table 2, comparison of arithmetic mean of fungi abundance in 1 g of seeds with seed moisture indicates that: with lower seed moisture, about 9.3–13.7 %, the abundance of microscopic fungi – *Micromycetes* – was lower than 1000 cfu $\cdot$ g^{-1} of seeds. In this moisture range the maximum amount of fungi (739 cfu $\cdot$ g^{-1} of seeds) occurred in winter-wheat 'Tonacja', which moisture ranged from 11.5 to 13.4 %. In case of increased seed moisture (15.3–21.4 %) the amount of fungi was a few to several times higher than in the previous group, reaching the maximum in the spring-wheat 'Tybalt' (3331 cfu $\cdot$ g^{-1} of seeds), which moisture was between 19.5 and 21.4 %.

Mycological analyses of 12 grain cultivars revealed presence of 22 fungi species. They belonged to the following genera: *Actinomucor* sp., *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Fusidium* sp., *Humicola* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Rhodotorula* sp., *Torulopsis* sp. and *Trichoderma* sp. Table 4 presents the frequency of their occurrence.

Genus *Aspergillus* was definitely the most frequently occurring group of fungi where the predominant species were *Aspergillus niger* which infected 72 % of the analysed grain seeds and *Aspergillus fumigatus* (61 %). Also *Penicillium* genus was numerous with the predominant species *Penicillium citrinum* (39 %), and *Mucor hiemalis*, that infected 42 % of the analysed seeds as well as *Rhizopus nigricans* (47 %). *Actinomucor elegans* (5 %), *Humicola grisea* (5 %) and *Trichoderma viride* (5 %) were the least frequently isolated fungi. Predomination of each mould fungi changed in time. During five months of the research the share of genus *Aspergillus* increased from in about 15 % in October (the beginning of the analyses) to about 75 % in February 2010. The increase of *Penicillium* percentage was definitely less rapid, and was about 18 %. In case of *Fusarium* and *Alternaria* the situation was different and their percentage decreased by about 45 % and 56 %, respectively. As much as 10 out of 22 isolated fungi genera were recognised to be toxigenic. From the analysed seeds 210 potentially toxigenic strains were isolated and all of them were subject to the biological test. Only 55 % were found to be toxigenic. The tested strains inhibited from 3 to 93 % of germination energy of the tested plant and germination ability was reduced by 32 to 93 % in relation to the control plant. Metabolites of the rest of the strains did not show toxic properties. Metabolites of

Table 4

Frequency of each fungi species in the analysed grain cultivars

No.	Fungi species	Spring-wheat			Winter-wheat			Spring-barley			Oat		
		Helia	Tybalt	Bombona	Rywalka	Tonacja	Turnia	Stratus	Poldek	Sezam	Dukat	Hetman	Krezus
1	*Actinomucor elegans*						++						
2	***Alternaria alternata***	+	+++	+	++	++	+					+	+
3	*Alternaria geophila*	+	+++	++	+++	++	++					+	+
4	***Aspergillus candidus***									+++			
5	***Aspergillus flavus***	+		++	+++	+++		++		++			+
6	***Aspergillus fumigatus***	+	++	+++	+++	++	+++	++	+++		++	+	
7	*Aspergillus niger*		++	+++	+++	+++	+++	+	+++	+++	++	++	+
8	***Fusarium graminearum***		++			+		+++			++		++
9	***Fusarium nivale***		++					+			+		+
10	***Fusarium oxysporum***		+++			++		+++			++		++
11	***Fusarium redolens***		+			+		++			+++		+
12	*Fusidium* sp.		+							+++			+++
13	*Humicola grisea*					+						+	
14	*Mucor hiemalis*	+++		++		++	++	+	+	++		+	+
15	***Penicillium citrinum***		+	+	+	+	++	+++	+++	++			
16	*Penicillium frequentans*			+	++		+	+++	+	++			+
17	*Penicillium notatum*					+		++					
18	*Penicillium oxalicum*			+	++			+++	+	+++	+		
19	*Rhizopus nigricans*	+++	+++	++	+++		+	+	+		++		+
20	*Rhodotorula glutinis*				+		+	+	+				
21	***Trichoderma viride***					++							
22	*Torulopsis* sp.				+		+	+	+				

Explanation: +++ very frequent (67–100 %); ++ frequent (34–66 %); + occasional (0–33 %); potentially toxicogenic species were bolded.

the following strains showed the strongest phytotoxic effects: *Aspergillus flavus* – strain isolated from the spring-wheat ‘Bombona’ (reduced germination energy by 82 % and germination ability by 93 %), *Penicillium citrinum* – strain isolated from the spring-barley ‘Poldek’ (reduced germination energy by 72 % and germination ability by 81 %) and *Fusarium graminarum* – strain isolated from the winter-wheat ‘Tonacja’ (reduced germination energy by 69 % and germination ability by 89 %). Metabolites of the following strains proved to have the weakest effect: *Fusarium redolns* – strain isolated from the spring-barley ‘Stratus’ (reduced germination energy by 39 % and germination ability by 42 %), *Aspergillus fumigatus* – strain isolated from the winter-wheat ‘Turnia’ (reduced germination energy by 48 % and germination ability by 51 %) and strain *Fusarium oxysporum* isolated from oat ‘Kresus’ (reduced germination energy by 30 % and germination ability by 35 %). Metabolites of the rest of the strains appeared to be of medium toxicity. Mycotoxins that showed very strong toxicity in many cases not only inhibited but totally destroyed viability of peas seeds.

Parametric Pearson’s correlation coefficient (r) and its statistical significance ($p \leq 0.05$) were calculated using Statistica software (v. 5.1 G) for Windows PL in order to evaluate the possible impact of environmental variables: moisture and storage on the number of microscopic fungi isolated from the analysed seeds. The statistical analysis revealed that the abundance of microscopic fungi was impacted by moisture. On the other hand statistically important relevance was not observed for the species of the analysed seeds and abundance of fungi on their surface.

Grain seeds, often collected in unfavourable weather, are kept in different grain stores where together with pollutants, pests and microorganisms they create an ecosystem of conserved and stored seeds. Proper care of such seeds bases on skillful inhibition of the seeds’ life processes which are the major cause of the loss and seeds’ damage [12]. As given by Trojanowska [13], mould fungi occurrence on seeds causes loss as much as 10–30 % of the whole material depending on the world region. Undoubtedly, seed moisture is one of the most important factors that influence seed storage conditions. The research proved that increased moisture of caryopses favoured the increase of pollution of seed samples with mould fungi. 15 % of seed moisture is the threshold above which mould develops due to fungi spore germination. This causes danger to the collected seed [14]. Mycotoxins produced by mould fungi are very dangerous to human and animal health. They cause damage of the most important organs: lungs, liver, kidneys, heart as well as nervous, circulatory, digestive and endocrine systems. They cause mucous membranes, placenta and skin damage. They perturb reproduction processes [15]. The only protection of seed against mycotoxins is its protection against mould fungi. There are no possibilities to remove mycotoxins from seeds or from feed. This is why the precaution and control of the basic parameters of seeds (especially temperature and moisture) as well as ventilation and seeds’ cooling processes throughout the whole period of seed storage is so important [16]. This is the reason why it is so crucial that the seeds are free from unwanted microorganism contamination, especially from mould fungi and mycotoxins.

Conclusions

1. Occurrence of microscopic fungi – *Micromycetes* was found in the samples of the analysed grain material stored in silos and in granaries.

2. The abundance of microscopic fungi – *Micromycetes* in the stored grain seeds was influenced by the following conditions: seed moisture, store type, grain type and storage period.

3. Seed moisture played the crucial role during storage. The higher the moisture was, the higher number of fungi infected the seeds. Moisture of 13.5–14 % was considered the threshold for safe seed storage.

4. Analysis of the seed samples revealed the presence of 22 mould fungi species. They belong to the following genera: *Actinomucor* sp., *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Fusidium* sp., *Humicola* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Rhodotorula* sp., *Torulopsis* sp. and *Trichoderma* sp.

5. As much as 10 out of 22 isolated fungi species were potentially toxicogenic: *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium graminearum*, *Fusarium nivale*, *Fusarium oxysporum*, *Fusarium redolens*, *Penicillium citrinum*, *Trichoderma viride.*

6. The biological test proved that 55 % of the analysed strains were toxigenic.

7. Metabolites of the following strains appeared to show the highest phytotoxic effect: *Aspergillus flavus* – strain isolated from the spring-wheat ‘Bambona’, *Penicillium citrinum* – strain isolated from the spring-barley ‘Poldek’ and *Fusarium graminearum* – strain isolated from the winter-wheat ‘Tonacja’.

References

[1] Kaczmarczyk S. Agrotechnika roślin uprawnych. Szczecin: Wyd Akad Roln w Szczecinie; 2005.

[2] Krężel R, Parylak D, Zimny L: Zagadnienia uprawy roli i roślin. Wrocław: Wyd Akad Roln we Wrocławiu; 1999.

[3] Rozbicki J. Produkcja i rynek zbóż. Warszawa: Wyd. “Wieś Jutra”; 2002.

[4] Kościelniak W, Dreczka M. Nowoczesna uprawa zbóż. Poznań: Wyd. Apra; 2009.

[5] Chełkowski J. Mikotoksyny, wytwarzające je grzyby i mikotoksykozy. Wyd. I. Warszawa: Skrypty SGGW-AR; 1985.

[6] Grajewski J. Mikotoksyny i grzyby pleśniowe – zagrożenia dla człowieka i zwierząt. Bydgoszcz: Wyd. Uniwer Kazimierza Wielkiego; 2006.

[7] Samson RA, Frisvad JC. Penicillium subgenus Penicillium; New taxonomic schemes, Mycotoxins and other Extrolites. Studies in Mycology. 2004;49:260 p.

[8] Klich MA. Identification of Common. Aspergillus species. Centraalburean voor Schimmelcultures, Utrecht. The Netherlands; 2002.

[9] Rotem J. The genus Alternaria. Biology, epidemiology and pathogenicity. APS Press, St. Paul, Minnesota: 1998;11-272.

[10] Fassatiowa O. Grzyby mikroskopowe w mikrobiologii technicznej. Warszawa: WNT; 1983.

[11] Mirczink TG. Mikrobiologia. 1957;26:78-86.

[12] Ryniecki A. Przegląd Zbożowo-Młynarski. 1998;10:31-32.

[13] Trojanowska K. Przegląd Zbożowo-Młynarski. 2002;2:9-12.

[14] Janowicz L. Przechowywanie ziarna pszenicy i jęczmienia w silosach BIN. Zboże wysokiej jakości: poradnik dla producentów. Warszawa: Biznes-Press; 2005.

[15] Janowicz L. Przechowywanie surowców rolniczych – zmiany w jakości ziarna. Agrotechnika: Poradnik Rolnika. 2007;5:37-40.

[16] Kośla T. Biologiczne i chemiczne zanieczyszczenia produktów rolniczych. Warszawa: Wyd. SGGW; 1999.

WYSTĘPOWANIE GRZYBÓW W TYM TOKSYNOTWÓRCZYCH W MAGAZYNOWYM ZIARNIE ZBÓŻ

Katedra Mikrobiologii
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Badania zostały przeprowadzone w okresie od października 2009 do lutego 2010 r. Próbki ziarna przeznaczone do badań mikrobiologicznych pobierano z różnych magazynów zbożowych zarówno podłogowych, jak i silosowych, występujących na terenie gmin: Brzeźnica, Czernichów i Tomice położonych w województwie małopolskim, z gospodarstw indywidualnych i szkół rolniczych.

Na liczebność grzybów mikroskopowych – *Micromycetes* w magazynowanym ziarnie zbóż miały wpływ następujące czynniki: wilgotność ziarna, rodzaj magazynu, rodzaj zboża i długość okresu przechowywania. Spośród 22 wyizolowanych gatunków grzybów, 10 gatunków należało do grzybów potencjalnie toksynotwórczych: *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium graminearum*, *Fusarium nivale*, *Fusarium oxysporum*, *Fusarium redolens*, *Penicillium citrinum*, *Trichoderma viride*. Najsilniejszym oddziaływaniem fitotoksycznym odznaczały się metabolity następujących szczepów: *Aspergillus flavus* – szczep wyizolowany z pszenicy jarej 'Bambona', *Penicillium citrinum* – szczep wyizolowany z jęczmienia jarego 'Poldek' oraz *Fusarium graminearum* – szczep wyizolowany z pszenicy ozimej 'Tonacja'.

Słowa kluczowe: ziarno zbóż, grzyby, fitotoksyczność

DOI: 10.2428/ecea.2012.19(03)028 ECOL CHEM ENG A. 2012;19(3):267-273

Jacek GRZYB[1], Joanna MICHAŁEK[1], Krzysztof FRĄCZEK[1]
and Dariusz ROPEK[2]

MICROBIAL AIR CONTAMINATION ON THE MARKETPLACES IN KRAKOW

MIKROBIOLOGICZNE ZANIECZYSZCZENIE POWIETRZA NA PLACACH TARGOWYCH W KRAKOWIE

Abstract: Microbiological air research was conducted on five different marketplaces in Krakow and on the Blonia meadows in different seasons. The air was sampled by using the collision method with a microbiological air-sampler MAS-100 (Merck). Abundance of bacteria ranged from 42 to 2300 CFU/m^3. Fungi were seldom present and ranged from 30 to 3460 CFU/m^3. Actinomycetes were most uncommon and their quantities ranged from 0 to 140 CFU/m^3, but quantities that ranged over 100 CFU/m^3 also caused heavy atmospheric air pollution. Research proved a tight correlation between microorganisms' occurrence and seasons. During summer bacteria appeared in larger numbers whereas fungi and actinomycetes had larger quantities during springtime.

Keywords: air microflora, marketplace, Krakow, bacteria, fungi, actinomycetes

Marketplaces that have existed for several hundred years in Krakow etched permanently into its landscape and history. They are places where not only all kinds of food, crops or flowers are sold but also where normal daily life goes. Many people consider food sold on such marketplaces directly by farmers to be fresh, healthy and nutritious. Quality of products sold on marketplaces is often influenced by their surroundings. Marketplaces are located in the city centres neighbouring crowded streets with plenty of cars and people [1].

Such locations may have negative impact on microbiological quality of the products, due to high concentration of air dust and bioaerosols containing various size dust particles that can transmit microorganisms. Bioaerosols may be of natural origin: plants, soil, open water reservoirs, or anthropogenic, such as waste, sewage, agriculture, animal husbandry, etc. [2–6].

---

[1] Department of Microbiology, University of Agriculture in Krakow, al. A. Mickiewicza 24/28, 30–059 Kraków, Poland, phone: +48 12 662 41 81, email: rrgrzyb@cyfronet.pl.

[2] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 24/28, 30–059 Kraków, Poland, phone: +48 12 662 44 02, email: rrropek@cyf-kr.edu.pl.

Currently literature lacks information about atmospheric air quality on marketplaces. The only available data concern air quality in supermarkets [7]. On the other hand, with respect to external environment, microbial quality of food sold on the streets of Sao Paulo in Brazil [8], or on Thailand marketplaces [9] was analysed, whereas microbial air quality was ignored.

The aim of the present research was to carry out microbiological analyses of air regarding microbial abundance (bacteria, actinomycetes, mould fungi) in crowded places where microbial bioaerosol can adversely affect food quality.

Material and methods

Air sampling was performed on five marketplaces at fixed locations and the control site was located at the Blonia meadows. The sampling sites were located in the centers of analysed objects. The air was sampled in 4 seasons (spring: 27th May 2009, summer: 28th July 2009, autumn: 26th October 2009, winter: 18th January 2010), before noon.

Air sampling was performed by MAS-100 (Merck) air sampler, at a height of 1.3 m above the ground – according to Polish Standard PN-89/Z-04008/08 [10]. Incubation conditions and applied media (according to Polish Standard: PN-89/Z-04111, sheets 02 and 03) [11, 12] are presented in the Table 1.

Table 1

Microbial culture conditions

Microorganisms	Temperature [°C]	Time of incubation [h]	Medium
Total no. of bacteria	37	24	Nutrient agar
Fungi	28	120	Wort agar
Actinomycetes	28	168	Gauss medium

Based on the guidelines of the above – mentioned standards (Tables 2 and 3) microbial air pollution was evaluated.

Table 2

Evaluation of bacterial air contamination [CFU per 1 m^3]

Total no. of bacteria	No. of actinomyces	Air pollution degree
< 1000	10	No pollution
1000–3000	10–100	Average pollution
> 3000	> 100	Heavy pollution

Table 3

Evaluation of fungal air contamination [CFU per 1 m^3]

Total no. of fungi per 1 m^3 of air	Air pollution degree
< 3000	No pollution
3000–5000	Average air pollution, especially in late-spring and early-autumn
5000–10000	Pollution that may adversely affect human environment
> 10000	Pollution that threaten human environment

Results and discussion

The following percentage of the analysed microorganisms on the marketplaces was indicated by the quantitative study:

- bacteria – 61.5 %;
- fungi – 35.5 %;
- actinomycetes – 3 %.

Considering the average number on all analysed marketplaces in all seasons, bacteria were most abundant in summer (1238 CFU/m^3), slightly less abundant in autumn (939 CFU/m^3) and in spring (936 CFU/m^3); the smallest average number of bacteria occurred in winter (447 CFU/m^3). In the research carried out in Torun Donderski et al [13] found seasonal variations in bacterial abundance; abundances found by them were significantly lower, but it resulted from the nature of the sampling sites.

Comparing with the control site at Blonia meadows bacterial numbers were higher in all analysed marketplaces, from nearly 3 times greater abundance in Plac Nowowiejski to nearly 8 times greater in Plac Imbramowski. Bacteria (Fig. 1) were the most abundant in Plac Imbramowski, particularly in summer (2130 CFU/m^3) and autumn (2305 CFU/m^3). High bacterial abundances on this marketplace result most probably from the close neighbourhood of a crowded street, large number of buyers as well as from the lack of green spaces in the neighbourhood that could retain dust and bacteria. Bacteria, on the other hand, were least numerous at Blonia meadows in autumn (15 CFU/m^3). Such high differences in bacterial abundance in the same season are mostly related to the sampling sites location. Similar results were obtained by Giorgio et al [14] in Marseille. Average bacterial abundance on all marketplaces was nearly 5 times higher than in the control site (890 in comparison to 179 CFU/m^3).

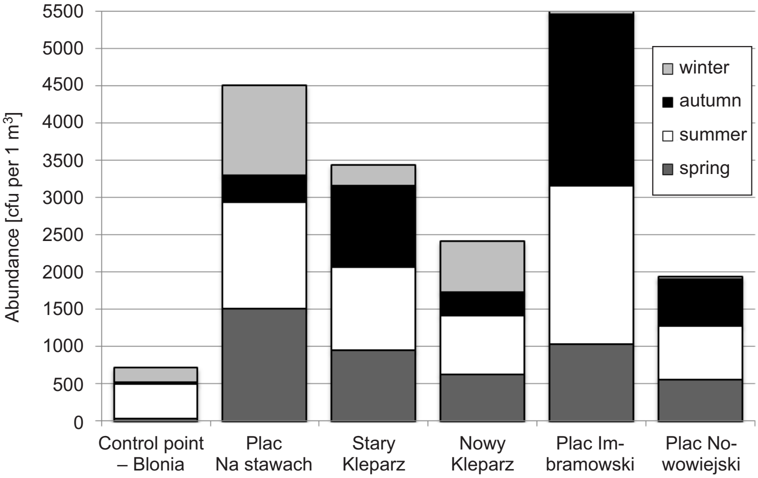

Fig. 1. The number of bacteria on marketplaces in different seasons

Bacterial air pollution was average according to Polish Standards [11] in 3 out of 5 analysed marketplaces (Plac na Stawach, Plac Imbramowski, Stary Kleparz, including 2 marketplaces in 3 seasons).

Large number of fungal spores (Fig. 2) occurred mostly in spring and summer, whereas fungi were less abundant in autumn. Stary Kleparz was the most polluted place concerning fungal abundance – 3460 CFU/m^3 were found there in spring. This result could be affected by numerous stalls with flowers and field vegetables such as potatoes or beets. Fungal spores are released from their surface and from the ground. High numbers of fungi were found at Blonia meadows in spring and in summer – higher than in the majority of the analysed marketplaces; such high abundance of fungi might have resulted from lush vegetation that could have promoted fungal development. Therefore, the average number of fungi on the marketplaces (513 CFU/m^3) was lower than in the control site (652 CFU/m^3). In the study of seasonal variations in fungal number in Krakow Medrela-Kuder [15] indicated their highest incidence in summer, half that number in spring and in autumn, and the lowest number in winter. On Nowy Kleparz, Plac Imbramowski and Plac Nowowiejski discovered number of fungi was similar to the studies presented by Medrela-Kuder [15]. Bacteria outnumbered fungi in the analysed marketplaces almost in all cases except summer measurements carried out in Stary Kleparz and in Plac Nowowiejski.

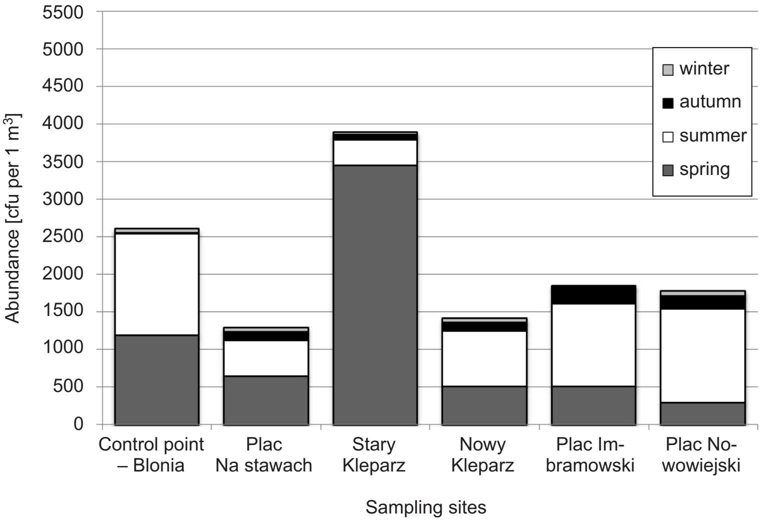

Fig. 2. The number of fungi on marketplaces in different seasons

According to Polish Standards [12] results of spring measurements revealed average mould fungi pollution of air only in Stary Kleparz.

Actinomycetes are widespread in the environment, especially in soil, on plant surface, or animals [16]. They were the least numerous among all studied microorganisms (Fig. 3), but their presence was found on each sampling site.

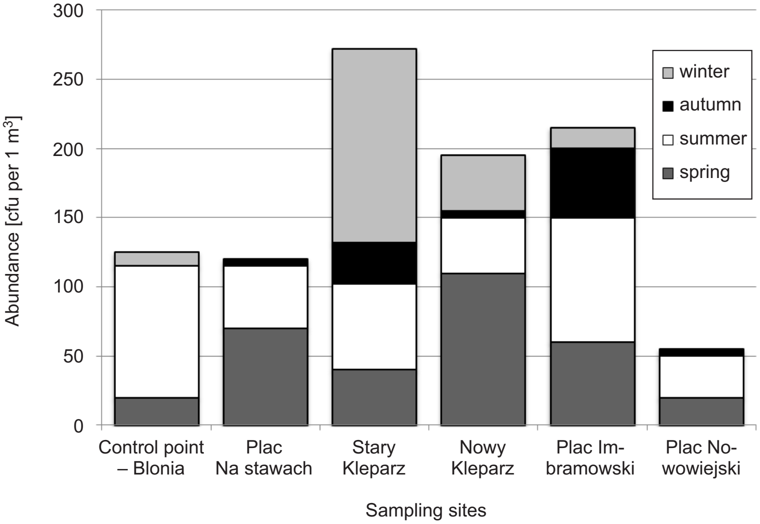

Fig. 3. The number of actinomyces on marketplaces in different seasons

Significant differences can be observed in abundance of actinomycetes – *eg* they occurred profusely on Stary Kleparz in winter (140 CFU/m^3), but they were absent on Plac Nowowiejski. Actinomycetes occurrence was frequent both in spring (Nowy Kleparz, Plac Na Stawach, Plac Imbramowski), and in summer (Plac Imbramowski, Błonia, Stary Kleparz). Their abundance was nearly twice lower on Plac Nowowiejski than on the control site, whereas opposite results were obtained on Stary Kleparz, where number of these microorganisms was over twice higher in relation to the control site. According to Marcinowska [16] actinomycetes abundance increases from spring to autumn and their highest numbers are detected in summer. This is consistent with the results obtained on Plac Imbramowski and Nowowiejski. Soil nearby the marketplaces or in flower pots or on vegetables can act as a source of actinomycetes.

According to Polish Standards [11] average actinomycetal air pollution was observed on all marketplaces as well as on the control site in spring and in summer; whereas heavy air pollution was observed on Nowy Kleparz in spring and on Stary Kleparz in winter.

Conclusions

1. On the studied marketplaces bacteria were the most numerous group of all microorganisms and mould fungi belonged to the second numerous group.

2. Quantity of the studied microorganisms showed seasonal variations. Bacteria were the most numerous in summer, whereas fungi and actinomycetes predominated in spring. In turn, minimum numbers of bacteria and fungi occurred in winter and minimum numbers of actinomycetes was detected in autumn.

3. Abnormally high number of actinomycetes was detected on Stary Kleparz (in winter) and on Nowy Kleparz (in spring).

4. Average air pollution occurred:

a. due to bacterial abundance: on Plac Na Stawach and on Plac Imbramowski in 3 seasons, on Stary Kleparz in 2 seasons,

b. due to fungal abundance: on Stary Kleparz in spring,

c. due to actinomycetal abundance: on all marketplaces in spring and in summer, on Stary Kleparz and on Plac Imbramowski in autumn, and on Stary and Nowy Kleparz and on Plac Imbramowski in winter.

5. The analysed marketplaces may be arranged in the following order of increasing total number of microorganisms: Plac Nowowiejski, Nowy Kleparz, Plac Na Stawach, Plac Imbramowski, Stary Kleparz.

References

[1] http://krakow.gazeta.pl/krakow/1,39245,4740286.html.

[2] Teltschande B, Katzenelson E. Airborne enteric bacteria and viruses from spray irrigation with wastewater. Appl Environ Microbiol. 1978;35:290-296.

[3] Lighthart B. Microbial aerosols: estimated contribution of combine harvesting to an airshed. Appl Environ Microbiol. 1984;47:430-432.

[4] Linderman J, Upper CD. Aerial dispersal of epiphytic bacteria over bean plants. Appl Environ Microbiol. 1985;50:1229-1232.

[5] Lighthart B, Shaffer BT. Bacterial flux from chaparral into the atmosphere in mid-summer at a high desert location. Atmos Environ. 1994;28:1267-1274.

[6] Wilson SC, Morrow-Tesch J, Straus DC, Cooley JD, Wong WC, Mitlöhner FM, McGlone JJ. Airborne microbial flora in a cattle feedlot. Appl Environ Microbiol. 2002;68:3238-3242.

[7] Guo H, Lee SC, Chan LY. Indoor air quality investigation at air-conditioned and non-airconditioned markets in Hong Kong. Sc Tot Environ. 2004;323:87-98.

[8] Hanashiro A, Morita M, Matté G R, Matté MH, Torres EAFS. Microbiological quality of selected street foods from a restricted area of São Paulo city, Brazil. Food Contr. 2005;16:439-444.

[9] Minami A, Chaicumpa W, Chongsa-Nguan M, Samosornsuk S, Monden S, Takeshi K, Makino S, Kawamoto K. Prevalence of foodborne pathogens in open markets and supermarkets in Thailand, Food Contr. 2010;21:221-226.

[10] Polska Norma, PN-89/Z-04008/08, Ochrona czystości powietrza. Pobieranie próbek powietrza atmosferycznego (imisja) do badań mikrobiologicznych metodą aspiracyjną i sedymentacyjną.

[11] Polska Norma, PN-89/Z-04111/02, Ochrona czystości powietrza. Badania mikrobiologiczne. Oznaczanie liczby bakterii w powietrzu atmosferycznym (imisja) przy pobieraniu próbek metodą aspiracyjną i sedymentacyjną.

[12] Polska Norma, PN-89/Z-04111/03, Ochrona czystości powietrza. Badania mikrobiologiczne. Oznaczanie liczby grzybów mikroskopowych w powietrzu atmosferycznym (imisja) przy pobieraniu próbek metodą aspiracyjną i sedymentacyjną.

[13] Donderski W, Walczak M, Pietrzak. Microbiological Contamination of Air within the City of Toruń. Pol J Environ St. 2005;14:223-230.

[14] Giorgio CD, Krempff A, Guiraud H, Binder P, Tiret C. Atmospheric pollution by air borne microorganisms in the city of Marseilles. Atmos Environ. 1996;30:155-160.

[15] Mędrela-Kuder E. Seasonal variations in the occurrence of culturable airborne fungi in outdoor and indoor air in Cracow, Int Biodeter Biodegr. 2002;52:203-205.

[16] Marcinowska K. Charakterystyka, występowanie i znaczenie promieniowców w przyrodzie. In: Aktywność Drobnoustrojów w Różnych Środowiskach. Kraków: Akademia Rolnicza im Hugona Kołłątaja; 2002;121-130.

MIKROBIOLOGICZNE ZANIECZYSZCZENIE POWIETRZA NA PLACACH TARGOWYCH W KRAKOWIE

Katedra Mikrobiologii, Katedra Ochrony Środowiska Rolniczego
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Badania mikrobiologiczne powietrza przeprowadzone zostały na pięciu placach targowych Krakowa i na miejskim terenie zieleni (Błoniach) – jako kontroli w różnych porach roku. Próbki powietrza pobierane były metodą zderzeniową za pomocą aspiratora MAS-100 firmy Merck. Liczebność bakterii w powietrzu badanych placów targowych wahała się od 42 do 2300 jtk/m^3, natomiast grzybów pleśniowych od 30 do 3460 jtk/m^3. Promieniowce występowały najrzadziej w ilościach od 0 do 140 jtk/m^3, lecz stwierdzone liczebności wynoszące ponad 100 jtk/m^3 również wskazywały na silne zanieczyszczenie powietrza atmosferycznego. Badania wykazały korelację występowania mikroorganizmów z porą roku. W okresie letnim maksimum swojego występowania miały bakterie, natomiast grzyby i promieniowce w okresie wiosennym.

Słowa kluczowe: mikroflora powietrza, plac targowy, Kraków, bakterie, grzyby, promieniowce

Lidia DĄBEK, Ewa OZIMINA
and Anna PICHETA-OLEŚ[1]

APPLYING THE COMBINED PROCESSES OF SORPTION AND OXIDATION TO REMOVE ORGANIC COMPOUNDS FROM AN AQUEOUS ENVIRONMENT USING THE EXAMPLE OF *p*-CHLOROPHENOL

ZASTOSOWANIE POŁĄCZONYCH PROCESÓW SORPCJI I UTLENIANIA DO USUWANIA ZWIĄZKÓW ORGANICZNYCH ZE ŚRODOWISKA WODNEGO NA PRZYKŁADZIE *p*-CHLOROFENOLU

Abstract: The efficiency of the removal of *p*-chlorophenol from an aqueous solution was tested to simulate the elimination of highly-hazardous soluble chloro-organic compounds from the environment. The methods selected to remove this pollutant were the consecutive and simultaneous processes of sorption and oxidation utilizing hydrogen peroxide and Fenton's reagent. Hydrogen peroxide was incapable of oxidizing *p*-chlorophenol, whereas in Fenton-driven oxidation the substance was decomposed immediately. Sorption on activated carbon proved to be an effective method for removing *p*-chlorophenol from an aqueous solution. Moreover, the *p*-chlorophenol-spent activated carbon was successfully regenerated by oxidation of the adsorbed substance applying hydrogen peroxide or Fenton's reagent and then reused as a sorbent. However, the regeneration process involved a considerable loss of mass of the activated carbon due to oxidation. A more effective method of removal of *p*-chlorophenol from an aqueous solution was oxidation with Fenton's reagent or hydrogen peroxide in the presence of activated carbon. Under such conditions, the processes of sorption and oxidation of the organic substance took place both in an aqueous solution and on the surface of the activated carbon, which contributed to the regeneration and reuse of the carbon.

Keywords: activated carbon, oxidation, sorption, hydrogen peroxide, *p*-chlorophenol

Pollution of the environment due to the release of organic compounds is still a serious problem despite the introduction of increasingly restrictive environmental

---

[1] Faculty of Civil and Environmental Engineering, Kielce University of Technology, al. Tysiąclecia Państwa Polskiego 7, 25–314 Kielce, Poland, phone: +48 41 342 45 35, fax: +48 41 344 29 97, email: ldabek@tu.kielce.pl

standards. The main reasons for such concern are high toxicity of these substances and numerous sources of their emission [1, 2]. Organic pollutants include chloro-organic compounds, which can be found in industrial and municipal waste arising from the activities of the chemical, petrochemical, pharmaceutical, paper-making and other manufacturing industries, agriculture (*eg* crop protection chemicals) and households (*eg* household chemicals). Since most chloro-organic compounds are well-soluble in water, they can easily migrate in the environment, posing a considerable threat to it. It is thus necessary that their emission be limited. In this context, treatment of industrial wastewater containing chloro-organic compounds is particularly important.

Treating industrial wastewater effectively and efficiently requires applying various methods, sometimes combined, because of the highly complex chemical composition of its organic constituents [3]. One of the solutions that much attention has recently been paid to is to apply sorption using activated carbon as a sorbent [4–6] and oxidation, with the latter being frequently carried out using *Advanced Oxidation Processes* (AOPs), where the oxidants are ozone, hydrogen peroxide and Fenton's reagent and the process is performed in the presence or absence of UV irradiation. The common feature of these methods is the formation of hydroxyl radicals in the reaction environment, which are some of the strongest radicals, having an oxidizing potential of 2.7 V [1]. The processes of sorption and oxidation with the AOP methods are highly efficient and effective. However, there may be a problem with disposal of spent sorbents, considerable amounts of oxidants required and a consequent increase in the volume of wastewater to be treated. Combining the two processes into a simultaneous event is being considered as an alternative [7, 8]. In such a case, organic compounds are removed in a two- or one-stage process, *ie* consecutive sorption and oxidation, which involves regeneration of activated carbon, or simultaneous sorption and oxidation, with the latter being a more favourable solution. The literature [7–9] suggests that activated carbon not only acts as a sorbent but also catalyzes the decomposition of oxidants, which contributes to the formation of hydroxyl radicals. Despite numerous studies on the subject [7, 8, 10], it is still unclear how the process proceeds. Some researchers [7] assume that organic compounds are first adsorbed on activated carbon, and then oxidized. Others, however, *eg* [10] claim that the catalytic role of activated carbon is the most significant, as it contributes to the formation of hydroxyl radicals in the reaction environment.

This paper provides a comparative analysis of the course and efficiency of the process of removal of soluble chloro-organic compounds from aqueous solutions, through sorption and oxidation, using the example of *p*-chlorophenol. Activated carbon, WD extra, was used as a sorbent. The process of oxidation was conducted using hydrogen peroxide or Fenton's reagent. The testing involved sorption of *p*-chlorophenol on activated carbon, followed by regeneration of the spent sorbent by means of hydrogen peroxide or Fenton's reagent so that it could be used again. The results were compared with those obtained for *p*-chlorophenol removed from an aqueous solution through simultaneous sorption and oxidation. The process was performed twice, using the same amount of activated carbon.

Materials and methods

The materials used for the analysis were:

– commercially available *granular activated carbon* (GAC), WD extra, produced by Gryfskand, whose physical and chemical parameters, and pore structure are shown in Tables 1 and 2,

– aqueous solution of *p*-chlorophenol with a concentration of 1 g/dm^3.

Table 1

Physical and chemical properties of the WDextra activated carbon [7]

Parameter	Value
Surface area [m^2/g]	1050
Bulk density [g/dm^3]	415
Water adsorption capacity [cm^3/g]	0.80
Mechanical strength [%]	96
Abrasion resistance [%]	0.5
Ash content [%]	21.8
Methylene blue index (MBI)	36
Iodine value [mg/g]	980

Table 2

Capillary pore distribution in WD extra carbon [7]

WD extra	Capillary pore radius [nm]					ΣΔV
	< 1.5	1.5–15	15–150	150–1500	1500–7500	
	Capillary pore volume, ΔV					
[cm^3/g]	0.3048	0.1727	0.1230	0.2716	0.0872	0.9530
[%]	32.0	18.1	12.9	28.5	8.5	100

1. Determining the parameters of the porous structure

The porous structure of the material was assessed by performing low-temperature adsorption of nitrogen (at 77 K). The adsorption and desorption isotherm was established with a volumetric method, using a Fisons Sorptomatic 1900 and a Micromeritics ASAP 2010. The dechlorination efficiency, the iodine value and the methylene blue index were estimated according to DIN 19603, PN-83/C-97555.04, and PN-82/C-97555.03, respectively.

2. Sorption isotherm

The isotherm of sorption of *p*-chlorophenol on activated carbon, WD extra, was determined for a system of 0.5 g of activated carbon and 100 cm^3 of *p*-chlorophenol solution with concentrations ranging from 200 to 1000 mg/dm^3. The time of contact required to achieve the adsorption equilibrium in the *p*-chlorophenol – WD extra system was 4 hours. The sorption was conducted at room temperature.

3. Removing *p*-chlorophenol from a solution

a) Sorption. 1 g samples of activated carbon, WD extra, were placed in conical flasks and treated with 300 cm^3 of an aqueous solution of *p*-chlorophenol with a concentration of 0.8 g/dm^3. The contents were stirred intensively. After 10, 30, 60 and 120 minutes, the sorption was stopped and the amount of *p*-chlorophenol remaining in the solution was determined.

b) Oxidation

– H_2O_2. 300 cm^3 of *p*-chlorophenol solution with a concentration of 0.8 g/dm^3 was treated with NaOH to obtain a pH of 8. Then, H_2O_2 was added, with the amount ensuring stoichiometric oxidation of *p*-chlorophenol. The contents were stirred intensively. After 10, 30, 60 and 120 minutes, a sample of the oxidizing mixture was taken and analyzed for the content of *p*-chlorophenol.

– *in Fenton's reaction* $Fe^{2+}/\ H_2O_2$. 300 cm^3 of *p*-chlorophenol solution with a concentration of 0.8 g/dm^3 was treated with H_2SO_4 to obtain a pH of about 4. Subsequently, H_2O_2 and iron(II) sulphate(VI) ($FeSO_4 \cdot 7H_2O$) were added, the former in the amount ensuring stoichiometric oxidation of *p*-chlorophenol). The $Fe^{2+}/\ H_2O_2$ ratio by weight (corresponding to the quantity of Fe^{2+}) was 1:5. The contents were stirred intensively. After 10, 30, 60 and 120 minutes, a sample of the oxidizing mixture was taken and the amount of the remaining *p*-chlorophenol was determined.

c) Sorption + oxidation

– *WD extra +* H_2O_2. 300 cm^3 samples of *p*-chlorophenol solution with a concentration of 0.8 g/dm^3 were weighed into conical flasks and treated with NaOH to obtain a pH of 8. Then, 1 g of activated carbon, WD extra, and H_2O_2 were added. The amount of the latter had to be adjusted to ensure stoichiometric *p*-chlorophenol oxidation. The contents of the flasks were shaken intensively. After 10, 30, 60 and 120 minutes, the oxidation was stopped and the amount of *p*-chlorophenol remaining in the reaction mixture was determined.

– *WD extra +* $Fe^{2+}/\ H_2O_2$. 300 cm^3 samples of *p*-chlorophenol solution with a concentration of 0.8 g/dm^3 were placed in conical flasks and treated with H_2SO_4 to obtain a pH of about 4. Subsequently, activated carbon (WD extra), H_2O_2 and iron(II) sulphate(VI) ($FeSO_4 \cdot 7H_2O$) were introduced into the solution, the first in the amount of 1 g, the second in the amount ensuring stoichiometric oxidation of *p*-chlorophenol and the third in the Fe^{2+}/H_2O_2 ratio by weight (corresponding to the quantity of Fe^{2+}) of 1:5. The flasks were then shaken intensively. After 10, 30, 60 and 120 minutes, oxidation was stopped and the amount of *p*-chlorophenol remaining in the reaction mixture was determined.

4. Regenerating *p*-chlorophenol-spent activated carbon

– *H_2O_2*. 5 g samples of activated carbon, WD extra, saturated with *p*-chlorophenol, were treated with 500 cm^3 of water. NaOH was added to achieve a pH of 8. Then, the solution was treated with H_2O_2, in the amount ensuring stoichiometric oxidation of *p*-chlorophenol. The contents were stirred for 20 minutes at room temperature and then the carbon was rinsed five times using 100 cm^3 of distilled water. The activated carbon regenerated with hydrogen peroxide was represented by the symbol WD extra/H_2O_2.

– *Fe^{2+}/H_2O_2*. 5 g samples of activated carbon, WD extra, saturated with *p*-chlorophenol, were treated with 500 cm^3 of water. The pH-value was reduced to about 4 by adding H_2SO_4. Then, H_2O_2 and iron(II) sulphate(VI) ($FeSO_4 \cdot 7H_2O$) were introduced into the reaction system, the former in the amount ensuring stoichiometric oxidation of *p*-chlorophenol. The Fe^{2+}/H_2O_2 ratio by weight (corresponding to the quantity of Fe^{2+}) was 1:5. The contents were stirred intensively for 20 minutes, and then the carbon was rinsed five times using 100 cm^3 of distilled water. The activated carbon regenerated using a Fenton-driven mechanism was represented by the symbol WD extra/Fe^{2+}/H_2O_2.

5. Determining the concentration of *p*-chlorophenol and chloride ions

The concentration of *p*-chlorophenol was determined using a chromatographic method by means of a Thermo Scientific Focus GC gas chromatograph. Prior to that, liquid to liquid extraction of the analyte was conducted using xylene according to the procedure developed by the authors in order to extract *p*-chlorophenol and other by-products of the oxidation taking place in the solution. The analyte recovered from the aqueous solution amounted to 92 %. The quantity of chloride ions, Cl^-, was determined by volumetric titration with a voltometer being part of a Behr CL10 AOX analyzer [11, 12].

Discussion of results

The analysis was conducted for activated carbon, WD extra, characterized by a large surface area of 1050 m^2/g, a pore volume of 0.95 cm^3/g, a micropore volume of 0.30 cm^3/g, an iodine value of 990 mg/g, a methylene blue index of 36 cm^3, and a detergent value of 21 mg/g (Tables 1 and 2). These parameters indicate that the selected activated carbon possesses high sorptive capacity. With regard to *p*-chlorophenol, it was 150 mg/g (Fig. 1). The carbon had a low dechlorination index of 2 cm, which was an evidence of good catalytic capacity with regard to the decomposition and reduction of chlorates to chloride ions, and, indirectly, also the decomposition of hydrogen peroxide, which contributes to the formation of hydroxyl radicals.

Activated carbon, WD extra, saturated with *p*-chlorophenol, was regenerated by oxidizing the adsorbed substance using hydrogen peroxide or Fenton's reagent. Prior to

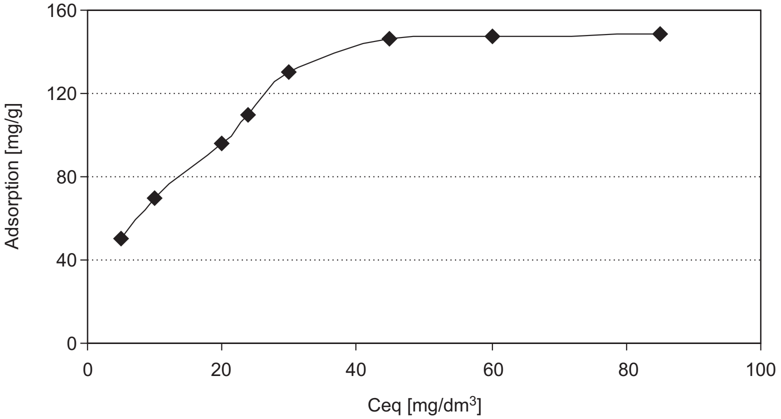

Fig. 1. Isotherm of sorption of *p*-chlorophenol from an aqueous solution on activated carbon, WD extra

that, it was essential to check the oxidation capacity of *p*-chlorophenol using H_2O_2 and Fenton's reagent. As shown in Fig. 2, the Fenton's reaction in which the hydroxyl radicals acted as the oxidizing agent led to immediate decomposition of this substance.

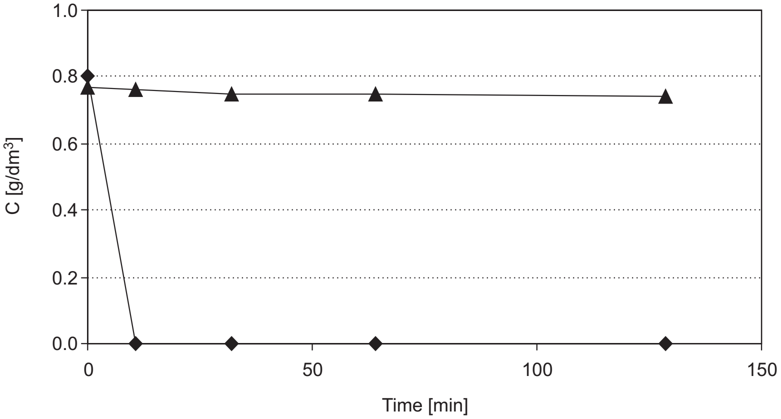

Fig. 2. Changes in the concentration of *p*-chlorophenol for both oxidants in the function of oxidation time

It was found that *p*-chlorophenol was not oxidized in the presence of hydrogen peroxide even though optimal reaction conditions were satisfied. From the literature [8, 11] it is clear, however, that the activated carbon catalyzes the decomposition of H_2O_2, which contributes to the formation of $OH^\bullet$ radicals. However, *p*-chlorophenol adsorbed on activated carbon was expected to be decomposed in the presence of Fenton's reagent or hydrogen peroxide, the result of which would be the regeneration of activated carbon. The regeneration efficiency was assessed by analyzing the changes in the concentration of chloride ions in an aqueous solution, being a product of the decomposition of *p*-chlorophenol, as well as the sorptive capacity of the regenerated activated carbon (Figs. 3 and 4). The contact of the carbon surface with the adsorbed *p*-chlorophenol resulted in gradual decomposition of *p*-chlorophenol in the oxidant solution, the evidence of which was an increase in the amount of chloride ions. After 10 minutes of contact, the chloride ions released into the solution amounted to 25.5 % of the adsorbed *p*-chlorophenol. After 30 minutes, the decomposition increased to 44 %. Extending the reaction time fourfold gave rise to a slight increase in the degree of decomposition of up to about 48 %, in the case of Fenton's reagent. It should be noticed that the decomposition efficiency of the *p*-chlorophenol adsorbed on carbon was comparable for both oxidants. However, the reaction in a solution was different in nature. The $OH^\bullet$ radicals responsible for the oxidation of organic compounds in a WD extra/*p*-chlorophenol/H_2O_2 system were generated in the same way as in the Fenton's reaction. Neither of the oxidants caused complete decomposition of the adsorbed *p*-chlorophenol. The decomposition efficiency could not be improved either by increasing the amount of the oxidant or extending the contact time. A side effect of the oxidation was a considerable loss of the carbon mass (approx. 20 %).

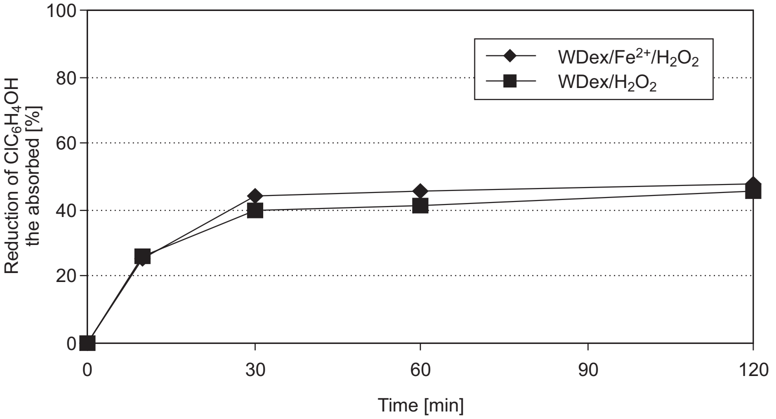

Fig. 3. Efficiency of the reduction of the adsorbed *p*-chlorophenol according to the oxidizing agent

The regenerated activated carbons were reused as sorbents to remove *p*-chlorophenol from an aqueous solution (contact time – 120 min). The results in Fig. 4 show that the removal efficiency of regenerated carbons, WD extra/H_2O_2-I and WD extra/Fe^{2+}/H_2O_2-I, with regard to *p*-chlorophenol removed from a solution was only slightly lower than that of the virgin carbon, despite incomplete decomposition of the originally adsorbed *p*-chlorophenol. The sorptive capacity of the carbon regenerated with H_2O_2 or Fenton's reagent was approx. 105 mg/g, whereas the capacity of the virgin carbon, WD extra, amounted to about 132 mg/g. Since the sorption of *p*-chlorophenol was still high and satisfactory, the carbons were regenerated again under the same conditions, to be reused as sorbents of *p*-chlorophenol. The removal efficiency of the twice-regenerated carbons, WD extra/H_2O_2-II or WD extra/Fe^{2+}/H_2O_2-II, with regard to *p*-chlorophenol,

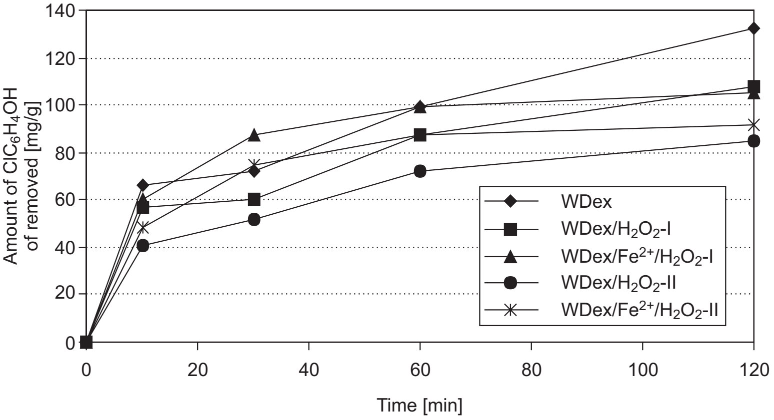

Fig. 4. Efficiency of the removal of *p*-chlorophenol from a solution for virgin carbon, WD extra, and regenerated carbons, WD extra/H_2O_2 and WD extra/Fe^{2+}/H_2O_2

was approx. 15% lower than that obtained for the once-regenerated carbon, but still high, reaching approx. 90 mg/g after the same contact time. The results indicate that the consecutive cyclic processes of sorption of *p*-chlorophenol and regeneration of activated carbon are effective and might be considered satisfactory except for the fact that every subsequent regeneration involves a loss of carbon mass. Applying many sorption/regeneration cycles is rather difficult. It should be emphasized that the problem has not been discussed in the literature on the subject.

The simultaneous processes of sorption and oxidation aiming at the removal of organic compounds from aqueous solutions are an alternative to the cyclic consecutive sorption/regeneration method. They allow regeneration of spent carbons and their reuse as sorbents. The analysis was performed using *p*-chlorophenol with a concentration identical to that observed during sorption, activated carbon, WD extra, and H_2O_2 or Fenton's reagent, the concentration of which was the same as for carbon regeneration. The carbon efficiency to remove *p*-chlorophenol from a solution was assessed by analyzing the changes in the concentration of *p*-chlorophenol and the amount of Cl^- ions, whose presence indicated oxidation of the substance (Table 3 and Fig. 5). In the presence of activated carbon, Fenton's reagent caused almost immediate reduction in the concentration of *p*-chlorophenol in the solution, accompanied by the formation of chloride ions, the amount of which ensured stoichiometric, *ie* total oxidation of *p*-chlorophenol (Table 3). The analysis performed under the same conditions for H_2O_2 (WD extra+H_2O_2-1) showed a 31 % loss of *p*-chlorophenol (72 mg/g) after 10 minutes of the reaction. 83 % of this was oxidized (which corresponded to the amount of chloride ions occurring in the WD extra+H_2O_2/Cl-1 solution), whereas the other 17 % of the removed *p*-chlorophenol was adsorbed on activated carbon. The oxidation to sorption ratio was assessed to be 83/17. Extending the reaction time up to 30 minutes resulted in the removal of 91 mg/g of *p*-chlorophenol, 76 % of which was oxidized and the rest was adsorbed on carbon. The proportion of sorption to oxidation increased in time and after 120 minutes it was 41 %.

Table 3

Efficiency of the removal of *p*-chlorophenol per 1 g WD extra in simultaneous oxidation/sorption in two consecutive cycles

Reaction time [min]	WD extra + Fe^{+2}/H_2O_2		WD extra + H_2O_2 – cycle I			WD extra + H_2O_2 – cycle II		
	ClC_6H_4OH removed [mg]	ClC_6H_4OH oxidized [%]	ClC_6H_4OH removed [mg]	ClC_6H_4OH oxidized [%]	ClC_6H_4OH remaining on WD extra (adsorbed) [%]	ClC_6H_4OH removed [mg]	ClC_6H_4OH oxidized [%]	ClC_6H_4OH remaining on WD extra (adsorbed) [%]
10	240	100	72	83	17	59	54	46
30	—	—	91	76	23	65	60	40
60	—	—	105	68	32	90	46	54
120	—	—	138	60	40	99	43	57

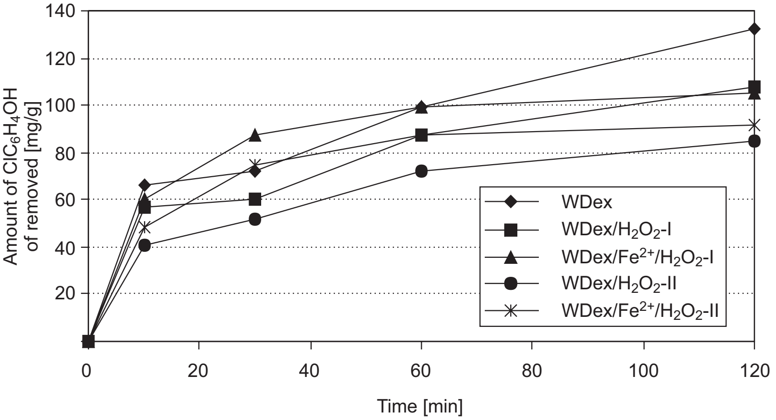

Fig. 5. Comparison of the amount of *p*-chlorophenol removed from a solution on 1 g of activated carbon, WD extra, in the presence of hydrogen peroxide in two successive cycles, WD extra/H_2O_2-1 and WD extra/H_2O_2-2, with the amount of oxidized *p*-chlorophenol estimated based on the concentration of Cl^- ions in the WD extra/H_2O_2/Cl-1 and WD extra/H_2O_2/Cl-2 solutions

As shown in Fig. 5, the activated carbon regenerated by simultaneous removal of *p*-chlorophenol using H_2O_2 was reused for another *p*-chlorophenol removal process (WD extra+H_2O_2-2 and WD extra+H_2O_2/Cl-2). The results indicate that the removal efficiency was about 35 % lower, which can be explained by the partial immobilization of the non-oxidized *p*-chlorophenol adsorbed at the carbon surface. The analysis of the reaction course shows that the removal efficiency with regard to *p*-chlorophenol in the subsequent simultaneous sorption/oxidation was increasingly high; after 120 minutes, it was 57 %.

The total amount of *p*-chlorophenol removed from a solution by double sorption and regeneration of activated carbon with hydrogen peroxide or Fenton's reagent is comparable with the amount removed by simultaneous sorption, oxidation and regeneration in a solution. In the latter case, however, there was no considerable mass loss of activated carbon (with a total loss of carbon of up to 10 %), as was the case during sorption and regeneration, where the loss was 20 % per cycle. The process thus contributes to an increase in the useful life of activated carbon.

Conclusion

The analysis shows that the selected activated carbon, WD extra, possesses high sorptive efficiency with regard to *p*-chlorophenol – 150 mg/g. The decomposition of *p*-chlorophenol by means of hydrogen peroxide under the pre-determined conditions resulted in no oxidation of this substance. Using a Fenton reaction was a more effective method of removing *p*-chlorophenol. Its drawbacks, however, included the formation of considerable amounts of iron compounds in the reaction system and a decrease in the pH-value. An alternative solution was to combine the processes of sorption and oxidation involving regeneration of activated carbon consecutively or simultaneously.

The sorptive capacity of the activated carbon regenerated with H_2O_2 or Fenton's reagent was found to be similar to that of virgin carbon. However, the process of regeneration resulted in a significant loss of mass of the activated carbon, especially when Fenton's reagent was used (approx. 20 %). The results for the removal of *p*-chlorophenol from an aqueous solution in the simultaneous processes of sorption and oxidation show that oxidation was the predominant process and included the decomposition of both *p*-chlorophenol and the activated carbon, which indicates the presence of chloride ions and a significant loss of the carbon mass. By extending the reaction time, it is possible to increase the proportion of sorption to oxidation for the removal of *p*-chlorophenol from a solution.

Acknowledgements

The authors acknowledge support from the Ministry of Science and Higher Education (project No. N N205 1993 33) and the Kielce University of Technology (statutory project No. 2.24/5.02).

The expenses of the conference participation of the co-author Anna Picheta-Oleś, a PhD student, have been paid from the funds of the Programme for the Development of the Educational Potential of the Kielce University of Technology: Education for Success, Agreement UDA-POKL.04.01.01-00-175/08-02, co-financed by the EU European Social Fund, Priority IV, Measure 4.1, Action 4.1.1. The student is a holder of a grant co-financed by the EU European Social Fund within the above mentioned project.

References

[1] Świderska R, Czerwińska M, Kutz R. Utlenianie zanieczyszczeń organicznych za pomocą odczynnika Fentona (Oxidation of organic pollutants using Fenton's reagent). VII Ogólnopolska Konferencja Naukowa pt.: „Kompleksowe i szczegółowe problemy inżynierii środowiska". ZN Politechniki Koszalińskiej, Koszalin: 2005;22:1-12.

[2] Wąsowski J, Piotrowska A. Rozkład organicznych zanieczyszczeń wody w procesach pogłębionego utleniania (Decomposition of organic pollutants using the advanced oxidation processes). Ochr Środow. 2002;2:27-32.

[3] Barbusiński K. Oczyszczanie ścieków przemysłowych metodami katalitycznymi z wykorzystaniem nadtlenku wodoru (Treatment of industrial waste water with catalytic methods using hydrogen peroxide). Chemik. 2001;2:31-36.

[4] Bansal RCh, Goyal M. Activated Carbon Adsorption. Baca Raton-London-New York-Singapore: Taylor & Francis Group; 2005.

[5] Gupta VK, Suhas V. Application of low-cost adsorbents for dye removal – A review. J Environ Manage. 2009;90:2313-2342.

[6] Santos VP, Pereira MFR, Faria PCC, Órfão JJM. Decolourisation of dye solutions by oxidation with H_2O_2 in the presence of modified activated carbons. J Hazard Mater. 2009;162:736-742.

[7] Toledo LC, Silva ACB, Augusti R, Lago RM. Application of Fenton's reagent to regenerate activated carbon saturated with organochloro compounds. Chemosphere. 2003;50:1049-1054.

[8] Huang HH, Lu MC, Chen JN, Lee CT. Catalytic decomposition of hydrogen peroxide and 4-chlorophenol in the presence of modified activated carbons. Chemosphere. 2003;51(9):935-943.

[9] Dąbek L, Ozimina E, Picheta-Oleś A. Sorptive and catalytic properties of activated carbon used for the removal of crystal violet from an aqueous solution in the presence of hydrogen peroxide. Ecol Chem Eng A. 2010;17(11):1423-1433.

[10] Mourand JT, Crittenden JC,Hand DW, Perram DL. Notthakun S.: Regeneration of spent adsorbents using homogeneous advanced oxidation. Water Environ Res. 1995;67(3):355-363.

[11] Vidic RD, Suidan MT, Sorial GA, Brenner RC. Effect of molecular oxygen on adsorptive capacity and extraction efficiency of granular activated carbon for three ortho-substituted phenols, J Hazard Mater. 1994;38:373-388.

ZASTOSOWANIE POŁĄCZONYCH PROCESÓW SORPCJI I UTLENIANIA DO USUWANIA ZWIĄZKÓW ORGANICZNYCH ZE ŚRODOWISKA WODNEGO NA PRZYKŁADZIE *p*-CHLOROFENOLU

Katedra Inżynierii i Ochrony Środowiska, Wydział Budownictwa i Inżynierii Środowiska
Politechnika Świętokrzyska w Kielcach

Abstarakt: W prezentowanej pracy prowadzono badania nad skutecznością usuwania *p*-chlorofenolu z roztworu wodnego, jako symulację eliminowania ze środowiska łatwo rozpuszczalnych związków chlorowcoorganicznych stanowiących jedno z groźniejszych zanieczyszczeń środowiska. Jako metody usuwania wybrano sorpcję, utlenianie nadtlenkiem wodoru i odczynnikiem Fentona oraz symultanicznie realizowany proces sorpcji i utleniania nadtlenkiem wodoru. Wykazano, że nadtlenek wodoru nie utlenia *p*-chlorofenolu, natomiast w reakcji Fentona następuje natychmiastowy rozkład tej substancji. Stwierdzono, że sorpcja na węglu aktywnym jest skutecznym sposobem usuwania *p*-chlorofenolu z roztworu wodnego. Ponadto, zużyty węgiel aktywny można z powodzeniem zregenerować poprzez utlenienie zaadsorbowanej substancji nadtlenkiem wodoru lub odczynnikiem Fentona i ponownie wykorzystać jako sorbent. Jednakże proces regeneracji łączy się ze znaczną stratą węgla aktywnego na skutek jego utlenienia. Znacznie skuteczniejszym rozwiązaniem jest usuwanie *p*-chlorofenolu z roztworu wodnego poprzez utlenienie odczynnikiem Fentona lub nadtlenkiem wodoru w obecności węgla aktywnego. W tych warunkach ma miejsce tak proces sorpcji, jak i utleniania substancji organicznych zarówno w roztworze wodnym, jak i zaadsorbowanych na węglu aktywnym, co równocześnie skutkuje jego regeneracją i umożliwia ponowne wykorzystanie.

Słowa kluczowe: węgiel aktywny, utlenianie, sorpcja, nadtlenek wodoru, *p*-chlorofenol

DOI: 10.2428/ecea.2012.19(03)030 ECOL CHEM ENG A. 2012;19(3):287-294

Dorota MIROSŁAW-ŚWIĄTEK[1] and Marlena ŻELAZOWSKA[1]

INFLUENCE OF LAND USE ON FLOOD WAVE PROPAGATION IN THE LOWER BIEBRZA BASIN

WPŁYW SPOSOBU UŻYTKOWANIA DOLINY NA PRZEPŁYW FALI WEZBRANIOWEJ W BASENIE DOLNYM BIEBRZY

Abstract: The status of riparian wetlands depends on water conditions, especially the main role is played by flooding which occurs almost every year on wetland areas. The aim of this work is the evaluation of the influence of land use on flood wave propagation in the Biebrza River floodplain. An 1D numerical model of river flow was applied to the Biebrza River floodplains covered with reeds, sedges, grasses, bushes, and trees. Various scenarios of land use with such activities as extensive agriculture, willow scrubs and birch trees cutting or no activity (natural succession) were analyzed to evaluate the influence of changes in vegetation structure on the flood wave propagation in the river valley. Simulations were performed for the maximum flood event in 1979 in the Biebrza Basin. The results were presented for selected river cross-sections.

The obtained results show the influence of floodplain vegetation type on the water stage hydrographs and flood duration in the Lower Biebrza Basin.

Keywords: 1D hydrodynamic model, The Lower Biebrza River Basin, Numerical Terrain Model, flood wave

The status of riparian wetlands is mostly connected with flooding and the appearing structure of valuable ecosystems here depends on annual floods of defined duration and frequency. The most important flood characteristics of plant communities development are: flooding area, flooding frequency, and average depth of flooding [1–3]. It indicates that there is a very strong relationship between a vegetation structure and water conditions [2]. On the other hand, floodplain vegetation significantly affects flood extent in the valley. This question was a main task of this paper. The influence of the different floodplain land use, described by changes in vegetation structure, on flood wave propagation in the *Lower Biebrza River Basin* (LBRB) was analyzed. The Biebrza River valley is an extremely valuable wetland site of a global significance protected by Ramsar Convention and annual flooding influence formation of a unique character of

---

[1] Department of Hydraulic Engineering and Environmental Restoration, Warsaw University of Life Sciences – SGGW, ul. Nowoursynowska 159, 02–776 Warszawa, Poland, email: dorotams@levis.sggw.pl

the study site. A zonal system of various plants reflects water conditions of the LBRB [4–6] and causes spatial division of the resistance to water flow.

Materials and methods

Study site

The Biebrza River valley is situated in the Northeast of Poland and designated as a wetland site of a global significance, which is under protection of Ramsar Convention. The analysed area is located in the Lower Biebrza River Basin (LBRB) is an ice-marginal valley where the floodplain (21 000 ha) is a predominant morphologic structure covered by mosaic vegetation starting from sedge, sedge-moss and reed communities to willow shrubs, black alder forest, swampy birch and peat coniferous forest (Fig. 1). In the LBRB the river valley is 30 km long and 12–15 km wide. The Biebrza River is the main channel of the hydrodynamic network of the Lower Basin and it is about 50 km long [7]. The river course winds and forms meanders, side streams, and old riverbeds which area activates only during periods of high water flows. The width of the river channel varies from 20 to 35 m.

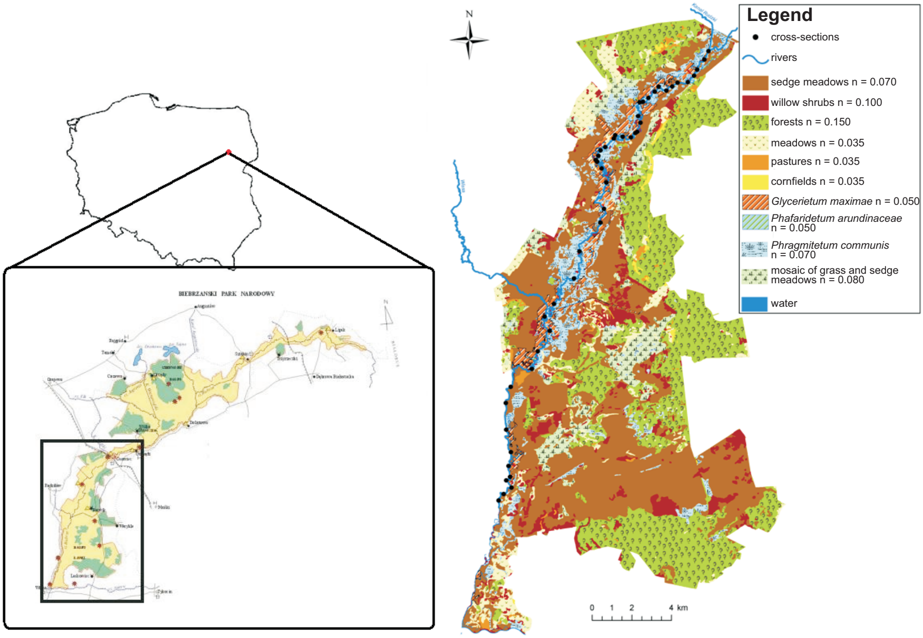

Fig. 1. Location of the Lower Biebrza River Basin (LBRB) and plant characteristic described by Manning's coefficient (*n* value) used in this study

The LBRB hydrological conditions, described by discharges calculated in upstream and downstream gauges Osowiec and Burzyn for maximum of annual discharges for a long term period from 1965 to 1996, are presented in Table 1.

Table 1

Discharges in Osowiec and Burzyn gauges calculated for a long term period from 1965 to 1996; *maximum of maximum* (WWQ), *mean of maximum* (SWQ) and *minimum of maximum* (NWQ) of annual discharges

Discharge	$Q_{Osowiec}$	Q_{Burzyn}
	$[m^3 \cdot s^{-1}]$	
WWQ	360.00	517.00
SWQ	82.06	131.89
NWQ	17.20	36.00

The most valuable ecosystems here are not only natural areas of peatlands but also large areas of open semi-meadows, which are the result of extensive agriculture use. The hydrogenic dependent habitats in the Biebrza marshes run on a stable ground water inundation or river flooding which occurs regularly and reinforce these representative water ecosystems every year. Changes of water conditions and discontinuance of extensive agriculture causes transformation of meadows and pastures into tall herb vegetation, reed and in the end the succession of shrubs and forest upon non-forest ecosystems of peatlands which can be observed in some places in the Biebrza River valley.

Scenarios determination

Various types of land use were determined for three scenarios and two of them present protective activities carried out nowadays in the National Park, which consist of preservation of open grasslands by mowing, elimination of biomass in selected areas, and mechanical scrubs cutting. In the first analyzed scenario (scenario I), wet meadows, sedge plant communities, and pastures are areas of an extensive agriculture. In this scenario, these areas are mowed for cattle bedding, used for hay production and as feeding areas. In the second scenario (scenario 2), there are willow scrubs and birch trees cutting, in addition to the former scenario. The overgrowing process in many cases seem to be a natural succession so the third scenario (scenario 3) allows the natural succession of willow shrubs and birch forest upon non-forest ecosystems in the valley.

Hydrodynamic model

In this work, a one-dimensional unsteady open-channel flow model UNET (One-Dimensional Unsteady Flow Through a Full Network of Open Channels) was applied to simulate flow in the LBRB. This program is a component of computer software HEC-RAS and it is a numerical solution of the mathematical model of river water flow based on the St. Venant equations [8]. In the topological discretization scheme of the flow, the LBRB and its floodplain are represented as a one-dimensional channel from Osowiec gauge (BD1) to Burzyn gauge (BD17). A geometry of the river channel and floodplain is described by 47 river cross-sections. The cross-sections were measured by manual sounding for the main channel part and the topography of the floodplain was calculated from the Digital Elevation Model [9]. A vegetation map was used to define spatial hydraulic roughness which was described by Manning's coefficient (n value) using Chow's tables [10], in the floodplain (Fig. 1) [11], and n values for the river channel were selected during the calibration process of the model. The LBRB model was calibrated and verified for the measured data and historical data of flood events in 1979, 1992, 1999. The upstream boundary condition is located at BD1 and formed by a flow hydrograph which is a sum of discharges at the Przechody and Osowiec gauges. The rating curve is used as a downstream boundary condition at the Burzyn gauge (BD17) [9]. The Wissa River is treated as a point lateral inflow and described by the flow hydrograph at the Czachy gauge.

The numerical calculations for the three scenarios [12, 13], which show changes in the land use, were made for the maximum flood event in the LBRB in 1979. In scenario I, Manning's coefficients for wet meadows, tall sedge plant communities, and pastures were given 0.035 n value. In the model scenario II, it was described by 0.035 coefficient for the floodplain area with willow scrubs. In the last simulation (scenario 3), Manning's coefficient for the study site was increased to 0.10 because of natural succession in this area.

Results and discussion

The results were analyzed in four river cross-sections which represent a variety of the hydromorphological shape of the main channel and floodplain in the Lower Biebrza River Basin. The distances from BD17, where the river course ends, to the selected cross-sections are 7.62 km, 29.62 km, 33.04 km and 39.96 km. Figures 2 and 3 show the calculated hydrographs of water stages and water stage profile of the maximum flood event of 3 April 1979 for the various land use scenarios.

The average depth in scenario I and scenario II was decreased by 0.25 m compared with the actual state (scenario 0). In scenario III, the average depth was increased by 0.20 m. Some differences in the water stage at the outlet (near BD17) are related to the downstream boundary condition which is formed by the rating curve. In computations, the influence of the vegetation structure on the rating curve was excluded which results in lack of influence of a land use type on a calculated water stage at BD17. Table 2 shows an average water depth in the floodplain, top flood width, and flood duration for the various land use scenarios.

The results for scenario I and scenario II are similar, because of willows scrubs covering a small area in the valley (Fig. 1). The highest changes of flooding time were calculated for P4 localized in the northern part of the Lower Basin, where the width of the river valley is lower than in the middle and southern parts of the research area. Time of flood in scenario I and II, when the resistance of flow is lower, was decreased by about 40 %. When the resistance of flow is higher because of growing vegetation (scenario III), flood duration was increased by 17 % compared with the actual state. An increasing valley width resulted in decreased flooding for scenarios I and II by 8 to 6 % along the river from P17 to BD14. In scenario III, flood duration was increased by 5, 7,

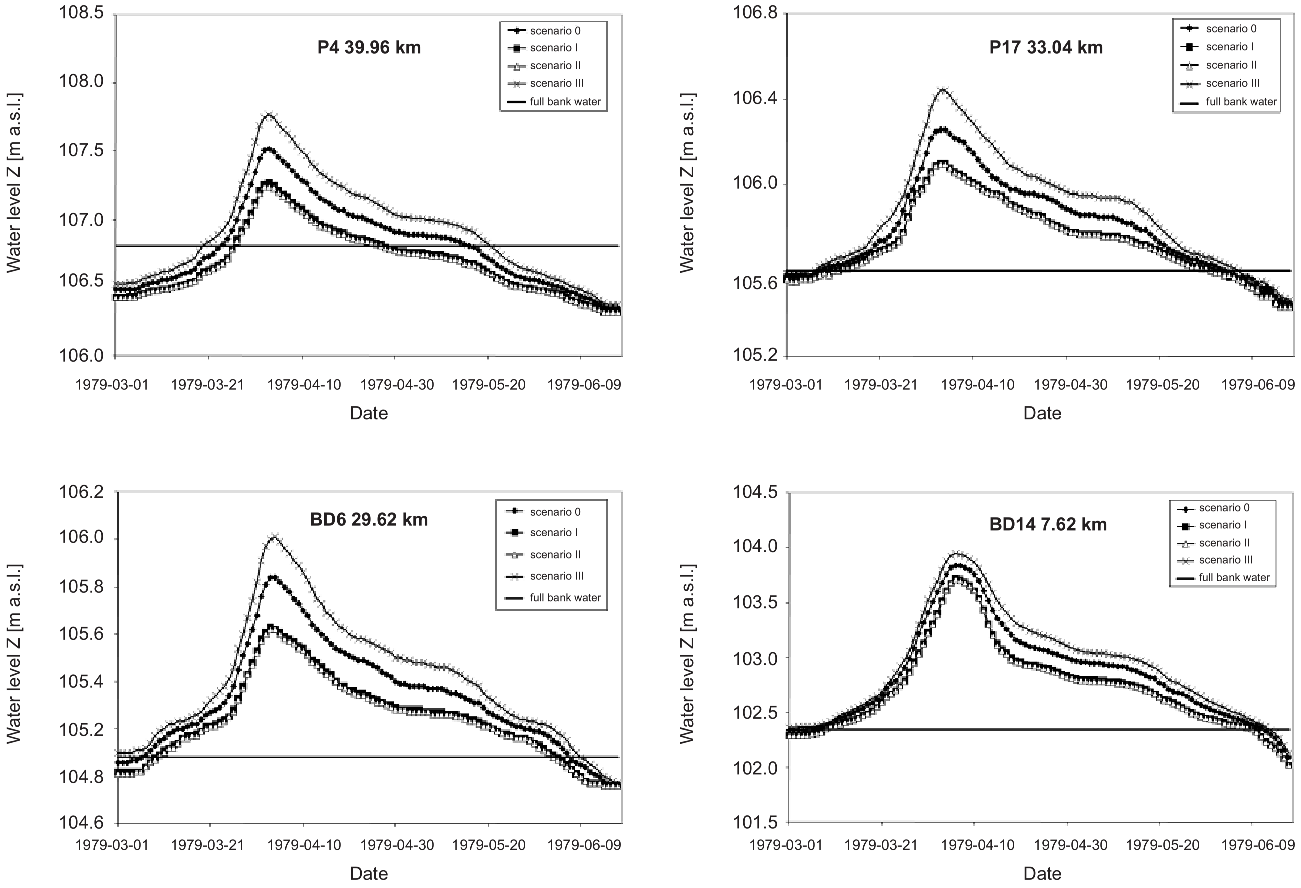

Fig. 2. Calculated stage hydrograph at select cross-sections for different land use scenarios

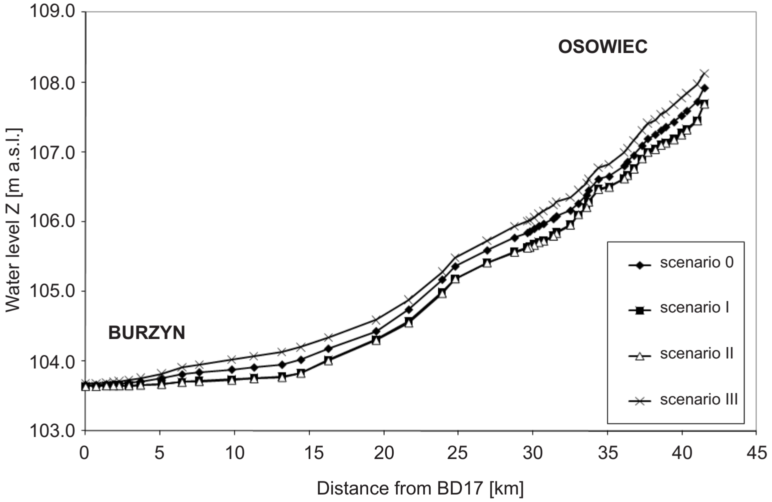

Fig. 3. Calculated water stage profile for different land use scenarios (3 April 2009)

Table 2

Variation of the average water depth on the floodplain, top flood width and flood duration for different land use scenarios

Number	Cross-section name	Distance along the river from BD17 [km]	Scenario	Average depth [m]	Top width [m]	Flood duration [days]
1	P4	39.96	0	0.58	2346	53
			I	0.51	1432	32
			II	0.51	1332	31
			III	0.64	2400	62
2	P17	33.04	0	0.24	3439	86
			I	0.19	3010	79
			II	0.19	3008	79
			III	0.29	3744	90
3	BD6	29.62	0	0.32	5103	92
			I	0.28	4685	86
			II	0.28	4666	85
			III	0.35	5367	100
4	BD14	7.62	0	0.49	6950	97
			I	0.44	5655	91
			II	0.44	5592	91
			III	0.53	7182	104

and 9 % for P17, BD6, and BD14 in comparison with the scenario 0. Changes in the average depth do not exceed 10 %. The variation of flood extent is connected with the cross-sections geometry. The highest decrease of width occurs at P4 for scenarios I and II and is about 40 %. In these scenarios it is about 10 % at P17 and BD6. The flood extent was increased by several percent in scenario III at the analyzed cross sections.

In the scenario III the flood extent, water depth and flood duration increased which means that the area of plant communities dependent from rich surface water from the river like *Phragmition* and *Magnocaricion* will increase at the cost of more valuable plant communities (like sedge-moss meadows). Long lasting inundation also does not support the development of woody vegetation except the alluvial forests. Using the hydrodynamic model it was impossible to predict how much changes in flooding characteristic will affect the vegetation, changing in the next turn its structure and location. One can only state here that secondary succession probably does not form a climax vegetation on the floodplain.

Conclusions

The obtained results show that vegetation structure on the floodplain influences the flood wave propagation in the LBRB. An intensive land use on the floodplain decreased the resistance of flow, which decreased flood duration, flood extent, and average flood water depth. These flood characteristics are strongly connected with the cross-sections geometry. The valley in the northern part is narrow, which causes the flood duration shortening; for an intensive land use on the floodplain the time was decreased by about 40 % compared with the actual state. In the other part of the Lower Basin the flood duration was shorter by less than 10 %. The influence of the natural succession on the flood wave is less significant than the influence of intensive agriculture. In this case, a flood duration changes by a few percent.

Achieved results were obtained with the use of a one dimensional hydrodynamic model which does not cover water flow across the river. It means that the used model represents simplified flow conditions in the Lower Biebrza River Basin, which is a wide area and for which this aspect is very important. The essential influence also has assigned ineffective flow areas that contain water which is not actively conveyed. Ineffective flow areas are used to described portions of a cross a section in which water ponds, but velocity of that water is close to or equal to zero. Moreover, the hydrodynamic model is an appropriate tool for assessment of various agricultural practices with regard to the effective management to protect the unique wetland site of the Biebrza National Park.

References

[1] Oświt J. Rola roślinności bagiennej w środowisku przyrodniczym doliny Narwi w aspekcie oczyszczania się wód. Zesz Probl Post Nauk Rol. 1980;234:87-102.

[2] Hooijer A. Floodplain hydrology: An ecologically oriented study of the Shannon Callows, Ireland. Amsterdam: Virje Universiteit; 1996.

[3] Zalewski M, Janauer GA, Jolankai G. A new paradigm for the sustainable use of aquatic resources. UNESCO IHP Technical Document in Hydrology 7. IHP-V Projects 2.3/2.4, UNESCO Paris. Ecohydrology. 1997;1-58.

[4] Okruszko H. Przyrodniczo-rolnicza charakterystyka doliny Biebrzy. Zesz Probl Post Nauk Rol. 1973;134:173-199.

[5] Oświt J. Strefowy układ zbiorowisk roślinnych jako odzwierciedlenie stosunków wodnych w dolinie dolnej Biebrzy. Zesz Probl Post Nauk Rol. 1968;83:317-232.

[6] Oświt J. Warunki rozwoju torfowisk w dolinie dolnej Biebrzy na tle stosunków wodnych, 1- 79. Polska Akademia Nauk, Wydział Nauk Rolniczych i Leśnych. Roczn Nauk Roln. 1973;Ser D: Monografie:143,1-80.

[7] Byczkowski A, Fal B. Wody Kotliny Biebrzańskiej [in:] Banaszuk H. (ed) Kotlina Biebrzańska i Biebrzański Park Narodowy. Białystok: Wyd. Ekonomia i Środowisko; 2004:113-183.

[8] River Analysis System, HEC-RAS, User's Manual, Version 3.0. US Army Corps of Engineers, Institute for Water Resources, Hydrologic Engineering Center, Davis, CA 2000.

[9] Świątek D, Chormański J. The verification of the numerical river flow model by use of Remote Sensing. In: Wetlands: monitoring, modelling and management: proceedings of the international conference w3m "Wetlands: modelling, monitoring, management", Wierzba, Poland, 22-25 September 2005. Okruszko T, Maltby E, Szatyłowicz J, Świątek D, Kotowski W, editors. London: Taylor & Francis; 2007:173-179.

[10] Chow Ven Te. Open-Chanel hydraulics. McGraw Hill Book Company: New York; 1959.

[11] Szporak S, Kozioł A, Mirosław-Świątek D, Kubrak J. Określenie charakterystyk roślin wykorzystywanych w obliczeniach przepustowości doliny rzeki Biebrzy. Przegl Nauk. Inż Kształt Środow. 2006;15(2):81-89.

[12] Świątek D, Szporak S, Chormański J, Ignar S. Influence of a different land use on a flood extent in the lower Biebrza valley. In: Fifth International Symposium on Environmental Hydraulics: December 4-7, 2007:1-7.

[13] Światek D, Szporak S, Chormański J. Hydrodynamic model of the Lower Biebrza River flow – a tool for assessing the hydrologic vulnerability of a floodplain to management practices. Ecohydrol & Hydrobiol. 2008;8(2-4):331-337.

WPŁYW SPOSOBU UŻYTKOWANIA DOLINY NA PRZEPŁYW FALI WEZBRANIOWEJ W BASENIE DOLNYM BIEBRZY

Katedra Inżynierii Wodnej
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Abstrakt: Stan ekosystemów mokradłowych jest uzależniony głównie od warunków zasilania ich wodami wezbraniowymi. W pracy przeanalizowano wpływ sposobu użytkowania doliny na propagację fali wezbraniowej na obszarze Biebrzańskiego Parku Narodowego (BPN) w Basenie Dolnym Biebrzy. W tym celu zastosowano jednowymiarowy model hydrodynamiczny przepływu wód rzecznych. Opracowany model wykorzystano do obliczeń symulacyjnych dla różnych typów użytkowania doliny. Występuje tu roślinność łąkowa, turzyce, szuwary, krzewy oraz olszyna bagienna i wysokopienny ols. Sposób użytkowania określono w trzech wariantach. W pierwszym scenariuszu przeanalizowano wpływ ekstensywnego użytkowania terenów łąk i pastwisk oraz intensyfikację użytkowania terenów zajmowanych przez szuwary i turzycowiska. W wariancie drugim zabiegi ochronne poszerzono o wycięcie w dolinie zakrzewień. Wariant trzeci opisywał stan, w którym nastąpiła naturalna sukcesja roślinności na terenach zalewowych. Obliczenia numeryczne wykonano dla maksymalnego wezbrania w dolinie rzeki Biebrzy z 1979 r. Wyniki symulacji opracowano w wybranych przekrojach obliczeniowych. Uzyskane rezultaty pokazały, że odpowiednie zagospodarowanie terenów zalewowych ma istotne znaczenie w Basenie Dolnym Biebrzy na przejście fali wezbraniowej.

Słowa kluczowe: model hydrodynamiczny, Basen Dolny Biebrzy, Numeryczny Model Terenu, wezbranie

DOI: 10.2428/ecea.2012.19(03)031 ECOL CHEM ENG A. 2012;19(3):295-300

Jadwiga WIERZBOWSKA[1], Teresa BOWSZYS
and Paweł STERNIK

EFFECT OF A NITROGEN FERTILIZATION RATE ON THE YIELD AND YIELD STRUCTURE OF MILK THISTLE (*Silybum marianum* (L.) Gaertn.)

WPŁYW POZIOMU NAWOŻENIA AZOTEM NA PLONOWANIE I STRUKTURĘ PLONU OSTROPESTU PLAMISTEGO (*Silybum marianum* (L.) Gaertn.)

Abstract: In the following experiment, the effect of nitrogen fertilization on the yield and biomass structure of two forms of milk thistle has been examined. A higher yield of achenes and biomass produced by milk thistle was obtained by growing a Polish population variety than the cultivar Silma. The yield of achenes rose proportionally to the increasing rates of nitrogen. The population variety responded better than cv. Silma to the increased nitrogen fertilization rates. The highest percentage of achenes in the total biomass structure was achieved when applying 2 g N per pot.

Keywords: milk thistle, yields, nitrogen

Introduction

Milk thistle (*Silybum marianum* (L.) Gaertn.) is one of the major medicinal plant species. Milk thistle extracts, which contain silymarin, are used in treatment of hepatic disorders. Silymarin protects liver cells from damaging agents and stimulates recovery processes [1, 2]. Moreover, milk thistle fruit (achenes) contain about 25 % of oil (including 63 % of linoleic acid and *ca* 20 % of oleic acid), 25–30 % of proteins, sterols (0.63 %) with tocopherol (0.038 %) and *ca* 2 % of flavonoids [3].

Owing to its relatively high content of proteins and oil containing the fatty acids beneficial to human health, milk thistle has drawn attention of animal feed and animal nutrition experts [4, 5], functional food producers and cosmetics manufacturers [6–8].

---

[1] Department of Agricultural Chemistry and Environment Protection, University of Warmia and Mazury in Olsztyn, ul. M. Oczapowskiego 8, 10–744 Olsztyn-Kortowo, Poland, phone: +48 89 523 32 31, email: jadwiga.wierzbowska@uwm.edu.pl, teresa.bowszys@uwm.edu.pl, pawel.sternik@uwm.edu.pl

With its abundant biomass production and low agronomic costs of cultivation, the plant can also serve as an energy crop.

The purpose of this study has been to evaluate the effect of a level of nitrogen fertilization on the yield and biomass production of two forms of the milk thistle.

Methods

The foregoing objective has been achieved in a two-factorial pot trial set up in a completely random design with four replications. The pots were filled with 10 kg of light soil each. The average content of available elements in the soil was as follows: 58 mg P $\cdot$ kg^{-1}, 97 mg K $\cdot$ kg^{-1} and 32 mg Mg $\cdot$ kg^{-1}. The soil reaction was slightly acid ($pH_{KCl} = 5.8$). A uniform PKMg fertilization regime (0.4 g P as $Ca(H_2PO_4)_2$; 1.5 g K as KCl and 0.3 g Mg as $MgSO_4 \cdot 7H_2O$ per pot) was combined with the following doses of nitrogen in the form of urea: N_0 – 0.0 g N; N_1 – 1.0 g N; N_2 – 2.0 g N; N_3 – 3.0 g N per pot. The PKMg fertilizers and 1 g of nitrogen (treatments N1, N2 and N3) were introduced to soil before sowing whereas the remaining doses of nitrogen (treatments N2, N3) were applied as a top-dressing treatment during the leaf rosette stage.

Two forms of the milk thistle were grown: cultivar Silma and a Polish population variety. Five plants were grown in each pot. The plants were harvested during the technological maturity phase, dissected into parts, dried and subjected to the following determinations: the weight of achenes, flower heads, stems and leaves per pot. In addition, a 1000 achenes weight as well as the number of achenes per plant and the harvest index were determined.

The results underwent analysis of variance and the significance of differences at $\alpha = 0.01$ was verified by Tukey's test.

Results and discussion

The two forms of milk thistle differed significantly in the weight of achenes, flower heads and stems (Table 1). The population line produced a 41 % higher yield of achenes and a 14 % higher yield of green parts than cv. Silma. The yield of the population plants was superior owing to the higher number of achenes (27 % more than the cultivar), which were also filled in better (a 9 % higher 1000 achenes weight). No such dependence was observed for the weight of leaves (Table 2). The population line produced the highest weight of leaves under the effect of 1 g N and the cultivar Silma achieved the highest leaf weight when fertilized with 3 g of nitrogen per pot. The fertilization treatment consisting of 3 g N per pot resulted in an over two-fold and three-fold increase in the yield of achenes produced by cv. Silma and the population variety, respectively, compared with the pots which had not received nitrogen fertilization. The most wholesome achenes from both forms of milk thistle were obtained when the plants had been fertilized with the highest nitrogen dose. Compared with the nitrogen unfertilized treatments, an increment in the weight of 1000 achenes from the plants receiving the highest nitrogen rate was about 11 % for cv. Silma and over 22 % for the population variety. These plants also formed twice as many achenes

Table 1

Mass of milk thistle organs (g per pot)

Variety	N0	N1	N2	N3	Mean	$LSD_{0.01}$
Mass of achenes						
Silma	11.57	15.02	24.69	26.05	19.33	A = 2.525; B = 3.570; C = 5.049
Population	11.47	30.96	32.89	34.03	27.34	
Mean	11.52	22.99	28.79	30.04	—	
Mass of flower heads						
Silma	11.14	12.82	24.52	27.16	18.91	A = 1.965; B = 2.779; C = 3.930
Population	10.38	28.32	28.51	32.16	24.84	
Mean	10.76	20.57	26.52	29.66	—	
Mass of stalks						
Silma	10.47	14.03	23.08	24.04	17.91	A = 2.283; B = 3.229; C = 4.567
Population	13.26	26.44	25.67	29.05	23.61	
Mean	11.87	20.24	24.38	26.55	—	
Mass of leaves						
Silma	31.84	44.66	53.93	59.22	47.41	A = n.s. B = 6.973; C = 9.861
Population	29.72	53.19	48.88	29.05	47.70	
Mean	30.78	48.93	51.41	59.11	—	
Vegetative mass						
Silma	53.45	71.51	101.54	110.42	84.23	A = 8.161; B = 11.547; C = 16.330
Population	53.36	107.96	102.86	120.20	96.10	
Mean	53.41	89.74	102.20	115.31	—	

A – $LSD_{0.01}$ variety; B – $LSD_{0.01}$ doses of nitrogen; C – $LSD_{0.01}$ interaction.

Table 2

Selected elements of milk thistle yield structure

Variety	N0	N1	N2	N3	Mean	$LSD_{0.01}$
1000 achenes weight [g]						
Silma	23.05	21.82	25.37	25.66	23.98	A = 1.356; B = 1.918; C = 2.713
Population	22.87	26.12	27.81	28.05	26.21	
Mean	22.96	23.97	26.59	26.86	—	
Number of achenes per plant						
Silma	100.39	135.90	194.62	203.06	158.49	A = 22.67; B = 32.06; C = 45.34
Population	100.26	226.65	236.45	244.55	201.98	
Mean	100.32	181.28	215.54	223.81	—	

A – $LSD_{0.01}$ variety; B – $LSD_{0.01}$ doses of nitrogen; C – $LSD_{0.01}$ interaction.

as the ones grown without nitrogen fertilization. In a study conducted by Andrzejewska and Sadowska [10], the yield of milk thistle fruit was significantly correlated with the plants' height before harvest, number of fruits in an inflorescence on the main stem and on branch stems, weight of 1000 achenes, number of all flower heads on a plant and number of flower heads with pappus.

Kozera and Nowak [11] observed a positive response of milk thistle to an increasing mineral fertilization rate. The highest yield of achenes was reported by these authors when 261 kg NPK ha^{-1} had been applied as fertilizer. In addition, the yield was found out to have increased significantly after micronutrients had been applied in a top-dressing treatment. Other authors as well indicate that the yield of achenes produced by milk thistle plants rose proportionally to the increased rates of nitrogen [12]. In turn, Geneva et al [13], who tested top-dressing application of NPK and micronutrients, achieved an over 70 % increase in the yield of milk thistle compared with the control, unfertilized treatment.

Some researchers suggest that achene yields produced by milk thistle are considerably affected by the weather conditions, the date when forecrops are sown and the way the soil is tilled [10, 14–16]. It has been demonstrated that milk thistle grown in a short-term monoculture yielded 40 % lower than in a crop rotation [17]. Some authors claim that the weight of 1000 achenes is related to the colour of achenes. Higher weight is attained by dark brown seeds [18].

The harvest index, *ie* the percentage of achenes in the biomass yield, depends on the nitrogen fertilization rate, which has been confirmed by high determination coefficients (Fig. 1). The percentage of achenes in the biomass yield of plants grown without nitrogen fertilization was similar for both forms of milk thistle, and reached *ca* 16.5 %. The highest value of this parameter (over 24 %) was obtained for the population plants fertilized with 2 g N per pot. The average value of the harvest index for each of the tested varieties was higher for the population line (over 21 %) than for cv. Silma.

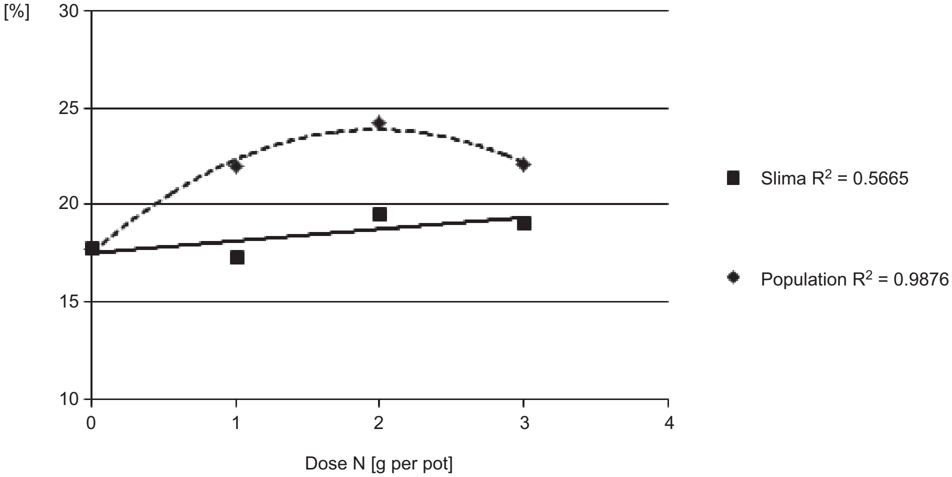

Fig. 1. Harvest index of milk thistle depending on the level of nitrogen fertilization

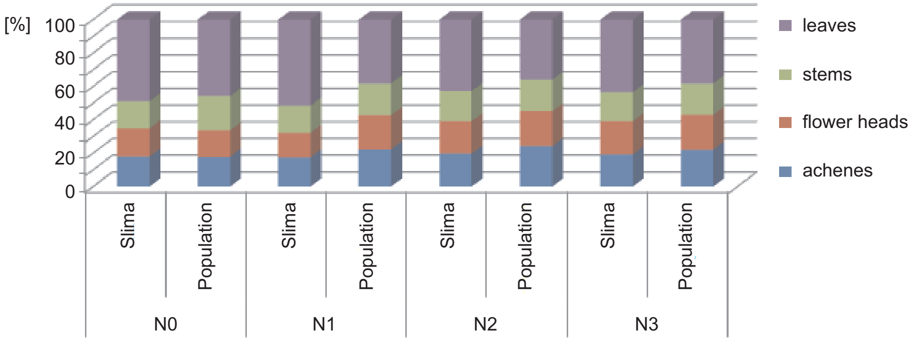

Fig. 2. Structure of biomass

The two forms of milk thistle were only slightly different in the structure of biomass (Fig. 2). In cv. Silma, depending on the fertilization rate, 43 to 52 % of the dry mass was accumulated in leaves and 16–18 % was in stems. With respect to the population line, leaves accumulated from 36 to 46 % of dry mass while stems contained 19–20 % of dry mass. In both forms, the highest share of achenes, and the lowest one of leaves, in the total dry mass accumulation were found after the application of 2 g N per pot.

Conclusions

1. Higher yields of achenes and green parts of milk thistle were obtained when growing the population line (on average 27.3 g) than cv. Silma (on average 19.3 g).
2. The yield of milk thistle achenes increased proportionally to the rates of nitrogen, and population plants responded more strongly to the increased nitrogen fertilization than the cultivar Silma.
3. The population line produced more achenes than cv. Silma. The average number of achenes on the population plant was 202 versus just 158 on a single plant of the cultivar Silma. Achenes produced by the population plants were also larger.
4. The highest share of achenes in the total biomass structure was obtained when the rate of nitrogen fertilization was 2 g per pot.

References

[1] Dvorak Z, Kosina P, Walterowa D, Simanek V, Bachleda P, Ulrichova J. Toxicol Lett. 2003;137:201-212.
[2] Niedworok J. Wiad Zielarskie. 1996;7-8(96):17-18.
[3] Szczucińska A, Kurzepa K, Kleczkowska P, Lipkowski AW. Rośliny Oleiste – Oilseed Crops. 2006;XXVII:57-366
[4] Łangowska A, Szymaś B, Przybył A. J Apic Sci. 2002;46(2):49-54.
[5] Urbanczyk J, Hanczakowska E, Swiatkiewicz M. Med Wet. 2002;11:887-889.
[6] Andrzejewska J, Skinder Z. Acta Sci Polon Agric. 2006a;5(1):5-10.
[7] Hadolin M, Skerget M, Knez Z, Bauman D. Food Chem. 2001;74:355-364.
[8] SzczucińskaA, KurzepaK, Grabowska A, Lipkowski AW. Tłusz Jadal. 2007;42(3-4):151-157.
[9] Sulas L, Ventura A, Murgia L. Options Mediterr Ser A Semin Mediter. 2008;79:487-490.

[10] Andrzejewska J, Sadowska K. Acta Sci Polon Agric. 2008;7(3):3-11.
[11] Kozera W, Nowak K. Ann UMCS Sec E. 2004;59(1):369-374.
[12] Omer E A, Refaat A M, Ahmed SS, Kamel A, Hammouda F M. J Herbs Spieces and Med Plants. 1993;1(4):17-23.
[13] Geneva M, Stancheva I, Sichanova M, Boychinova M, Georgiev G, Dolezal M. Gen Appl Plant Physiol Special. 2008;34(3-4):309-318.
[14] Andrzejewska J, Skinder Z. Herba Polon. 2006b;4:11-17.
[15] Andrzejewska J, Sadowska K, Mielcarek S. Ind Crop Prod. 2011;33:462-468. DOI: 10.1016/j.indcrop.2010.10.027.
[16] Habán M, Otepka P, Kobida L, Habánová M. Hort Sci. (Prague, CZ). 2009;36(2):25–30
[17] Andrzejewska J, Skinder Z. Herba Polon. 2007;53(1):5-10.
[18] Dyduch J, Najda A. Herba Polon. 2007;53(3):331-336.

WPŁYW POZIOMU NAWOŻENIA AZOTEM NA PLONOWANIE I STRUKTURĘ PLONU OSTROPESTU PLAMISTEGO (*Silybum marianum* (L.) Gaertn.)

Katedra Chemii Rolnej i Ochrony Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

Abstrakt: W pracy badano wpływ poziomu nawożenia azotem na plonowanie i strukturę biomasy dwóch form ostropestu plamistego. Większy plon niełupek i masy wegetatywnej ostropestu plamistego uzyskano, uprawiając wyselekcjonowaną populację krajową niż odmianę Silma. Plon niełupek wzrastał proporcjonalnie do dawek azotu. Rośliny krajowej populacji silniej reagowały na wzrost poziomu nawożenia azotem niż odmiana Silma, ponadto rośliny krajowej populacji wytwarzały więcej i bardziej dorodnych owoców niż odmiana Silma. Największy udział niełupek w strukturze biomasy uzyskano, stosując nawożenie w dawce 2 g N na wazon.

Słowa kluczowe: ostropest plamisty, plonowanie, azot

DOI: 10.2428/ecea.2012.19(03)032 ECOL CHEM ENG A. 2012;19(3):301-309

Krystyna HOFFMANN[1], Jakub SKUT[1],
Tomasz SKIBA[1] and Józef HOFFMANN[1]

LIFE CYCLE ASSESSMENT FOR INDUSTRIAL PROCESSESS ON THE EXAMPLE OF PARTIALLY ACIDULATED PHOSPHATE ROCKS

ANALIZA CYKLU ŻYCIA DLA PROCESÓW PRZEMYSŁOWYCH NA PRZYKŁADZIE PRODUKCJI FOSFORYTÓW CZĘŚCIOWO ROZŁOŻONYCH

Abstract: The aim of work was life cycle assessment (LCA) of simple inorganic PAPR (partially acidulated phosphate rock) type fertilizer. PAPR type fertilizers are specified as products of partial dissolution of grinded phosphate rock with usage of sulfuric or phosphoric acid, containing as a main components monocalcium phosphate, tricalcium phosphate and calcium sulfate. LCA was made by using the model of PAPR type fertilizer production process produced under the laboratory conditions. Model was based on the research provided for assessment of product physical properties, phosphorus content expressed as a P_2O_5 soluble in mineral acids and water, mass balance and emissions from the process. Results, supplemented with reference data were used as a input data for GaBi 4 software which constitutes the computer support to perform LCA.

Keywords: partially acidulated phosphate rocks (PAPR), Life Cycle Assessment (LCA), phosphate fertilizers

Intensified efforts to evaluate the impact of industrial plants and enterprises on the environment are observed as a response to increasing degree of environmental awareness. Currently, public concern regarding the phenomenon of global depletion of natural resources results in the search for effective strategies and systems that limit the human adverse impact on the environment. Many companies, to counteract the global environmental threats, introduced to the market "greener" products, defined by the manufacturer as environmentally friendly, which are produced using "greener" technology. Environmental characteristics of products and manufacturing processes has become a major contributor to the development of enterprises in the field of investigation of methods for minimizing their adverse impact on the environment. Recently, various businesses use the advantages of applying pollution prevention

---

[1] Institute of Inorganic Chemistry and Mineral Fertilizers, Wroclaw University of Technology, ul. J. Smoluchowskiego 25, 50–372 Wrocław, Poland, phone: +48 71 320 29 94, fax: +48 71 328 29 40, email: jozef.hoffmann@pwr.wroc.pl

strategies and environmental management systems in order to gain a marketing supremacy over competitors. *Life Cycle Assessment* – LCA is one of tools used for these purposes. This method presents a comprehensive approach to the environmental aspects of a product life cycle "cradle-to-grave". It begins with the process of natural resources extraction in order to manufacture the product and ends at the point where all materials are re-utilized [1–3].

LCA evaluates all stages of product life cycle from the perspective of their total interdependence, thereby understanding that every successive operation leads to the next. LCA enables the estimation of the total environmental impact resulting from all stages of product life cycle, often including impacts not considered in more traditional studies (*eg* raw material extraction, material transportation, final disposal of the product, *etc.*). Taking into account the impact on the environment, therefore raw materials and energy consumption, wastes, effluents and emissions, which is connected with the production, usage and ultimately the management of waste product (recycling, storage, scrapping), LCA provides a comprehensive view of the environmental aspects of the product or process and more accurate product selection and its manufacturing process. In addition to the materials and energy flows, also information on financial flows are often collected. This process is referred to LCC – *Life Cycle Cost* [4, 5]. LCA has its origin in an ISO standards of 14000 – Environmental Management series. Life cycle assessment is presented in detail in the standards starting from 14040 to 14043. Both definitions specific to the subject and discussion on the particular stages of the environmental life cycle assessment were included within these reference documents [6–8].

Implementation of environmental life cycle assessment is time-consuming process and often requires large amounts of data about the processes and materials included in the manufacturing system. Performing the LCIA stage and the final report involves conducting the arduous and complicated calculations and specifications. One of the commercially available software tools may be used to simplify performing LCA. Computer programs such as GEMSIS, SimaPro, GaBi are the most popular. The use of computer support also allows making quick alterations in created project (sensitivity analysis) and keeping standardized, comparable results.

PAPR-type (*partially acidulated phosphate rocks*) fertilizers are produced in contrast to conventional superphosphate fertilizers as a result of the partial acidulation of ground phosphate rock with sulfuric or phosphoric acid [9]. They contain monocalcium phosphate, tricalcium phosphate and calcium sulfate as a main components. The parameter, which classifies the PAPR-type fertilizer formulations is the degree of PAPR stoichiometric norm (η_{PAPR}) expressed by the following formula:

$$\eta_{\mathrm{PAPR}} = \frac{\eta^{a}_{\mathrm{min.\,ac.}}}{\eta^{s}_{\mathrm{min.\,ac.}}}$$

where: η_{PAPR} – degree of PAPR stoichiometric norm;
$\eta^{a}_{\mathrm{min.\,ac.}}$ – actual amount of mineral acid used for acidulation [kg];
$\eta^{s}_{\mathrm{min.\,ac.}}$ – amount of mineral acid resulting from stoichiometry of phosphate rock acidulation reaction [kg] [10].

Values of η_{PAPR} in the range 0.1–0.5 are commonly used in industry. The idea of the PAPR fertilizer manufacturing is mainly attributable to economic reasons. Lower acid consumption reduces the unit price of the total content of phosphorus compounds in the product in relation to the fertilizer produced by the complete acidulation of raw material (*single superphosphate* – SSP or *triple* – TSP). Another advantage is the tolerance of the manufacturing process for low-grade raw phosphate rocks, which are not suitable for use in the manufacture of superphosphate. In addition, a number of experiments on various crops showed that fertilizers produced by this method represent the same, and under certain conditions, higher efficiency than superphosphates, in particular the SSP [11,12]. As an explanation for this phenomenon the following chemical reactions occurring in the soil have been proposed [13]:

$$Ca(H_2PO_4)_2 \cdot H_2O + xH_2O \rightleftharpoons CaHPO_4 \cdot 2H_2O + H_3PO_4 + (x - 1)H_2O$$

$$Ca_{10}(PO_4)_6F_2 + 14H_3PO_4 + 10H_2O \rightleftharpoons 10Ca(H_2PO_4)_2 \cdot H_2O + 2HF$$

As a result of hydrolysis of monocalcium phosphate molecule free phosphoric acid is formed, which subsequently reacts with the prior unreacted part of phosphate rock, forming of dicalcium phosphate and further less soluble compounds [14]. The rate of each reaction is different. The first one is faster, a second slower. The rate of the second reaction is limited by the phosphorus uptake by plants, which successively increases the amount of soluble compounds. Therefore, phosphorus contained in the structure of the PAPR has a prolonged release profile in the soil environment.

Emissions are important aspect in terms of life cycle assessment of PAPR fertilizers. As the production of SSP and PAPR is based on the same raw materials and the same reaction, both processes are characterized by emission of the same type of pollutants into the environment. The first type are dusts released into the atmosphere during the reloading, milling and transportation of phosphate rocks. Their amount depends on the fineness of the raw material. Another type of emission are volatile fluorine and silicon compounds. They are formed as a by-product of phosphate rock acidulation with sulfuric acid. The last type of emissions are dusts generated by the crushing and transportation of a final product.

Materials and methods

The investigated PAPR-type fertilizer preparations were obtained under laboratory scale, applying for calculations of the amount of sulfuric acid resulting from the stoichiometry of the acidulation reaction of phosphate rock ($\eta^{s}_{min.\ ac.}$) procedure recommended by the U.S. Department of Agriculture (1964) expressed by the following formula [15]:

$$\frac{\text{kg } H_2SO_4 \text{ } 100\%}{100 \text{ kg phosphate rock}} = 1.749(\%\ CaO) + 0.962(\%\ Al_2O_3) +$$

$$+ 0.614(\%\ Fe_2O_3) + 2.433(\%\ MgO) + 1.582(\%\ Na_2O) +$$

$$+ 1.041(\% \; K_2O) - 0.691(\% \; P_2O_5) - 1.225(\% \; SO_3)$$

The investigations were conducted for the degree of PAPR stoichiometric norm values of 0.3, 0.4, 0.5 and 1.0 – as a control batch of single superphosphate (SSP). Morocco phosphate rock 68 BPL samples with a declared content of P_2O_5 min. 31 % w/w and 95 % w/w sulfuric acid supplied by POCH manufacturer were used for the experiments. Fertilizer products of assumed degree of PAPR stoichiometric norm values were prepared in a model-type apparatus produced by Atlas Syrris Ltd which was equipped with automatic control of process parameters such as temperature, stirring rate and time. The applied apparatus allowed manufacturing of fertilizers by batch processing, while the conditions have been adjusted to imitate the continuous process for PAPR and SSP fertilizers production.

The first is the short contact time of reactants (2–3 min). Under operating conditions of the process it was achieved through intensive mixing of the reactants together – stirring rate was 600 rpm. Taking into consideration small volume of the reaction mixture pre-heating of the reactor before adding the reactants to about 90–100 ^{o}C was implemented. Diluted sulfuric acid at a concentration of 70 % w/w and temperature of about 70 ^{o}C was introduced into the reactor, while the temperature of the process, measured inside the reactor was 110 ^{o}C ± 5 ^{o}C. Exhaust gases were absorbed in distilled water in a scrubber, and subsequently analyzed for the fluoride ions content using Orion ion-selective electrode. The extraction of phosphate in the form of total P_2O_5 (soluble in mineral acids) and water-soluble P_2O_5 was performed in accordance with the procedures enclosed in Regulation (EC) No. 2003/2003 of the European Parliament and of the council of 13 October 2003 relating to fertilizers at 0, 4, 7, 14 and 28 day after production [9]. Extracted forms of phosphorus were determined using a spectrophotometric method according to the recommendations contained in PN-88/C-87015 based on creation of yellow complexes between metavanadate-molybdate and an orthophosphate ion [16].

The assessment of environmental impact of the PAPR industrial manufacturing process was performed using GaBi 4 from PE International GmbH as a software supporting LCA. The process of industrial production of such fertilizer formulation includes the following unit operations: phosphate rock unloading, dilution and cooling the sulfuric acid, fertilizer acidulation (reaction with sulfuric acid), crushing the product, absorption of gases and dusts, product storage. Unloading raw materials, product storage and outputs from the absorption process were considered as system boundaries. One ton of the final product was assumed as a functional unit and a mixture of raw materials and mineral acid was as a reference stream.

Results and discussion

In comparison with conventional superphosphate fertilizers, the PAPR products obtained under laboratory conditions are characterized by shorter solidification time. In connection with the observed lack of significant changes in contents of various forms of phosphates during curing process, further processing of the PAPR fertilizer formulations with elimination of this process is possible, which in the case of SSP and TSP constitutes crucial requirement to achieve a product with demanded physical properties and content of available phosphorus.

The Life Cycle Assessment was performed for PAPR fertilizer $\eta_{PAPR} = 0.5$, which meets the requirements imposed to partially acidulated phosphate rocks by European legislation regarding the percentage of phosphorus (20 % P_2O_5 Phosphorus expressed as P_2O_5 soluble in mineral acids, at least 40 % of the declared content of P_2O_5 being water soluble) [1].

1. LCI

The analysis of a set of inputs and outputs were based on data collected from all unit operations included in the product manufacturing system. The streams of Inputs/Outputs of substances, emissions and energy were assigned. Streams values calculated on the basis of assumptions that were concluded from experimental part or discovered from bibliographic resources were depicted in Table 1.

Table 1

Balance sheet of calculated streams values.

	Inputs		Outputs	
Name of unit process	Name of stream	Value	Name of stream	Value
Phosphate rock unloading	Phosphate rock (32 % w/w P_2O_5)	658.66 kg	Phosphate rock (32 % w/w P_2O_5)	658.6 kg
			Phosphate dusts	0.06 kg
Sulfuric acid dilution and cooling	Sulfuric acid (VI) 96 % w/w	227.6 kg	Sulfuric acid (VI) 64 % w/w	455.2 kg
	Water	113.80 kg	Cooling water	373.9 kg
	Cooling water	373.9 kg	Residual heat	78.3 MJ
	Electric power consumption	0.21 kWh		
Phosphate rock acidulation	Sulfuric acid (VI) 64 % w/w	341.4 kg	PAPR 0.5	998.64 kg
	Phosphate rock (32 % w/w P_2O_5)	658.6 kg	Fluorides	0.11 kg
	Electric power consumption	9.14 kWh	Phosphate dusts	1.25 kg
PAPR crushing	PAPR 0.5	998.64 kg	PAPR 0.5	997.8 kg
	Electric power consumption	2.35 kWh	Fluorides	0.07 kg
			Phosphate dusts	0.77 kg
Absorption	Fluorides	0.18 kg	Water after absorption	100 kg
	Phosphate dusts	2.02 kg	Fluorides	0.0018 kg
	Water for absorption	100 kg	Phosphate dusts	0.0202 kg
	Electric power consumption	20 kWh	Fluorides (sewages)	0.1782 kg
			Phosphate dusts (sewages)	1.9998 kg
Storage	PAPR 0.5	997.8 kg	PAPR 0.5	997.8 kg
	Land area	1500 m^2		

The data collected during LCI stage were entered into the program. The data constituted the basis for preparation of a plan for PAPR η_{PAPR} = 0.5 fertilizer manufacturing system. Screenshot of the GaBi Software was demonstrated on Fig. 1.

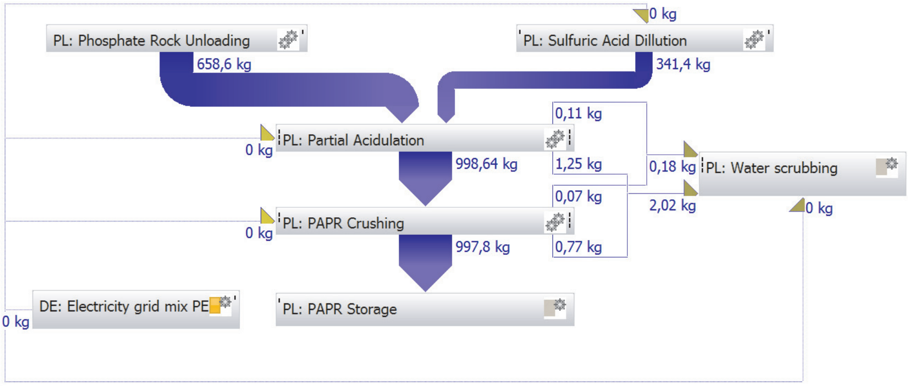

Fig. 1. Plan of the PAPR η_{PAPR} = 0.5 product manufacturing system carried out in GaBi 4 software

Names and values of input and output streams were supplemented, allocated and appropriate streams were labeled as tracked ones. This allowed their connection with the subsequent processes included within the plan composition. Creating a stream required the pre-qualification. For example, in this case, among streams called "phosphates" a group "air emissions" rather than "non-renewable resources" were selected. Energy demand was 396.3 MJ.

2. LCIA

After constructing the plan of manufacturing system based on data collected from the LCI stage and connecting the respective streams between processes the balances were created. These operations allowed the calculation of impact of the PAPR manufacturing process on the environment. All streams within the manufacturing system were assigned to the appropriate category of raw materials and energy resources (renewable, non-renewable), emissions (organic, inorganic; into water, soil, air). Classification and selection of the category indicator were executed automatically after selection of model of impact category, and indirectly by defining streams at the LCI stage. For the purposes of described case the following categories were selected from multiple models of the impact categories included in the GaBi 4 program:

- Mass balance;
- Energy balance;
- CML2001, Eutrophication Potential (EP);
- CML 2001, Global Warming Potential (GWP100 Years);
- EDIP 1997, Human toxicity air;

– EDIP 1997, Human toxicity soil;

– EDIP 1997, Human toxicity water;

– EI 99, EA, Ecosystem quality, Land conversion.

Successive LCA analysis was based on selection of the appropriate option of outputs presentation. The influence of manufacturing system on greenhouse effect was evaluated. For this purpose according to the path "Quantity" => "CML 2001, Global Warming Potential 100 Years" option was selected. In the balance window only those flows and processes that contribute to the greenhouse effect were displayed. In this case, with given values, it was only electric power introduced into the process. Normalization method was chosen each time to the appropriate category of influence. Normalization did not include material and energy balances of the process.

Conclusions

Manufacturing process of PAPR $\eta_{PAPR} = 0.5$, defined according to presented paper, have impact on the environment in range of electric power consumption and operations involving phosphate rock processing. The results of analyzes enabled the evaluation of process operations, which have the most adverse impact on the environment, and suggested that product system have impact on the environment on different levels:

– Global Warming Potential – manufacture of functional unit is connected with electricity consumption taking effect in emission of 22.976 kg CO_2 equivalent;

– Eutrophication Potential – manufacture of functional unit is connected with emission of 2.084 kg phosphate equivalent, in particular from absorption installation, phosphate rock unloading and electricity consumption;

– Human toxicity water – 82.545 m^3 of water, in particular due to electric power consumption;

– Human toxicity air – $9.74 \cdot 10^5$ m^3 of air, in particular due to electric power consumption;

– EDIP 1997, Human toxicity soil – 1.944 m^3 of soil, in particular due to absorption process of volatile fluorine compounds and phosphate dusts;

– Ecosystem quality, Land conversion – consumption of storage area is connected with 37740 PDF $\cdot$ m^2.

The results of LCA analysis demonstrated number of environmental aspects that require consideration during designing or optimization of the PAPRs manufacturing process. it is necessary to eliminate "weak points" of the product system such as reduction of phosphate dusts emissions, preferably by the recirculation, utilizing fluorine and silicon compounds from the process (production of fluorosilicic acid) and water recirculation during absorption process. GaBi built-in options enabled automatically detection of so called "weak points" of product manufacturing system. The indication was proceeded by highlighting those streams that have the greatest share in appropriate category of influence. The evaluated process demonstrates the following environmental threads:

– Electric power consumption – high energy demand results in a significant impact on global warming, despite none of the unit processes in itself does not cause greenhouse gases emissions;

– Absorption of volatile fluorine compounds and phosphate dusts;

– Dilution of sulfuric acid – emission of the residual heat into environment.

To solve the problems the following procedure should be taken into account:

a) Reduction of electric power consumption by the apparatus and equipment used in the manufacturing process.

b) Recirculation of water stream in the absorption unit and rerouting the recovered fluorine compounds for the production of fluorosilicic acid. Such a solution is suggested by the reference documents as the *Best Available Technology* for the phosphate fertilizers industry [17].

c) Recycling of phosphate dusts in the process, which are emitted during the operation of acidulation, transportation and crushing of the product.

Residual heat management by its utilization *eg* heating water for sanitary purposes on the premises. It may be necessary to redesign the sulfuric acid dilution unit to obtain a higher temperature gradient between the water which cools heat exchanger and heated water for its further application.

Acknowledgement

This scientific work was funded as a research project No. NN209 213 138 by finance for science in the years 2010–2013.

References

[1] Scientific Applications International Corporation (SAIC). Life Cycle Assessment: Principles and Practice. Rep no 68-C02-067. P 7, Reston; 2006.

[2] Curran MA, editor. Environmental Life Cycle Assessment. ISBN 0-07-015063-X, McGraw-Hill: 1996.

[3] Hoffmann JN, Radosiński E. Environmental management concepts supported by information technology, Pol J Chem Technol. 2007;9(4), 8-13.

[4] Kowalski Z, Kulczycka J, Góralczyk M. Ekologiczna ocena cyklu życia procesów wytwórczych (LCA). Warszawa: PWN; 2007.

[5] Kulczycka J. Ekologiczna ocena cyklu życia (LCA) nową techniką zarządzania środowiskowego. Kraków: Wydawnictwo Sigmie PAN; 2001.

[6] International Standards Organization. 1997. Environmental Management – Life Cycle Assessment – Principles and Framework ISO 14040.

[7] International Standards Organization. 1998. Life Cycle Assessment – Impact Assessment ISO 14042.

[8] International Standards Organization. 1998b. Environmental Management – Life Cycle Assessment – Life Cycle Interpretation ISO 14043.

[9] Rozporządzenie (WE) No 2003/2003 Parlamentu Europejskiego i Rady z dnia 13 X 2003 r. w sprawie nawozów. Dziennik Urzędowy Unii Europejskiej 21 XI 2003 r., L 304.

[10] Skut J, Hoffmann J, Hoffmann K. Perspektywy zastosowania częściowego rozkładu surowców fosforowych metodą PAPR w przemysłowych instalacjach nawozowych. Przem Chem. 2010;89(4):534-539.

[11] Hagin J, Katz J. Effectiveness of partially acidulated phosphate rock as a source to plants in calcareous soils, Fertilizer Res. 1985;8(2):117-127.

[12] Grzebisz W, Potarzycki J. Partially acidulated phosphate rocks (PAPRs) as an alternative source of phosphorus in agriculture of Poland, New agrochemicals and their safe use for health and environment. Czech-Pol Trade. 2004;86-90.

[13] Rajan SSS. Partial acidulation of an "unground" phosphate rock: 1. Preparation and characteristics. Nutrient Cycling in Agroecosystems. 1985;8(2):147-155.
[14] McLean EO, Wheeler RW. Partially acidulated rock phosphates as a source of phosphorus to plants, Soil Sci Society of America J. 1964;28:545-550.
[15] US Department of Agriculture and Tennessee Valley Authority, Superphosphate: Its History, Chemistry, and Manufacture, US Government Printing Office, Washington DC, 1964.
[16] PN-88/C-87015, Nawozy sztuczne, Metody badań zawartości fosforanów, 1989.
[17] European Commission. 2006. European Integrated Pollution Prevention and Control Bureau (EIPPCB). Reference Document on Best Available Techniques in Large Volume Inorganic Chemicals – Ammonia, Acids and Fertilizers. Seville: EIPPCB.

ANALIZA CYKLU ŻYCIA DLA PROCESÓW PRZEMYSŁOWYCH NA PRZYKŁADZIE PRODUKCJI FOSFORYTÓW CZĘŚCIOWO ROZŁOŻONYCH

Instytut Technologii Nieorganicznej i Nawozów Mineralnych
Politechnika Wrocławska

Abstrakt: Celem niniejszej pracy była analiza cyklu życia nawozu nieorganicznego prostego typu PAPR (*Partially Acidulated Phosphate Rock* – fosforyty częściowo rozłożone). Nawozy typu PAPR definiowane są jako produkty otrzymywane w wyniku częściowego rozłożenia zmielonego fosforytu kwasem siarkowym lub fosforowym, zawierające jako składniki główne fosforan jednowapniowy, fosforan trójwapniowy oraz siarczan wapnia. Ocenę cyklu życia wykonano na podstawie modelu procesu produkcji preparatów nawozowych typu PAPR uzyskanego w warunkach laboratoryjnych. Opracowany model bazował na badaniach uwzględniających ocenę właściwości fizycznych produktu, zawartość fosforu w przeliczeniu na P_2O_5 rozpuszczalny w kwasach mineralnych oraz w wodzie, a ponadto bilans masowy i emisje z procesu. Uzyskane wyniki, uzupełnione danymi literaturowymi posłużyły jako dane wejściowe dla programu GaBi 4, który stanowi komputerowe wspomaganie wykonywania analizy LCA.

Słowa kluczowe: fosforyty częściowo rozłożone (PAPR), ocena cyklu życia (LCA), nawozy fosforowe

DOI: 10.2428/ecea.2012.19(03)033 ECOL CHEM ENG A. 2012;19(3):311-320

Ireneusz KRUKOWSKI[1], Marcin WIDOMSKI[1],
Małgorzata IWANEK[1] and Grzegorz ŁAGÓD[1]

EXFILTRATION FROM SANITATION PIPES AND TRANSPORT OF CHOSEN POLLUTANTS – A MODEL STUDY

PROCES EKSFILTRACJI Z PRZEWODÓW KANALIZACYJNYCH A ROZPRZESTRZENIANIE SIĘ WYBRANYCH ZANIECZYSZCZEŃ – BADANIA MODELOWE

Abstract: Sanitation systems exploitation may result in the possibility of sewage exfiltration. The exfiltering sewage, very often containing the high concentration of organic and inorganic pollutants may cause the clear danger for the groundwater and soil environment. The qualitative and quantitative monitoring of wastewater leaving the damaged sewage pipe is quite difficult and often, in practice – impossible. Recently, the numerical modeling of pollutants transport in groundwater and soil has gained the increased popularity. This paper presents the results of numerical calculations of chosen pollutants (cadmium and chromium) exfiltering from the damaged gravitational sewer system pipe located in the profile of Nadstawna St., Lublin, Poland. The numerical calculations were conducted by FEFLOW 5.2, WASY, Germany software. Soils' transport parameters as well as initial and boundary conditions were obtained by results of laboratory, field and literature studies. The results of our researches enables the analysis of sanitation pipe failure effect on soil environment and groundwater. However, the empirical verification of the modeling results in order to verify the validity of initial and boundary conditions is required.

Keywords: exfiltration, pollutants transport, numerical modeling

Heavy metals naturally appearing in the environment do not cause hazard for plants and animals. However, some of anthropogenic activities, particularly the metallurgical, textile, tanner and chemical industry burden the sewer system with enlarged concentration of the chromium and cadmium in the transported sewage [1].

The chromium appears commonly in two oxidation states as Cr(III) and Cr(VI). The chromium(III) occurs naturally in the environment and is indispensable for living beings. The trivalent chromium is relatively insoluble. Whereas Cr(VI) is comparatively soluble, so can be easily transported to groundwater and soil environment. Up to 90 %

---

[1] Faculty of Environmental Engineering, Lublin University of Technology, ul. Nadbystrzycka 40B, 20–618 Lublin, Poland, phone: +48 81 538 4431, email: I.Krukowski@wis.pol.lublin.pl

chromium pollutants are Cr(VI) [2]. Because of its strong mutagenic and carcinogenic attributes it is an essential threat for the natural environment. It causes, among others, many illnesses such as: damage of the liver and kidney, problems with the respiratory system and the ulceration of the skin.

Cadmium appears naturally in the environment as the effect of the erosion of rocks and soils as well as eruptions of volcanoes and fires of forests. Cadmium in sewage is equally dangerous as chromium. It has a carcinogenic and teratogenic activity and accumulates in the human organism mainly in kidney. Cadmium in sewage is connected directly to the intensive anthropogenic activity, mainly in metallurgical and chemical industry. Transport, concentration and the toxicity of Cr(VI) and cadmium in the environment depends on many physical, chemical and biological processes occurring in groundwater and soil [3, 4].

In order to define the range of the influence of chromium and cadmium pollutants outflowing the damaged gravitational sewer system numerical models are built. They allow the comprehensive simulation for different computational variants. The aim of this paper was to present the spread of chosen pollutants (cadmium, chromium) leaving the damaged sewage pipe located in the profile of Nadstawna St., Lublin, Poland.

Materials and method

To our numerical calculations the program FEFLOW 5.2, WASY GmbH Germany was chosen [5–7] and MPWiK Sp. z. o.o. Lublin delivered such information and data sources as:

1. The map with marked points of the conscription of samples of the ground (with wells) to laboratory-research in the scale 1:500.
2. The numeric map of sewage system and water-supply system in the scale 1:500, dated on 2007-10-17.
3. The results of inspection of sewage pipes in Nadstawna St., Lublin, Poland (The report from inspection No. /607/T/06 dated on 2006-11-15).
4. Materials from surveys of the construction of the existing road surface and the ground basis. The reconstruction of the water supply and sanitary sewage system with parachannels in Targowa St., Nadstawna St., Nowy Plac Targowy St. in Lublin, made by the Road-Laboratory "LABDROG" Grygowej 23 St., Lublin in 2004-09-03 and 2005-04-06.
5. Composition of raw sewage obtained on 2007.

On the basis of materials made accessible by MPWiK Sp. z o.o. Lublin (1–3) as the source of the pollutants (chromium and cadmium) to research the ceramics pipe of the sanitary gravitation sewage system was chosen. It had the 250 mm diameter and was located in Nadstawna Street in Lublin. These (4) materials allowed also to accept to research the soil profile which is presented in Fig. 1.

Research of the soil profile in Nadstawna St., Lublin conducted by the Road-Laboratory "LABDROG" allowed to specify eight layers in the accepted profile. The composition of individual layers was presented in the Table 1. Simultaneous research showed that groundwater did not appear to the depth 5.20 m under the level of road. In

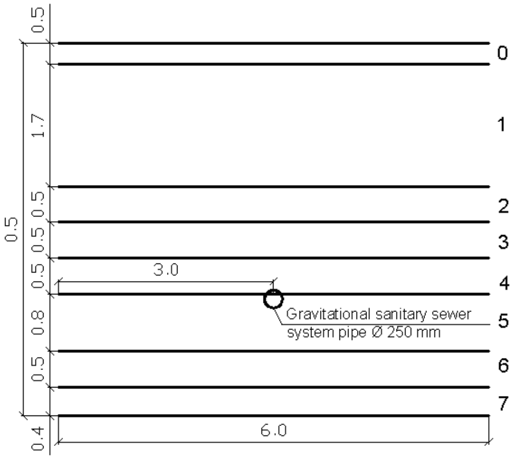

Fig. 1. Scheme of modeled profile accepted to studies

the model the fact, that the profile was covered by impermeable tight asphalt-surface was also taken into account. The profile of 6.0 m width, with sewage pipe was situated centrally on the depth 3.7 m (the level of pipe's bottom) was accepted to research. For individual distinguished layers in the profile the net of triangular finite elements was developed.

Table 1

Composition of individual layers of the profile

No. layer	Type and colour of the soil
0	mass mineral – asphalt, the rag, the layer draining off base coal
1	not building embankment – clayey sand impure stones and grey-beige bricks
2	not building embankment – clay, grey bricks
3	not building embankment – humic clay with admixtures of stones
4	not building embankment – dusty humic clay and grey bricks
5	not building embankment – dusty humic clay, dust, grey bricks
6	not building embankment – dusty humic clay, dust, grey bricks
7	dusty humic clay, dust, grey bricks

The physical-water parameters for individual layers of the profile were accepted on the basis of literature studies [8–10] and put together in the Table 2.

As basic pollutants leaving the damaged sewage pipe chromium and cadmium was chosen. The concentration of these elements in raw sewage was accepted on the basis of research carried out in 2007 by the Central Laboratory of MPWiK Lublin (5). For chromium the 0.1 mg · dm^{-3} concentration was accepted, whereas for cadmium 0.337 mg · dm^{-3}. It was also suitable to establish the boundary conditions. Parameters of adsorption and dispersion, presented in the Table 3, were accepted basing on numerous literature studies [11–15]. Physical-water parameters of the ground and parameters of the adsorption and the dispersion were assigned to the partuicular layers of the profile.

Table 2

Physical parameters of soils in individual layers of the studied profile

No. layer	Parameters of water retention curve		Saturated hydraulic conductivity K_{sat} [$\mathrm{m \cdot s^{-1}}$]	Porosity ε [-]	Maximum saturation S_s [-]	Residual saturation S_r [-]
	A [$\mathrm{cm^{-1}}$]	n [-]				
0	4.100	1.100	$1.00 \cdot 10^{-9}$	0.100	1.0	0.000
1	0.075	1.890	$1.23 \cdot 10^{-5}$	0.410	1.0	0.159
2	0.036	1.560	$2.89 \cdot 10^{-6}$	0.430	1.0	0.181
3	0.0065	1.325	$1.78 \cdot 10^{-7}$	0.430	1.0	0.000
4–7	0.019	1.310	$7.17 \cdot 10^{-7}$	0.410	1.0	0.232

To numerical calculations was necessary to accept initial and boundary conditions. For the water flow the initial condition of the profile's saturation 70 % was accepted. Whereas the initial concentration of pollutants in the ground 0.0 mg $\cdot$ $\mathrm{dm^{-3}}$ was assumed. Boundary conditions were founded on the surface of the street in the top of the profile which assured the tightness of the street pavement. This caused that flow of water and pollutants to the studied profile does not exist. For the water flow in nodes of the finite elements net the second kind condition 0.0 m $\cdot$ $\mathrm{d^{-1}}$ as well as a value of 0.0 m $\cdot$ $\mathrm{d^{-1}}$ for species mass transport inflow were set. In the bottom of the profile the gradient boundary condition was accepted, enabling free outflow of water and pollutants inside the profile.

Table 3

Parameters of adsorption and dispersion for the individual layers of the profile

	No. layer	Coefficient				
		Henry's coefficient	molecular diffusion [$\mathrm{m^2 \cdot s^{-1}}$]	longinear dispersion [m]	transverse dispersion [m]	first-order reaction decay constant [$\mathrm{s^{-1}}$]
Cadium	1	79.57	$1.0 \cdot 10^{-9}$	4.00	0.5	$1.727 \cdot 10^{-8}$
	2	29.70	$1.0 \cdot 10^{-9}$	36.00	0.5	$1.727 \cdot 10^{-8}$
	3	27.94	$1.0 \cdot 10^{-9}$	2.50	0.5	$1.727 \cdot 10^{-8}$
	4–7	28.16	$1.0 \cdot 10^{-9}$	1.50	0.5	$1.727 \cdot 10^{-8}$
Chromium	1	2.43	$1.0 \cdot 10^{-9}$	4.00	0.5	$2.886 \cdot 10^{-7}$
	2	0.20	$1.0 \cdot 10^{-9}$	36.00	0.5	$2.886 \cdot 10^{-7}$
	3	0.191	$1.0 \cdot 10^{-9}$	2.50	0.5	$2.886 \cdot 10^{-7}$
	4–7	0.192	$1.0 \cdot 10^{-9}$	1.50	0.5	$2.886 \cdot 10^{-7}$

The water transport in the soil profile is described by the Darcy Law (1) [5]:

$$q = -[K \cdot \nabla(\Psi + z)] \cdot n, \ [\mathrm{m \cdot d^{-1}}] \qquad (1)$$

where: K – hydraulic conductivity coefficient, [$\mathrm{m \cdot d^{-1}}$],
∇ – Hamilton's operator, [-],

Ψ – soil water potential [m],
z – height of position [m],
n – normal vector for Hamilton's operator, [-].

In order to conduct the simulation of water and pollutants outflow from the damaged gravitational sewer system pipe in place of its location the boundary conditions were set.

For the water flow the first kind condition of Head = 0.2 m was treated as the pressure of sewage in pipe, whereas for the flow of pollutants the first kind condition was understood as the concentration of the pollutant. The water flow in the ground is described in the program by Darcy's and Richard's equations [16], and the hydraulic conductivity in unsaturated state by modified version of the Van Genuchten's equation [5]:

$$K = K_{sat} \cdot S^{l}\left[1-\left(1-S^{\frac{1}{m}}\right)^{m}\right]^{2}, \ [\mathrm{m} \cdot \mathrm{s}^{-1}] \tag{2}$$

where: K_{sat} – saturated hydraulic conductivity coefficient, $[\mathrm{m} \cdot \mathrm{s}^{-1}]$,
S – effective saturation, [-],
l – exponent related with the logic diagram of pores, [-], $l = 0.5$,
$m = l - A/n$, $A = 1$ according to Mualem [17],
n – coefficient being the measure of the schedule of the pores size, [-].

The saturation in the model is described by the equation [5]:

$$S = \frac{\theta - \theta_r}{\theta_s - \theta_r}, \ [-] \tag{3}$$

where: θ – volumetric content of water in the ground $[\mathrm{m}^3 \cdot \mathrm{m}^{-3}]$,
θ_r – residual volumetric water content $[\mathrm{m}^3 \cdot \mathrm{m}^{-3}]$,
θ_s – saturated volumetric water content $[\mathrm{m}^3 \cdot \mathrm{m}^{-3}]$.

In order to read the concentrations of pollutants accepted to research in chosen places of the profile six reference points were established (Fig. 2). Points No. 1, 3 and 5 were

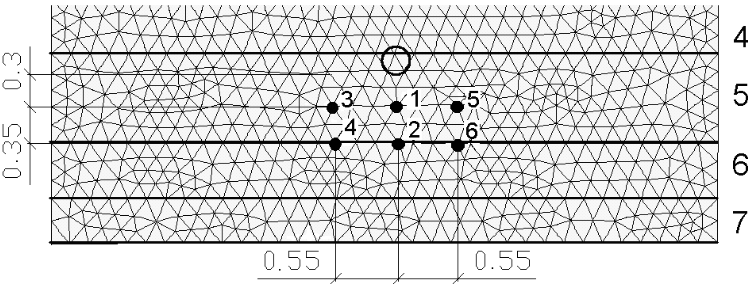

Fig. 2. Scheme of the profile with reference points

situated 0.3 m under the studied pipe and points No. 2, 4 and 6 were situated 0.55 m under this pipe.

The physical-water parameters of the soils, parameters of adsorption and dispersion introduced to the model and accepted initial and boundary conditions made possible the numerical calculations. The reference points enabled reading the pollutants concentrations in dependence on the time of the simulation.

Results

Our calculations were conducted for three different variants: variant I a period of the failure 1 day, variant II a period of the failure 2 days and variant III a period of the failure 3 days. Simulation calculations were conducted for 14 days for each variant. For each variants time-varying boundary conditions for water flow (head of 0.2 m) and for mass transport (cadmium – 0.337 mg · dm^{-3}, chromium – 0.1 mg · dm^{-3}) were set depending on the duration of the failure.

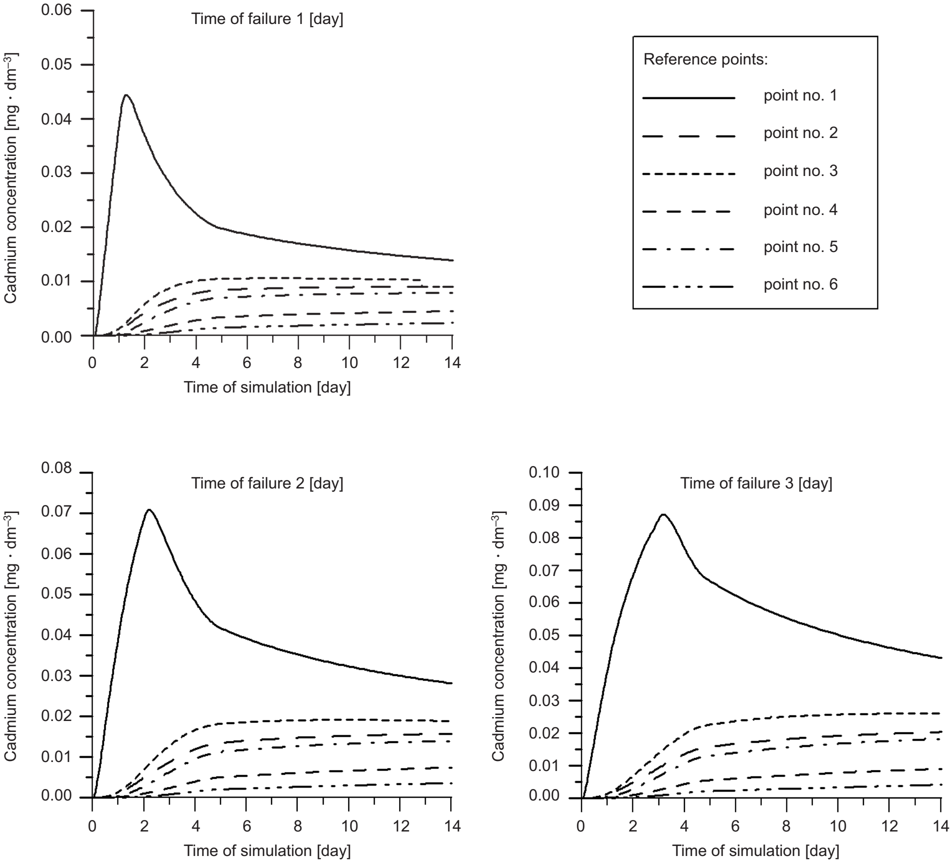

Fig. 3. Changes of the concentrations of cadmium in time

Individual computational variants allowed to reach different concentrations of chosen pollutants in the accepted reference points.

Changes of cadmium concentrations in time for accepted reference points are presented in Fig. 3. For each computational variants in point No. 1 concentration of cadmium rapidly grows, and after overrun the duration of the failure, with slight delay, gradually decreased, which was not observed in the rest of points. In remaining points the cadmium concentration after the achievement of the certain value is not prone to greater changes. Moreover, the longer duration of the failure the highest values of cadmium concentrations were obtained in the reference point No. 1. For the failure lasting 1 day concentration was 0.044 mg · dm^{-3} after the time of 1.28 day from the beginning of the simulation, for the failure lasting 2 days – 0.071 mg · dm^{-3} after the time 2.21 days, and for the failure lasting 3 days – 0.087 mg · dm^{-3} after the time of 3.19 days from the beginning of the simulation. The lowest values of the concentration of cadmium were obtained for reference points No. 6 and for the duration of the failure 1 day it was $3.86 \cdot 10^{-5}$ mg · dm^{-3} after the time of 1.28 day from the beginning of the simulation.

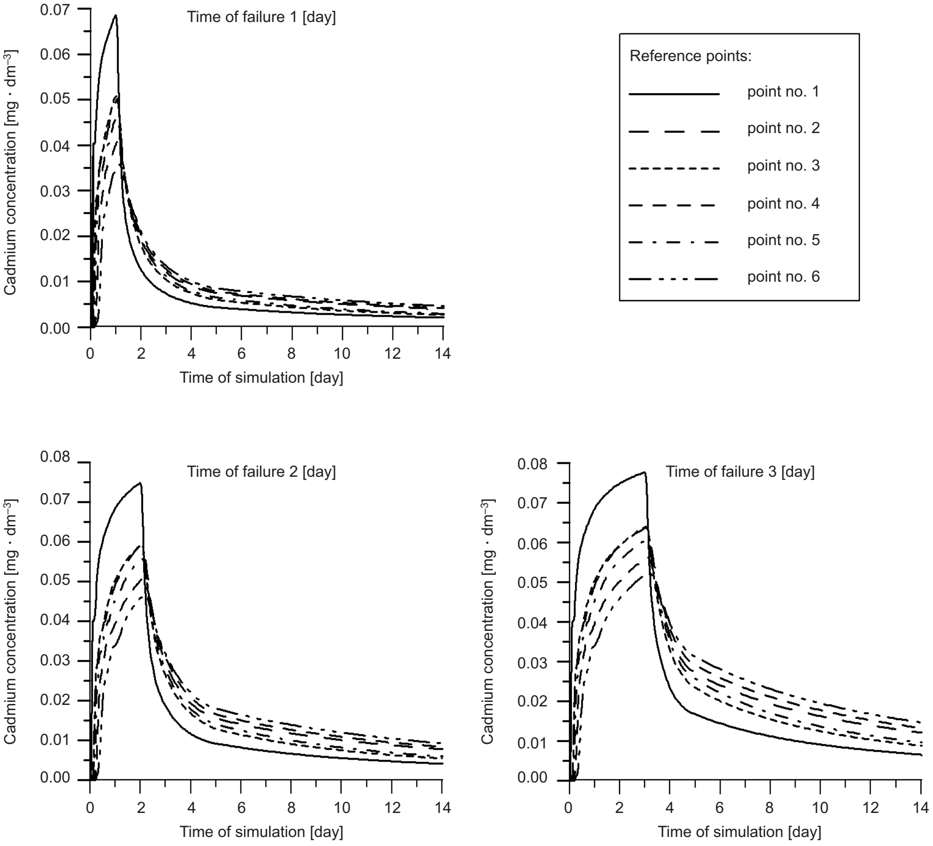

Fig. 4. Changes of the concentrations of the chromium in time

Changes of the chromium concentrations depending on the duration of the failure for the reference points are presented in Fig. 4. In each reference point, the chromium concentration in the beginning of the simulation rapidly grows, and after reaching the duration of the failure, with delay, it clearly decreases. More rapid increase causes equally significant decrease of chromium concentration. The more rapid initial increase of simulated chromium concentration, the higher later decrease of this concentration. The greatest values of concentrations aside from the computational variant were observed in the reference point No. 1 which was situated centrally under the studied pipe. For the failure lasting 1 day the highest chromium concentration was 0.69 mg · dm^{-3}, noted exactly after the time of 1 day from the beginning of the simulation. In variant No. 2 the greatest value of chromium concentration was 0.075 mg · dm^{-3} after the time 2 days, and for the variant 3 – 0.078 mg · dm^{-3} after the time 2.99 days. The least concentrations of the chromium were observed in reference point No. 6 and for the variant No. 1 after the time 1 day from the beginning of the simulation was 0.034 mg · dm^{-3}.

Conclusions

The obtained results of conducted numerical calculations allowed to draw the following conclusions:

1. Numerical calculations enable the analysis of the influence of pollutants (cadmium and chromium) leaving from the damaged gravitational sewer system pipe on the ground – water environment.
2. Numerical calculations showed that together with the growth of the duration of the failure the concentration of pollutants in the reference points increased. The longer duration of the failure, the greater concentration of pollutants in the reference points were observed. For each pollutant the highest value of the concentration was obtained at the failure lasting 3 days, for cadmium it was 0.087 mg · dm^{-3} , and for chromium 0.078 mg · dm^{-3}.
3. Obtained results of numerical calculations showed that chromium concentrations were greater in comparison with cadmium concentrations despite the fact that the chromium concentration leaving from the damaged gravitational sewer system pipe was above three times smaller than cadmium.
4. For chromium pollutant the rapid increase and the decrease of the concentration in the reference points are observed which means that it easily shifts in the ground centre and in small extent undergoes to the process of the sorption.
5. On the basis of obtained results it can be ascertained that the cadmium pollution accumulates in ground medium more than the chromium.
6. The empirical verification of the modeling results in order to verify the validity of initial and boundary conditions is highly required.

References

[1] Fonseca B, Teixeira A, Figueiredo H, Tavares T. Modelling of the Cr(VI) transport in typical soils of the North of Portugal. J Hazard Mater. 2009;167:756-762.

[2] Hossain A. Modeling nonlinearly transient zero-order bioreduction and transport of Cr(VI) in groundwater. Environ Modeling and Assess. 2005;10:315–321.

[3] Lichner L, Dlapa P, Sir M, Cipakova A, Houskova B, Fasko P, Nagy V. The fate of cadmium in field soils of the Danubian lowland. Soil & Tillage Res. 2006;85:154–165.

[4] Yolcubal I, Akyol NH. Retention and Transport of Hexavalent Chromium in Calcareous Karst Soils. Turkish J Earth Sci. 2007;16:363–379.

[5] Diersch HJG. FEFLOW 5.3 Finit Element Subsurface Flow & Transport Simulation System. Reference Manual, Berlin: WASY GmbH; 2005.

[6] Diersch HJG. FEFLOW 5.3 Finite Element Subsurface Flow & Transport Simulation System. User's Manual, Berlin: WASY GmbH; 2006.

[7] Trefry MG, Muffels Ch. FEFLOW: a finite-element ground water flow and transport modeling tool. Ground Water. 2007;45(5):525-528.

[8] Basile A, Mele G, Terribile F. Soil hydraulic behaviour of a selected benchmark soil involved in the landslide of Sarno 1998. Geoderma. 2003;117:331-346.

[9] Van Dam J, Huygen J, Wesseling J, Feddes R, Kabat P, Van Walsum P, Groenendijk P, Van Diepen C. Theory of SWAP version 2.0. Simulation of water flow, solute transport and plant growth in the Soil-Water Atmosphere-Plant environment. Wageningen: Wageningen Agricultural University and DLO Winand Staring Centre; 1997.

[10] Wessolek G, Plagge R, Leij F, Van Genuchten M. Analysing problems in describing field and labolatory measured soil hydraulic properties. Geoderma. 1994;64:93-110.

[11] Baker FG, Pavlik HF. Characterization and modelling of groundwater flow in a heterogeneous aquifer system to evaluate contaminant migration, in Fifth Canadian/American Conference on Hydrogeology. Bachu S, editor. National Water Well Association. 1990;453-458.

[12] Dunnivant FM, Jardine PM, Taylor DL, McCarthy JF. Cotransport of cadmium and hexachlorobiphenyl by dissolved organic carbon through collumns containing aquifer material. Environ Sci Technol. 1992;26(2):360–368.

[13] Huyakorn PS, Anderson PF, Motz FJ, Guver O, Melville JG. Simulations of two-well tracer tests in stratified aquifers at the Chalk River and the Mobile sites. Water Resour. Res. 1986;22(7):1016-1030.

[14] Lau LK, Kaufman WJ, Todd DK. Studies of dispersion in a radial flow system, Canal Seepage Research: Dispersion Phenomena in Flow Through Porous Media. Progress Rep. 3, I.E.R. Ser. 93, issue 3, Sanit. Eng. Res. Lab., Dep. of Eng. and School of Public Health, Berkeley: Univ of Calif; 1957.

[15] Vukovic M, Biscan JA. A contribution to environmental risk assessment for transport of cadmium through groundwater layers. Case study of the Sava River Region. Wat Res. 1998;32(12):3765-3771.

[16] Zaradny H. Matematyczne metody opisu i rozwiązań przepływu wody w nienasyconych i nasyconych gruntach i glebach, Praca IBW PAN 23, 1990.

[17] Mualem Y. A new model for predicting the hydraulic conductivity of unsaturated porous media. Wat Resouces Res. 1976;12(3):513-522.

PROCES EKSFILTRACJI Z PRZEWODÓW KANALIZACYJNYCH A ROZPRZESTRZENIANIE SIĘ WYBRANYCH ZANIECZYSZCZEŃ – BADANIA MODELOWE

Wydział Inżynierii Środowiska
Politechnika Lubelska

Abstrakt: Eksploatacja sieci kanalizacji sanitarnej związana jest z możliwością wystąpienia eksfiltracji ścieków z przewodu kanalizacyjnego do otaczającego środowiska gruntowego. Eksfiltrujące ścieki zawierające często duże stężenia zanieczyszczeń organicznych i nieorganicznych mogą stanowić zagrożenie zarówno dla jakości wód gruntowych, jak i środowiska naturalnego gleby. Monitoring jakościowo-ilościowy wód wydostających się z uszkodzonego przewodu kanalizacyjnego jest bardzo trudny, a często w warunkach terenowych niemożliwy. W związku z tym coraz większą popularność zdobywa numeryczne modelowanie rozprzestrzeniania się zanieczyszczeń w środowisku gruntowo-wodnym. Niniejsza praca przedstawia wyniki obliczeń numerycznych rozprzestrzeniania się wybranych zanieczyszczeń (kadm, chrom) wydostających się z uszkodzonego przewodu kanalizacji sanitarnej grawitacyjnej, zlokalizowanego w profilu ul. Nadstawnej w Lublinie. Obliczenia numeryczne zostały wykonane za pomocą oprogramowania FEFLOW 5.2, WASY, Niemcy. Parametry transportowe gruntów, warunki początkowe oraz warunki brzegowe zostały wyznaczone na podstawie wyników badań literaturowych, laboratoryjnych i terenowych. Dane z obliczeń umożliwiają analizę wpływu awarii przewodów kanalizacyjnych na środowisko glebowe i wody gruntowe. Obliczenia symulacyjne należy poddać weryfikacji empirycznej w celu potwierdzenia prawidłowości przyjętych warunków początkowych i brzegowych.

Słowa kluczowe: eksfiltracja, rozprzestrzenianie się zanieczyszczeń, modelowanie numeryczne

DOI: 10.2428/ecea.2012.19(03)034 ECOL CHEM ENG A. 2012;19(3):321-326

Elżbieta HUZAR[1], Alicja WODNICKA[1]
and Małgorzata DZIĘCIOŁ[1]

WINTER WINDSCREEN WASHING LIQUIDS AS A SOURCE OF HUMAN EXPOSURE TO ALCOHOLS

ZIMOWE PŁYNY DO SPRYSKIWACZY JAKO ŹRÓDŁO NARAŻENIA CZŁOWIEKA NA DZIAŁANIE ALKOHOLI

Abstract: Analyses of 12 winter windscreen washing liquids available on the home market were performed. Ethanol, methanol and 2-propanol were determined. Results of the investigations indicated that in the all samples ethanol was present. Its content in the majority of tested winter windscreen washing liquids was about 30–50 %. In four samples methanol was determined. Methanol content in one sample was very high and achieved nearly 40 %. One sample contained about 11 % of 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. People generally spend more than 80 % of their time in an indoor environment such as residential and office buildings, shopping centers, etc [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.

The air pollution accumulated inside a car cabin very often consists of gasoline and diesel exhaust. This toxic mixture of gases, aerosols, and microscopic particles includes carbon monoxide, nitrogen oxides, particulate matter, and a host of other hazardous chemicals, such as formaldehyde, 1,3-butadiene, and aromatic hydrocarbons (benzene, toluene, ethylbenzene and xylenes, BTEX) [5]. All the substances may influence harmfully the environment and human health. According to accessible literature, in car's cabin the other volatile substances such as acetone, ethanol, limonene, 2-propanol,

---

[1] Institute of Organic Chemical Technology, West Pomeranian University of Technology, al. Piastów 42, 71–065 Szczecin, Poland, phone: +48 91 449 45 12, email: elzbieta.huzar@zut.edu.pl

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.

During intensive use of these liquids the concentrations of alcohols inside a car cabin may reach high values. The above compounds have harmful impact on a human body that may give 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 endangers 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 thermostat chamber at the temperature of 50 ^oC. 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 chromatograph equipped with a *flame-ionization detector* (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 ^oC, injector 120 ^oC, detector 150 ^oC. 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, $\geq$ 99.9 %). The identification of the alcohols was confirmed using an Agilent 6890N gas chromatograph with a 5973N mass selective detector, equipped with a 7683 series injector. In order to prepare samples for the GC-MS analyses, the windscreen washing liquids were dissolved in butyl acetate. A HP-5MS capillary column with 5 % phenylmethylpolysiloxane, 30 m × 0.25 mm I.D. and 0.25 μm film thickness was used with the following temperature program: 50 ^oC (hold 5 min), 10 $^oC\ min^{-1}$ to 280 ^oC (hold 2 min). Helium was used as carrier gas at a constant flow rate of 1.2 $cm^3\ min^{-1}$. The injection port temperature was 250 ^oC, 2 mm^3 of solutions was injected via split (1:10) injection. The mass selective detector conditions were as follows: electron impact ionization, full scan mode (10–200 m/z), MSD transfer line temperature: 280 ^oC, MS quad: 150 ^oC; MS source: 230 ^oC. The chromatographic data of the windscreen washing liquids components are shown in Table 1.

Table 1

Retention time and mass fragmentation patterns of the windscreen washing liquids components

Substance	Retention time [min]		Mass/charge (relative intensity)
	GC-FID	GC-MS	
Methanol	2.50	1.38	32 (M^+, 72), 31 (100), 29 (60), 15 (12)
Ethanol	6.10	1.44	46 (M^+, 24), 45 (67), 43 (15), 31 (100), 29 (31), 27 (22)
2-Propanol	12.50	1.50	59 (M^+–1, 5), 45 (100), 43 (23), 41 (9), 39 (8), 29 (9), 27 (9)

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. The aim of this was to eliminate the errors resulting from adsorption on the walls of the container. In order of that, 5 mm^3 of liquid mixture containing methanol, ethanol, 2-propanol (0.13, 0.13, and 0.26 g · cm^{-3}, respectively) was injected into 1114 cm^3 container, tightly closed with the screw cap containing the silicon membrane. The further procedure was as for the samples of the windscreen washing liquids (evaporation at 50 °C, stabilization at the room temperature). After equilibration the gas mixture of 0.4; 0.8; 1.2 and 1.6 cm^3 volume were drawn with a Hamilton gas-tight syringe and analyzed on a gas chromatograph. Correlation coefficients of calibration curves for determined compounds were in the range of 0.9953–0.9967.

Alcohols content C in the examined products was calculated from the following equation:

$$C = \frac{m \cdot V_C}{V_S \cdot V_R \cdot d} \cdot 100 \text{ [\% vol.]}$$

where: m – mass of alcohol calculated from calibration curve [mg],
V_C – volume of the container [cm^3],
V_S – volume of gas sample injected to the chromatographic column [cm^3],
V_R – volume of windscreen washing liquid injected into container [mm^3],
d – density of alcohol [g · cm^{-3}].

Results and discussion

The analyses of 12 winter windscreen washing liquids available on home market were performed. Results of the quantitative analyses of alcohols content in the examined samples are given in Table 2.

Results of the investigations indicated that in the all samples ethanol was present. Its content in the majority of tested 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. Application of windscreen washing liquids with high ethanol content may influence the cabin air quality. High ethanol concentration in air may have an effect on decrease of concentration and reaction time extension of drivers.

Table 2

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

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 Fig. 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 %). WL6 sample was a concentrate to mix with water, which helps to prevent windscreen washer freeze. Without water addition, it protects to temperature –70 ^{o}C. All remaining winter windscreen washing liquids can be used to temperatures –20, –22 and –25 ^{o}C.

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 composi-

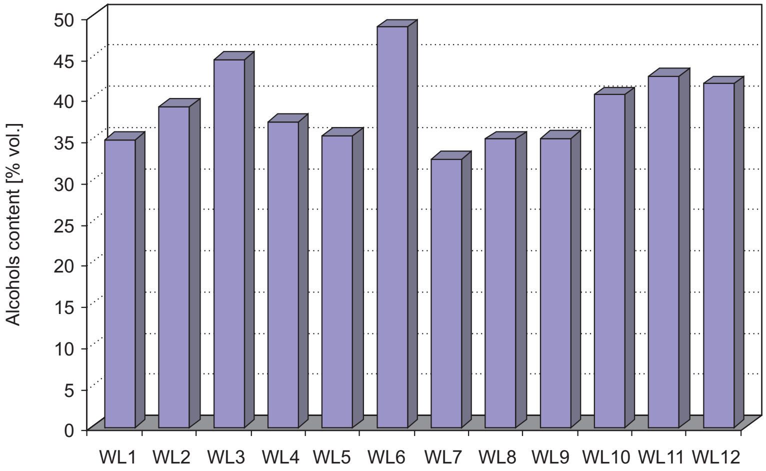

Fig. 1. Summary alcohols (methanol, ethanol and 2-propanol) content in winter windscreen washing liquids

tion 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 3.

Table 3

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

The mean alcohols recovery for the analysed products was 101.49 %. The lowest recovery was obtained for 2-propanol (94.76 %). The average recoveries of methanol and ethanol were a bit higher and achieved respectively 106.43 and 99.46 %. It may be caused by higher boiling temperature of 2-propanol than boiling temperatures of methanol and ethanol. Another cause of the lowest 2-propanol recovery may be its low content in a sample.

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 %.

References

[1] Li FB, Li XZ, Ao CH, Lee SC, Hou MF. Chemosphere. 2005;59:787-800.

[2] Zabiegała B, Partyka M, Namieśnik J. Jakość powietrza wewnętrznego – analityka i monitoring. In: Nowe horyzonty i wyzwania w analityce i monitoringu środowiskowym. Gdańsk: Centrum Doskonałości Analityki i Monitoringu Środowiskowego; 2003.

[3] Wang S, Ang HM, Tade MO. Environ Int. 2007;33:694-705.

[4] Nagda NL, Rector HE. Indoor Air. 2003;13:292-301.

[5] In-Car Air Pollution The Hidden Threat to Automobile Drivers, Report No. 4, An assessment of the Air Quality Inside Automobile Passenger Compartments, The International Center for Technology Assessment, Washington 2000.

[6] Toksykologia. Seńczuk W, editor. Warszawa; Wyd. Lekarskie PZWL: 2002.

ZIMOWE PŁYNY DO SPRYSKIWACZY JAKO ŹRÓDŁO NARAŻENIA CZŁOWIEKA NA DZIAŁANIE ALKOHOLI

Instytut Technologii Chemicznej Organicznej
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Abstrakt: Przedmiotem badań było 12 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. Jego zawartość w większości zimowych płynów do spryskiwaczy wynosiła 30–50 %. Metanol zidentyfikowano w czterech próbkach, przy czym w jednej z próbek jego zawartość była bardzo duża i wynosiła prawie 40 %. Jeden z produktów zawierał około 11 % 2-propanolu. 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

Varia

Invitation for ECOpole ’12 Conference

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 21st annual Central European Conference ECOpole ’12, which will be held in 11–13 X 2012 (Thursday–Saturday) at the Conference Center “Rzemieslnik” in Zakopane, PL.

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

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

The Conference language is English.

Contributions to the Conference will be published as:

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

Additional information one could find on the Conference website:

ecopole.uni.opole.pl

The deadline for sending the Abstracts is **15th July 2012** and for the Extended Abstracts: **1st October 2012**. The actualized list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one can find (starting from **31st July 2012**) on the Conference website.

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

At the Reception Desk each participant will obtain abstracts of the Conference contributions as well as the Conference Programme recorded on electronic media (the Programme will be also published on the ECOpole '12 website).

Further information is available from:
Prof. dr hab. eng. Maria Wacławek
Chairperson of the Organising Committee
of ECOpole '12 Conference
Opole University
email: Maria.Waclawek@o2.pl
and mrajfur@o2.pl
phone +48 77 455 91 49 and +48 77 401 60 42
fax +48 77 401 60 51

Conference series

1. 1992 Monitoring '92 Opole
2. 1993 Monitoring '93 Turawa
3. 1994 Monitoring '94 Pokrzywna
4. 1995 EKO-Opole '95 Turawa
5. 1996 EKO-Opole '96 Kędzierzyn Koźle
6. 1997 EKO-Opole '97 Duszniki Zdrój
7. 1998 CEC ECOpole '98 Kędzierzyn-Koźle
8. 1999 CEC ECOpole '99 Duszniki Zdrój
9. 2000 CEC ECOpole 2000 Duszniki Zdrój
10. 2001 CEC ECOpole '01 Duszniki Zdrój
11. 2002 CEC ECOpole '02 Duszniki Zdrój
12. 2003 CEC ECOpole '03 Duszniki Zdrój
13. 2004 CEC ECOpole '04 Duszniki Zdrój
14. 2005 CEC ECOpole '05 Duszniki Zdrój
15. 2006 CEC ECOpole '06 Duszniki Zdrój
16. 2007 CEC ECOpole '07 Duszniki Zdrój
17. 2008 CEC ECOpole '08 Piechowice
18. 2009 CEC ECOpole '09 Piechowice
19. 2010 CEC ECOpole '10 Piechowice
20. 2011 CEC ECOpole '11 Zakopane

GUIDE FOR AUTHORS

A digital version of the manuscript should be sent to:

Prof dr hab. Witold Wacławek,
Editor-in-Chief of Ecological Chemistry and Engineering A
Uniwersytet Opolski
ul. kard. B. Kominka 6
45–032 Opole
Poland
phone +48 77 401 60 42, +48 77 455 91 49
Fax +48 77 401 60 51
email: maria.waclawek@o2.pl
mrajfur@o2.pl

The Editor assumes, that an author submitting a paper for publication has been authorised to do that. It is understood the paper submitted to be original and unpublished work, and is not being considered for publication by another journal. After printing, the copyright of the paper is transferred to *Towarzystwo Chemii i Inżynierii Ekologicznej* (*Society for Ecological Chemistry and Engineering*).

"*Ghostwriting*" and "*guest authorship*" are a sign of scientific misconduct. To counteract them, please provide information, for the Editor, on the percentage contribution of individual Authors in the creation of publications (including the information, who is the author of concepts, principles, methods, etc.). Editorial Board believes that the main responsibility for those statements bears the Author sending the manuscript.

In preparation of the manuscript please follow the general outline of papers published in *Ecological Chemistry and Engineering A*, available on the website:

tchie.uni.opole.pl

a sample copy can be sent, if requested.

Papers submitted are supposed to be written in English language and should include a summary and keywords, if possible also in Polish language.

Generally, a standard scientific paper is divided into:

– Introduction: you present the subject of your paper clearly, indicate the scope of the subject, present state of knowledge on the paper subject and the goals of your paper;

– Main text (usually divided into: Experimental – you describe methods used; Results and Discussion);

– Conclusions: you summarize your paper;

– References.

The first page should include the author's (authors') given name(s) without titles or scientific degrees like Prof., Ph.D., etc., their affiliations, phone and fax numbers and their email addresses however, with the corresponding author marked by an asterisk.

It is urged to follow the units recommended by the *Systéme Internationale d'Unites* (SI). Graph axis labels and table captions must include the quantity units.

Symbols recommended by the International Union of Pure and Applied Chemistry (Pure and Appl Chem. 1979;51:1-41) are to be followed. Graphics (drawings, plots) should be supplied in the form of digital vector-type files, *eg* CorelDraw v.9, Excel, Inkscape or at least in a bitmap format (TIF, JPG) 600 DPI. In the case of any query please feel free to contact with the Editorial Office. Footnotes, tables and graphs should be prepared as separate files. References cited chronologically should follow the examples given below:

[1] Lowe DF, Oubre CL, Ward CH. Surfactants and cosolvents for NAPL remediation. A technology practices manual. Boca Raton: Lewis Publishers; 1999.

[2] Fasino CR, Carino M, Bombelli F. Oxidant profile of soy standardized extract. In: Rubin R, Stryger CS, editors. Joint Meeting 2001 – Book Abstracts '2001 Year of Natural Products Research'. New York: Harper and Row; 2001.

[3] Wosiński S. Effect of composition and processing conditions of ceramic and polymer composites on the electric field shielding ability [PhD Thesis]. Poznań: Poznan University of Technology; 2010.

[4] Trapido M, Kulik N, Veressinina Y, Munter R. Water Sci Technol. 2009;60:1795-1801. DOI: 10.2166/wst.2009.585.

[5] Cañizares P, Lobato J, Paz R, Rodrigo MA, Sáez C. Chemosphere. 2007;67:832-838. DOI: 10.1016/j.chemosphere.2006.10.064.

[6] Hakala M, Nygård K, Manninen S, Huitari S, Buslaps T, Nilsson A, et al. J Chem Phys. 2006:125:084504-1-7. DOI: 10.1063/1.2273627.

[7] Simeonov D, Spasov L, Simeonova P. Statistical calibration of model solution of analytes. Ecol Chem Eng A. Forthcoming 2012.

Journal titles should preferably follow the Chem. Abst. Service recommended abbreviations.

Each publication is evaluated by at least two independent reviewers from outside of the unit.

Receipt of a paper submitted for publication will be acknowledged by email. If no acknowledgement has been received, please check it with the Editorial Office by email, fax, letter or phone.

REDAKTOR TECHNICZNY
Halina Szczegot

SKŁAD I ŁAMANIE
Jolanta Brodziak

PROJEKT OKŁADKI
Jolanta Brodziak

Druk: „Drukarnia Smolarski”, Józef Smolarski, 45–326 Opole, ul. Sandomierska 1. Objętość: ark. wyd. 14,25, ark. druk. 11,75 Nakład: 350 egz. + 5 nadb. aut.