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Redakcja

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SPIS TREŚCI

VARIA

Vol. 17, No. 4 2010

Joanna ZEMBRZUSKA[1*] and Henryk MATUSIEWICZ[1]

DETERMINATION OF SELECTED SELENOAMINO ACIDS IN BEER BY CAPILLARY ISOTACHOPHORESIS

OZNACZANIE WYBRANYCH SELENOAMINOKWASÓW W PIWIE TECHNIKĄ IZOTACHOFOREZY

Abstract: A rapid, simple and reliable capillary isotachophoresis (ITP) with conductivity detection was used to determine the trace quantities of organic compounds of selenium - selenomethionine (Se-Met) and selenocystine (Se-Cys) - in various types of beer. The content of Se-Met in beer is between $59 \cdot 10^{-3}$ g dm^3 (Czarne beer) and $510 \cdot 10^{-3}$ g dm^3 (Żywiec Porter beer), while the content of Se-Cys ranges from $84 \cdot 10^{-3}$ g dm^3 (Czarne) to $247 \cdot 10^{-3}$ g dm^3 (Żywiec Porter).

Keywords: isotachophoresis, beer, selenomethionine, selenocystine

Selenium is found in organisms as a trace element, though it is indispensable to their correct functioning. It is supplied in organic form, mainly as selenomethionine and selenocysteine, as well as inorganic form, as selenate(VI), M_2SeO_4 and selenate(IV), $MSeO_3$. However, various selenium derivatives of sulphuric amino acids have been detected in plants and animals [1]. Selenium facilitates assimilation of vitamin E and regulates its physiological functions. This element is indispensable in the work of the heart muscle and blood vessels, stimulates the immune system and retards tissue aging processes. Besides taking part in enzymatic reactions protecting cells from the effects of free radicals, selenium has immunodulatory, anti-inflammatory and antiviral effects. It protects the organism from poisoning by heavy metals, eg Fe, Cd or Pb, by forming metal selenides (M_2Se) with them. It has also been found to have anticarcinogenic (antitumor) properties, so it plays an important role in the prevention of neoplasmic diseases [2].

In organisms selenium plays mainly a biochemical role, as a component of enzymic proteins. There is no more comprehensive evidence for potential protection against tumours by different diet components than that which concerns selenium [3]. In over 90% of scientific research on the anticarcinogenic effects of selenium, selenate(IV) or

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selenomethionine (Se-Met) are used. It has been proved that Se-Met is much less toxic than inorganic selenium compounds [4].

In 1996 Clark et al [5] discovered that supplementing the diet of a group of people with selenized brewer's yeast caused a decrease of nearly 50% in overall cancer morbidity and mortality.

Organisms take selenium primarily with their food. Popular products considered rich in selenium are yeast and garlic [6]. Moreover, in natural food products selenium most often occurs in chemical combination with proteins, so food products with high protein content, such as meat, fish, fish products and especially offal, are the richest sources of selenium. Selenium-rich food products most often mentioned in other studies include tomatoes, cucumbers, broccoli, cabbage, celery, onion, egg yolk, bran, wheat, barley and shellfish [3].

Due to their anticarcinogenic effects, selenoamino acids constitute a highly significant group of compounds, so their determination in foods is very important. Nearly all separation techniques are used to analyse selenoamino acids. Good results are achieved using such techniques as gas chromatography (GC), high performance liquid chromatography (HPLC), and capillary electrophoresis (CE) [7]. Many papers describe the coupling techniques used to separate selenoamino acids, mainly selenomethionine and selenocystine, in such products as garlic, onion or yeast. These are GC coupled to mass spectrometry (MS) [8] and HPLC coupled to mass spectrometer and inductively coupled plasma (ICP MS) [9-14]. Techniques used for selenium speciation are mainly ion chromatography with a variety of detectors: atomic absorption spectrometry (AAS) [15], inductively coupled plasma emission spectrometry [4, 16], hydride generation atomic absorption spectrometry (HG-AAS) [17], ultraviolet treatment-hydride generation atomic fluorescence spectrometry (UV-HG-AFS) [18-20] or hydride generation inductively coupled plasma mass spectrometry [21] and capillary electrophoresis coupled mainly with ICP MS [1, 22-25] and electrospray ionisation mass spectrometry (ESI MS) [22, 26].

Isotachophoresis is an electroseparation technique based on differences of migration velocities of analytes in an electrical field. In the ITP mode, only cations or anions can be separated. After analytes are introduced between two different boundaries, a leading electrolyte (LE) and a terminating electrolyte (TE), the sharp focsusing of individual zones is observed. If a steady state is attained, and zones are separated, all zones will be moving at a constant migration velocity [27].

This technique is used to a much lesser extent, despite its advantages of low cost, minimal or no sample preparation, especially in the case of the samples with complex matrix, and easy miniaturization.

TPI is a powerful technique for analyzing not only simple inorganic or organic samples but also biochemical samples. For example this technique has been applied to the analysis of amino acids. In most cases analysis has been performed on acidic amino acids as anions. A wide range of amino acids were investigated with a variety of aqueous electrolyte systems with alkaline pH levels [7, 28, 29].

In the literature on using ITP in determining amino acids there is only one paper [28], concering selenoamino acids. Using a ITP - capillary zone electrophoresis (CZE) coupling technique miniaturized system they determined Se-Met, selenoethionine (Se-Et), and Se-Cys, at a level of several micromoles per litre. The isotachophoresis was used to distribute and preseparate the sample components.

In the literature on selenium content in food there are no publications concerning selenoamino acids concentration in beer, despite the fact that beer is undoubtedly a rich source of this compound. For this reason the present study aimed to work out an analytical method using isotachophoresis, a relatively fast and low-cost technique, to determine Se-Met and Se-Cys in selected types of beer.

Materials and methods

Apparatus, reagents and samples

Isotachophoretic separations were performed using the Electrophoretic Analyser EA 102 (Villa Labeco, Spisska Nova Ves, Slovakia) equipped with column-coupling system consisting of two capillaries with conductivity detectors. The analytical capillary (160 x 0.3 mm ID) was connected with a preseparation capillary (90 x 0.8 mm ID). Capillaries of fluorinated ethylene-propylene copolymer were used. The analyser was equipped with a sample valve of 30 mm^3 fixed volume. Separations were performed at a room temperature.

5-bromo-2,4-dihydroxybenzoic acid and seleno-L-methionine (98%) (Se-Met) from Sigma-Aldrich (Steinheim, Germany), ethanolamine, 1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP), Mowiol (40-88), β-alanine and seleno-L-cystine (98%) (Se-Cys), sodium hydroxide (30%) all from Fluka (Buchs, Switzerland), methanol from J.T. Baker (Holland) and barium hydroxide from Chem-Lab (Belgia). All chemicals were of analytical grade. Deionized bidistilled water was used in the preparation of the electrolyte systems, stock solutions of all standards and beer samples.

Beers in bottles (Table 1) were purchased from local retail outlets.

Table 1

Beers

Name	Kind	Producer (importer)	Brewing place and racking	Extract contents [%]
MILLER	light	Brewing Company S.A.	Italy	11.2
PILSNER URQUELL	light	Brewing Company S.A.	Czech Republic	11.8
PERONI NASTRO AZZURRO	light	Brewing Company S.A.	Italy	lack of data
LECH PREMIUM	light	Brewing Company S.A.	Poland	11.1
DOG IN THE FOG	light	Brewing Company S.A.	Poland	lack of data
TYSKIE	light	Brewing Company S.A.	Poland	11.7
REDD'S	light	Brewing Company S.A.	Poland	lack of data
ŻYWIEC PORTER	dark	Brewing Group Żywiec S.A.	Poland	22
CZARNE	dark	Brewing „FORTUNA" Sp.zo.o.	Poland	12.7

Isotachophoretic conditions

Composition of the electrolyte system used for performing isotachophoretic separation is shown in Table 2.

Table 2

Composition of the electrolyte system used for performing isotachophoretic selenoamino acids separations

Leading electrolyte	Leading ion	4 mM 5-bromo 2,4-dihydroxybenzoic acid
	Counter ion	ethanolamine
	Co-counter ion	2 mM BTP
	Additive*	0.05% Mowiol (40-88)
	pH	9.4
Terminating electrolyte	Terminating anion	10 mM β-alanine
	Counter ion	Ba^{2+}[added as $Ba(OH)_2$]
	pH	10.5

*To suppress the electroosmotic flow

Prior to use, all buffer solutions were degassed with an ultrasonic bath Model Polsonic 3 (Polsonic, Poland). The separations performed were achieved using control program shown in Table 3.

Table 3

Separation program used for performing ITP

Step	Time [s]	Current [μA]
1	300	250
2	300	250
3	70	250
4	150	50
5	700	50

Sample preparation

Standard solutions of selenomethionine and selenocystine were prepared by dissolving $2 \cdot 10^{-3}$ g of each preparation in 10 cm^3 of water. Working standards solutions (from $1 \cdot 10^{-3}$ to $15 \cdot 10^{-3}$ $g \cdot dm^{-3}$) were freshly prepared daily by diluting appropriate aliquots of the stock solutions in water.

For ITP analysis beer samples were diluted 1:20 for bright beers and 1:40 for dark beers respectively, degassed in an ultrasonic bath for 70 min to remove CO_2 and adjusted to pH 9.5 by addition of 30% NaOH. Then the water was added to give a final volume of 10 cm^3.

Results and discussion

Choosing electrolytes

For isotachophoretic determination of selenoamino acids the electrolyte system developed by Evearst et al [7] was used for amino acids separation and later used for preliminary separation of synthetic mixtures of these compounds by Grass et al [28] using ITP technique. Since pH of these solutions is high there is a possibility of carbon dioxide absorption from the air leading to carbonate formation during isotachophoretic separation. This problem (to minimize interference due to this absorption) was solved adding 5-bromo-2,4-dihydroxybenzoic acid (leading ion of a mobility similar to carbonates) to the leading buffer while the terminating buffer was prepared using barium ions from barium hydroxide being the counter ion.

During preliminary experiments it was found that time elapsed since the preparation of electrolytes influenced the shape of a blank test curve on the recorded isotachophoregrams. The longer the time the smaller the difference of height between leading and terminating electrolyte showing additional steps most probably being products of electrolyte decomposition. This problem was solved by freezing fresh electrolytes and taking each time only the amount necessary for current experiments.

Isotachophoretic analysis

For the chosen electrolyte system (Table 2) isotachophoretic separation conditions for selenoamino acids mixture were optimized. Figure 1 shows recorded isotachophoregram with steps corresponding to selenocystine and selenomethionine, respectively. Other steps correspond to impurities of buffer solutions. The relative step heights (RSHs), counted as the ratio of the step height of the analyte to the step height of the terminator, values being the base for qualitative analysis were determined for both selenoamino acids. For Se-Met RSH is 0.60 while for Se-Cys RSH is 0.17.

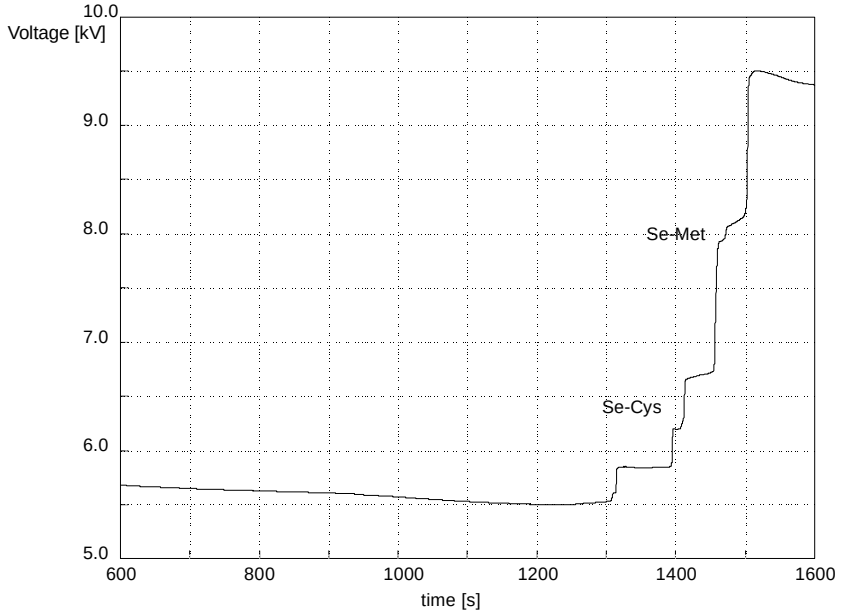

Fig. 1. Isotachopherogram of sample containing $1 \cdot 10^{-4}$ g·dm^{-3} Se-Met and Se-Cys. The experimental conditions employed are detailed in Tables 2 and 3

Quantitative analysis was performed using standard addition method that minimizes the matrix influence on results of determination. Usually selenoamino acid content was first estimated using calibration curve.

Calibration using standard addition method was performed for beers preparing beer solutions with 25, 50 and 100% addition of Se-Met and Se-Cys.

Following validation parameters were determined using calibration curves (standard solutions concentrations 1, 2.5, 5, 7.5, 10, $15 \cdot 10^{-3}$ g·dm^{-3}): linearity, detection limit and

quantification limit. Though calibration curves for Se-Cys and Se-Met (Table 4) show high correlation coefficients suggesting linearity of detector response in the selenoamino acids concentration range examined, linearity of detector response towards these analytes was determined. For this purpose for each signal recorded during preparation of calibration curves concentrations of selenoamino acids were calculated using calibration curve equations. Calculated concentrations were compared with expected values (known concentrations of the solutions used in preparation of calibration curve). Calculated values show that standard solutions having concentration $1 \cdot 10^{-3}$ and $2.5 \cdot 10^{-3}$ g·dm^{-3} are outside linearity range of the detector being calibrated.

Table 4

Regression parameters calculated from Se-Cys and Se-Met calibration curve

	Se-Cys	**Se-Met**
Parameter	Value	Value
Slope [s/10^{-3}g·dm^{-3}]	0.89	0.91
y-intercept [s]	1.14	1.08
Correlation coefficient	0.97	0.99
Standard deviation for slope coefficient [10^{-3}g·dm^{-3}]	0.03	0.02
Standard deviation for y-intercept [s]	0.26	0.16
Residual standard deviation [10^{-3}g·dm^{-3}]	0.81	0.51

Detection limits for Se-Cys and Se-Met were calculated using calibration curves. Regression parameters in Table 4 were calculated using appropriate functions of MS EXCEL program.

Using calibration curve parameters and the following equation: LOD = (3.3s)/b (where b is the slope coefficient of the calibration curve, s is the residual standard deviation for calibration curve (s_{xy}) or the residual standard deviation for y-intercept of calibration curve (s_a)), the following values of LOD were calculated taking into account residual standard deviation for y-intercept - s_a as well as residual standard deviation for slope coefficient - s_{xy}: s_a - LOD = $0.96 \cdot 10^{-3}$ g·dm^{-3} Se-Cys, s_a - LOD $0.59 \cdot 10^{-3}$ g·dm^{-3} Se-Met, s_{xy} - LOD = $2.99 \cdot 10^{-3}$ g·dm^{-3} Se-Cys, s_{xy} - LOD = $1.84 \cdot 10^{-3}$ g·dm^{-3} Se-Met.

Apparent difference between the calculated values is correct. Taking into account residual standard deviation gives higher LOD value because in this case not only y-intercept variation but also variation of slope coefficient is taken into consideration. Therefore, the mean value of measurements can be used as the determined LOD value: LOD ≈ $2 \cdot 10^{-3}$ g·dm^{-3} Se-Cys, LOD ≈ $1.2 \cdot 10^{-3}$ g·dm^{-3} Se-Met.

Using these values the limit of quantification (LOQ), ie the smallest amount or concentration of analyte that can be quantified using this method was calculated according to the equation: LOQ = 3 LOD. The calculated limit of quantification is $6 \cdot 10^{-3}$ g·dm^{-3} for Se-Cys and $3.6 \cdot 10^{-3}$ g·dm^{-3} for Se-Met. These values explain the source of so high deviation from linearity for calibration curve points in the low concentration range.

Selenoamino acids determination in various beers

The aim was to determine selenoamino acids concentrations in various beers. Before isotachophoretic analysis beer was prepared according to the procedure described in section

Sample preparation. Examples of isotachophoregrams for Miller and Żywiec Porter beer are shown in Figures 2 and 3. Se-Cys and Se-Met concentration was determined using on the basis of standard additions. Results obtained for Se-Cys and Se-Met are given in Table 5.

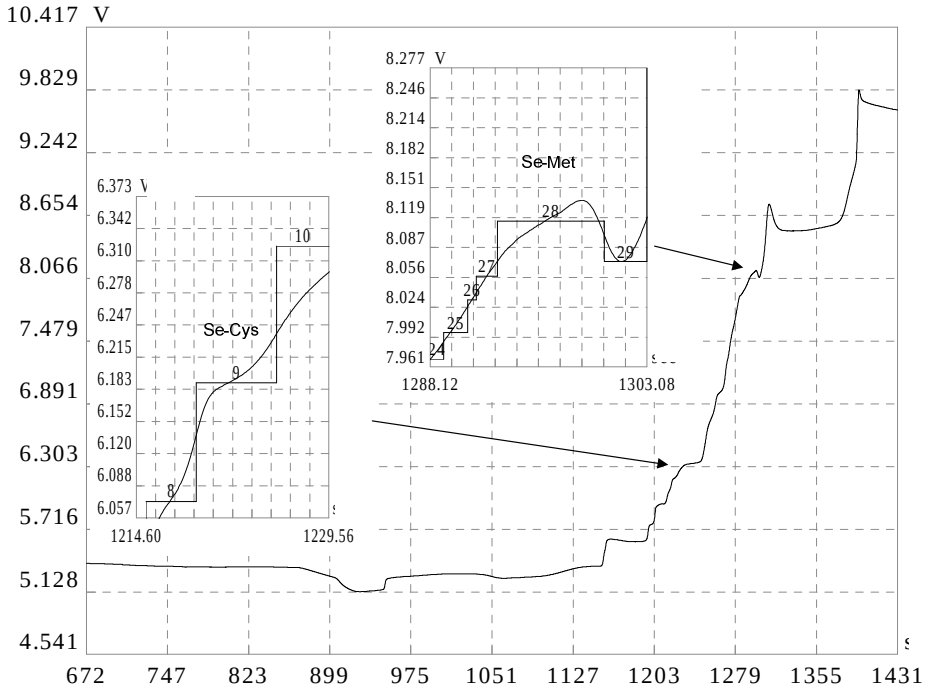

Fig. 2. Isotachopherogram of Miller beer, records of analytical capillary. The experimental conditions employed are detailed in Tables 2 and 3

Analyzing concentrations of Se-Cys obtained it was found that Redd's is the beer containing lowest concentration of this amino acid ($43.5 \cdot 10^{-3}$ g·dm^{-3}). Traditional light beers brewed in Poland, namely Lech Premium and Tyskie Se-Cys concentration was $100 \cdot 10^{-3}$ g·dm^{-3}. No such correlation (similar Se-Cys concentration in the same kind of beer from the same country) was found for Italian beers Miller ($120 \cdot 10^{-3}$ g·dm^{-3}) and Peroni Nastro Azzuro ($85 \cdot 10^{-3}$ µg·dm^{-3}). The picture is the same for sweet beers brewed in the same brewery: Dog In The Fog ($220 \cdot 10^{-3}$ g·dm^{-3}) and Redd's ($44 \cdot 10^{-3}$ g·dm^{-3}). The highest content of Se-Cys was found in dark beer Żywiec Porter ($247 \cdot 10^{-3}$ g·dm^{-3}).

A correlation between Se-Cys concentration and malt content can be observed (Table 1). Beers having malt content of 11% have Se-Cys concentration about $110 \cdot 10^{-3}$ g·dm^{-3} while beers having 22% of malt have about $240 \cdot 10^{-3}$ µg·cm^{-3} of Se-Cys.

The best repeatability was found for Miller beer having standard deviation of ± 2%, while the worst repeatability was found for Redd's beer with residual standard deviation ± 19%.

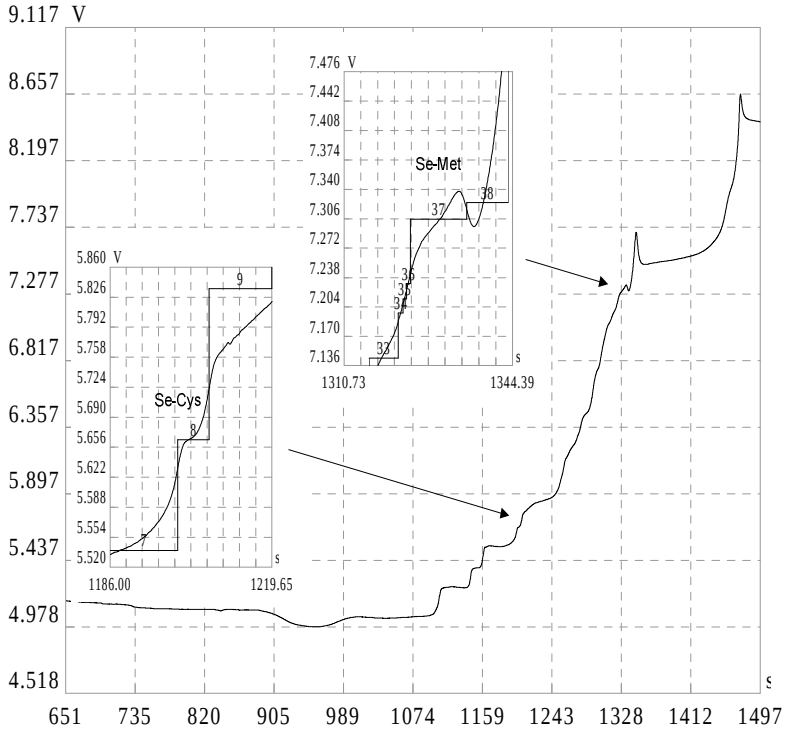

Fig. 3. Isotachopherogram of Żywiec Porter beer, records of analytical capillary. The experimental conditions employed are detailed in Tables 2 and 3

Table 5

Comparison of the content of Se-Met and Se-Cys in different types of beer

Beer	Se-Met			Se-Cys		
	Concentration [10^{-3} g·dm^{-3}]	SD	RSD [%]	Concentration [10^{-3} g·dm^{-3}]	SD	RSD [%]
MILLER	139.5	6.95	5.0	119.8	1.91	1.6
PILSNER URQUELL	165.6	8.67	5.2	132.3	7.66	5.8
PERONI NASTRO AZZURRO	131.5	19.41	14.8	84.6	9.08	12.1
LECH PREMIUM	145.1	7.40	5.1	99.4	5.4	5.4
DOG IN THE FOG	234.2	4.16	1.8	219.5	10.13	4.6
TYSKIE	83.5	10.86	13.0	92.5	4.69	5.1
REDD'S	88.8	10.05	11.3	43.5	8.29	19.1
ŻYWIEC PORTER	510.4	21.9	4.3	246.5	11.0	4.5
CZARNE	58.7	10.39	17.7	84.0	7.30	8.7

In the case of Se-Met (Table 5) Czarne is the beer having the lowest content of that substance ($59 \cdot 10^{-3}$ g·dm^{-3}) while dark beer Żywiec Porter which had the highest content of

Se-Cys has the highest content of that Se-Met too ($510 \cdot 10^{-3}$ g·dm^{-3}). Much similar content of Se-Met was found in beers brewed in Italy: Miller ($140 \cdot 10^{-3}$ g·dm^{-3}) and Peroni Nastro Azzurro ($132 \cdot 10^{-3}$ g·dm^{-3}), Czech beer Pilsner Urquell ($166 \cdot 10^{-3}$ g·dm^{-3}) and Polish beer Lech Premium (145 g·dm^{-3}). High variation in content of the examined amino acid was found for tow sweet beers from the same brewery: Dog In The Fog ($234 \cdot 10^{-3}$ g·cm^{-3}) i Redd's ($89 \cdot 10^{-3}$ g·dm^{-3}). The same as in the case of Se-Cys it was found that the higher the malt content the higher Se-Met concentration. The only beer that does not conform to that 'rule' is Czarne beer. Despite the high extract content in that beer (12%) only $59 \cdot 10^{-3}$ g·dm^{-3} of Se-Met was found.

In the case of quantification of that selenoamino acid repeatability of results is much worse than that for Se-Cys. Relative standard deviation is between ± 2% up to as much as ± 18%. The best repeatability was achieved for Miller beer and the worse was for Redd's beer. In the case of Pilsner Urquell and Peroni Nastro Azzurro it was impossible to select 7 most similar times, that had negative influence on repeatability of results, especially for the beer brewed in Italy.

It was also found that almost all the beers examined contain more Se-Met than Se-Cys. In seems to be justified by literature reporting high Se-Met content in beer production substrates [30].

Conclusions

An analytical procedure for simultaneous determination of Se-Cys and Se-Met in beer as anions using ITP technique having short time of analysis and low operating cost (chemicals) was developed.

The determined concentrations of Se-Cys are between $43 \cdot 10^{-3}$ g·dm^{-3} for Redd's beer and $247 \cdot 10^{-3}$ g·dm^{-3} for Żywiec Porter beer while Se-Met content flash in the range of $59 \cdot 10^{-3}$ g·dm^{-3} for Czarne beer to $510 \cdot 10^{-3}$ g·dm^{-3} for Żywiec Porter beer. In almost all beers Se-Met content is higher than Se-Cys content.

It was found that the concentrations of selenoamino acids under examination are highly dependent on malt content.

Determination and quantification limits found were: Se-Cys: LOD = $2 \cdot 10^{-3}$ g dm^{-3}, LOQ = $6 \cdot 10^{-3}$ g·dm^{-3}, Se-Met: LOD = $1.2 \cdot 10^{-3}$ g·dm^{-3}, LOQ = $3.6 \cdot 10^{-3}$ g·dm^{-3}.

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OZNACZANIE WYBRANYCH SELENOAMINOKWASÓW W PIWIE TECHNIKĄ IZOTACHOFOREZY

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Abstrakt: W pracy przedstawiono sposób oznaczania organicznych związków selenu - selenocystyny i selenometioniny - w różnych gatunkach piwa za pomocą techniki izotachoforezy (ITP). Na podstawie otrzymanych wyników stwierdzono, że technika izotachoforezy pozwala w stosunkowo krótkim czasie i przy małych kosztach analizy oznaczyć śladowe ilości selenocystyny (Se-Cys) i selenometioniny (Se-Met) w próbkach piwa. Wyznaczona zawartość Se-Met w piwie waha się w granicach od ok. $59 \cdot 10^{-3}$ g·dm^{-3} (piwo Czarne) do ok. $510 \cdot 10^{-3}$ g·dm^{-3} (piwo Żywiec Porter), a zawartość Se-Cys od ok. $84 \cdot 10^{-3}$ g·dm^{-3} (dla piwa Czarne) do ok. $247 \cdot 10^{-3}$ g·dm^{-3} (piwo Żywiec Porter).

Słowa kluczowe: izotachoforeza, piwo, selenometionina, selenocystyna

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EVALUATION OF THE ENVIRONMENTAL DAMAGES CAUSED BY NITROGEN OXIDES FORMED IN POWER PLANTS USING LIGNITE AS ENERGY CARRIER

OSZACOWANIE SZKÓD ŚRODOWISKOWYCH SPOWODOWANYCH PRZEZ TLENKI AZOTU POWSTAJĄCE W ELEKTROWNIACH CIEPLNYCH STOSUJĄCYCH LIGNIT JAKO NOŚNIK ENERGII

Abstract: A high proportion of the electricity produced in the world is based on lignite-fired power plants. However, these plants produce, among other pollutants, nitrogen oxides (NO_x), which are harmful to the ecosystems. One of the most widely accepted common denominator today for the comparison of the different electricity production technologies and their environmental impacts is the external cost, ie the monetary value of damages caused by electricity production. This approach is being used in this paper as well in order to quantify the external cost of NO_x formed during electricity generation in lignite-fired thermal power plants in Greece. This cost has been calculated up to 2030 and the obtained results confirm that it is comparable to the corresponding conventional private cost. This finding is very important since lignite is a principal energy source for Greece and many other countries as well.

Keywords: nitrogen oxides, external cost, electricity generation, lignite, Greece

The major conventional technologies of producing thermal and electrical energy at power plants, especially at thermal power stations, are based on burning carbon-containing fuel. As a result, it is the power units that are the major polluters of the air, and the source of carbon dioxide (CO_2), sulphur dioxide (SO_2) and nitrogen oxides (NO_x) [1]. Progress of mankind is impossible without growing power consumption, and, consequently, without increasing fuel consumption, and without growth of discharge accompanying such technologies. Moreover, all these polluting emissions are harmful to the health of humans, animals and plants. The emissions of carbon oxides (CO and CO_2) and nitrous oxide (N_2O) are directly related to the greenhouse effect. The negative effects of sulphur oxides (SO_x) and nitrogen oxides (NO_x) are their contribution towards acid rain. Global warming, caused by the increase of the amount of CO_2 in the atmosphere, has increased the world's concern for reducing these emissions, mainly in developed countries, which are the greatest

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polluters. The most widely accepted common denominator today for the comparison of the different electricity production technologies and fuels and their environmental impacts is the external cost, ie the monetary value of damages caused by electricity production. Therefore, the total cost of an energy consumption process is not only the conventional private cost, but also the external cost usually imposed on the society. In this context, the present work is concerned with the external cost of NO_x formed during electricity generation in lignite-fired thermal power plants in Greece. Specifically, in these plants the burning process produces nitrogen oxides (NO_x) where more than 95% are NO, the remainder being NO_2. In the atmosphere, because of the combination of NO with oxygen under the influence of ultraviolet rays, NO is transformed into NO_2, and this, either in this form or in the form of N_2O, is very harmful to living organisms directly. Also, N_2O has a greater influence, about 200-300 times more than CO_2 in the greenhouse effect [2]. For all these reasons, there is no doubt that NO_x is among the most harmful components of the combustion gases, and therefore the present analysis is fully justified.

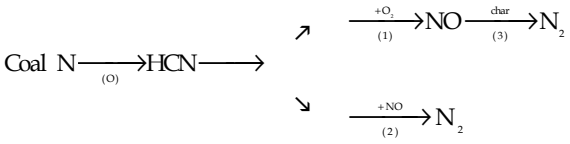
The NO_x formation framework

Generally, coal fired power plants operating with coal and other hydrocarbons like lignite generate the biggest problem of environmental pollution, in particular atmospheric pollution. Combustion gases contain, almost exclusively, harmful components, which affect the life of humans, animals and plants directly. Indirectly, it has negative effects as a result of the components CO_2 and NO_x, which generate the so-called greenhouse effect. The most important harmful components in the combustion gases are the carbon oxides (CO and CO_2), sulphur oxides (SO_2 and SO_3, denoted as SO_x) and nitrous oxides (NO and NO_2, generally denoted as NO_x). These are the results of the use of any type of fuel, as well as the solid particles (ash) resulting from the use of any solid fuel, although less results from the use of oil and natural gas. Also, there are some other harmful components in the combustion gases, such as heavy metals, dioxins etc., which, because of their very small concentration, are disregarded in all analyses regarding the ecological impact of coal fired power plants today. The other components mentioned above are considered more important because of their large quantity of emissions into the atmosphere. The environmental problem of fossil fuel energy systems is connected mainly to the emission of pollutants, such as CO, NO_x, SO_x and CO_2. The widely accepted approach to face them is to use abatement devices to reduce their emitted mass flows to less than the limits sufficient to minimize health damages [2, 3]. The investment in such devices is influenced by the environmental regulations and policies of each country, like subsidies, emission allowance trading schemes etc [4].

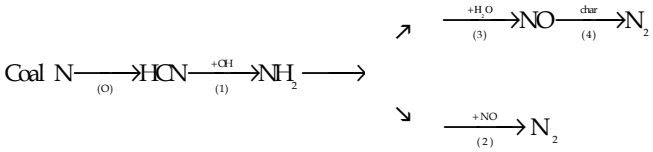
On the other hand, a high proportion of the electricity produced in Europe and indeed in the world is based on pulverized coal fired utility boilers and a considerable amount of this is from the combustion of lignite. There are considerable reserves of brown coal and lignite in the world and this will have to be utilized efficiently and with minimum emission of pollutants and carbon dioxide. This will continue for many decades and there will be the driving force to reduce emissions of acidic gases (SO_x, NO_x) and particulates, and to improve cycle efficiency so as to reduce CO_2 emissions. These needs are focused in the European Commission strategy, particularly with regard to the near- and medium-term objectives to improve the competitiveness and environmental impact of fossil fuel fired power production processes [5, 6]. More precisely, nitrogen oxides are formed in the fossil

fuel combustion process and emitted into the atmosphere directly from the stack. Their dispersion is governed by chemical and physical atmospheric conditions. The most important are wind speed, pressure, mixing boundary height and stability class, which set conditions for atmospheric transport. At the same time, the majority of pollutants undergo some chemical transformations, which are governed by temperature, insulation, humidity, background ambient concentrations and other atmospheric properties. Nitrogen oxides participate in chemical processes in which nitrate aerosols are formed [7]. The formation of NO_x in the coal combustion process is briefly the following. NO_x includes two principals components: NO and NO_2. According to Hirzi [8], NO_2 can be neglected compared to NO. The latter is formed by three separate reaction processes in the gas phase, which are classified as: *thermal NO, prompt NO* and *fuel NO*. Thermal NO is formed by the oxidation of atmospheric nitrogen and is usually associated only with high temperatures (above 1600 K) and in fuel lean environments [9]. Specifically, *thermal NO* is formed according to the Zeldovich mechanism, ie a reaction between atomic oxygen and molecular nitrogen and the continuing reaction of the resulting atomic nitrogen with oxygen molecules. Prompt NO is formed by hydrocarbon fragments (resulting from the devolatilization process) attacking molecular nitrogen near the reaction zone of the flame. Prompt NO is important in rich hydrocarbon flames. On the contrary, the prompt-NO fraction of the total nitrogen oxide emissions is very low and can be neglected in coal combustion [10]. Fuel NO is formed from the homogeneous oxidation of nitrogen constituents released during devolatilization or from heterogeneous oxidization of nitrogen compounds in the char following devolatilization. Fuel NO can also be reduced by both homogeneous and heterogeneous reactions. It generally accounts for 70÷90% of the total NO formed [6]. Two models have been developed for the description of NO formation: the model of Fenimore [11] and the model of De Soete [12]. This is realized according to the following reaction paths [13]:

DeSoete



Fenimore



Both models assume that all nitrogen, which leaves the coal during devolatilization and char burnout is directly transformed to HCN in the gas phase. Their difference appears mainly in the choice of oxidizer substance (O_2 for De Soete vs H_2O and OH for Fenimore). Most of these reactions are catalytic. In addition to coal, soot and coke particles, the coal ash and other bed materials have a catalytic effect. In fluidised bed combustion, addition of limestone for SO_2 retention results in higher NO_x emissions, ie NO_x formation due to

oxidation of N-containing components (NH and CN) is favoured by CaO. On the other hand, nitrogen oxide reduction reactions are catalytically accelerated as well. CaO, char and ash favour NO reduction by CO. Nitrous oxide decomposition is enhanced over calcium oxide, magnesium oxide and iron oxide. Other substances (ie SiO_2, Al_2O_3 and $CaSO_4$) have no or minor catalytic effect. Therefore the effect of lime decreases during sulphating. The influence of the ash on NO_x formation has scarcely been investigated hitherto [10]. The mathematical formulation of these two NO_x formation models is given below. In order to find the mass fraction of any individual species throughout a reaction chamber, the species continuity equation should be solved. This equation is shown below in cylindrical, axisymmetric coordinates (x-r) where m_i is the time mean mass fraction of species i (i = HCN, NO, or i = HCN, NH_2, NO for De Soete's and for Fenimore's model, respectively). The W_i term in this equation represents the time mean net rate of formation of species i and is the difference between the rate of formation and depletion of species i from chemical reactions

$$\frac{\partial(\rho u m_i)}{\partial x}+\frac{\partial(\rho v m_i)}{\partial r}+\frac{\partial}{\partial x}(\mu_{eff}\frac{\partial m_i}{\partial x})-\frac{1}{r}(\frac{\partial}{\partial r}(r\mu_{eff}\frac{\partial m_i}{\partial r}))=W_i \quad (1)$$

One of the largest problems in solving the species continuity equation is obtaining a value for W_i. Here, the simplest approximation is adopted: the time mean reaction rate is obtained using the time mean properties. It should be noted, however, that, as it is well known, due to the no linearity of this term this assumption could be lead to significant errors [14, 15].

For the model of Fenimore, simple inspection of the previous reaction path indicates the existence of 5 reactions, each having the following reaction rate:

$$w_0=\frac{2}{M_{N_2}}S_p m_{N_2}\ [\text{kmol/m}^3\text{/s}] \quad (2)$$

$$w_1=25.10^{12}e^{(-10\,\text{kcal}/RT)}10^3\ [\text{kmol/m}^3\text{/s}] \quad (3)$$

$$w_2=5.10^{12}[NO].[NH_2]10^3\ [\text{kmol/m}^3\text{/s}] \quad (4)$$

$$w_3=w_2 700e^{(-50\,\text{kcal}/RT)}\frac{[H_2O]}{[NO]}10^3\ [\text{kmol/m}^3\text{/s}] \quad (5)$$

$$w_4=4,18.10^4 e^{(-34,7\,\text{kcal}/RT)}p_{NO}A_E 10^{-3}\ [\text{kmol/m}^3\text{/s}] \quad (6)$$

The m_{OH}, m_{NH_2} mass fractions are taken from the following equilibrium assumptions:

$$2H_2O \rightleftarrows H_2+2OH \quad (7)$$

$$OH+NH_3 \rightleftarrows NH_2+H_2O \quad (8)$$

The overall reaction rates are:

$$W_{NO}=(w_3-w_2-\frac{w_4}{V})M_{NO}\ [\text{kg/m}^3\text{/s}] \quad (9)$$

$$W_{HCN} = (w_0 - w_1)M_{HCN} \ [kg/m^3/s] \tag{10}$$

$$W_{NH_3} = (w_1 - w_2 - w_3)M_{NH_3} \ [kg/m^3/s] \tag{11}$$

For the model of De Soete we have:

$$w_0 = \frac{2}{M_{N_2}} S_p m_{N_2} \ [kmol/m^3/s] \tag{12}$$

$$w_1 = 10^{10} x_{HCN} x_{O_2}^b e^{(-67 kcal/RT)} \frac{\rho}{M_m} \ [kmol/m^3/s] \tag{13}$$

$$w_2 = 3.10^{12} x_{HCN} x_{NO} e^{(-60 kcal/RT)} \frac{\rho}{M_m} \ [kmol/m^3/s] \tag{14}$$

$$w_3 = 4,18.10^4 e^{(-34,7 kcal/RT)} p_{NO} A_E 10^{-3} \ [kmol/m^3/s] \tag{15}$$

The overall reaction rates are:

$$W_{NO} = (w_1 - w_2 - \frac{w_3}{V})M_{NO} \ [kg/m^3/s] \tag{16}$$

$$W_{HCN} = (w_0 - w_1 - w_2)M_{HCN} \ [kg/m^3/s] \tag{17}$$

where: S_p - rate of coal weight loss due to devolatilization and char burnout [kg/m^3/s], m_{N_2} - mass fraction of N_2 in coal, M_i - molecular weight of species i [kg/kmol], M_m - molecular weight of the mixture [kg/kmol], x_i - mole fraction (= $m_i M_m/M_i$), p_{NO} - partial pressure of NO [atm], A_E - char specific external area [m^2/g], [i] - $pm_i/(M_i \cdot 10^3)$, V - cell volume [m^3], x - axial direction coordinate [m], r - radial direction coordinate [m], u - axial velocity component [m/s], v - radial velocity component [m/s], ρ - density [kg/m^3], μ_{eff} - mass diffusion coefficient [kg/ms], and b - coefficient given by De Soete [12].

For both models the last reaction can be neglected because its influence is small. The models have been tested and results have been obtained in simplified cases. It has been seen that the model of Fenimore is very sensitive to temperature distribution and water concentration while the model of De Soete gives generally higher results than this of Fenimore [13].

Projection of NO_x external cost

The lignite fuel cycle presents significant similarities to the coal fuel cycle and can be decomposed into the same stages. More specifically, the Greek lignite fuel cycle includes the following stages:

- Lignite extraction
- Transportation processes
- Operation of the power plant
- Disposal of wastes

In our case, the power generation stage is being examined. In general, most lignite coal-firing units in Europe are equipped with tangential-firing combustion systems with jet burners, where the flame is stabilized in the central fireball instead of individual vortex burners. Normally lignite has high moisture content (20÷70%) and the ash content varies from 1 to 40%. Because of the high moisture content, lignite-firing boilers are equipped with special drying and grinding systems. For grinding, the hammer type or fan type of mills are normally used, and for the drying, instead of air, a mixture of hot flue-gas (800÷1000ºC) and air is used. Because of the high amount of hot flue-gas and only low amount of air, to comply with the safety conditions, for drying of lignite, the O_2 content in the carrier gas is low. Typically the O_2 content of the carrier gas is 8÷15%, which is significantly lower compared with bituminous coal-ring systems. The final moisture-content of lignite after drying is about 7÷20%, which is also higher than for bituminous coal. Low O_2 contents of the carrier gas and high moisture contents of the fuel have negative effects on the coal ignition, causing low combustion efficiencies and high NO_x emissions. Existing lignite-firing units use very simple burners, which are actually not burners but coal injectors. This means that lignite coal ignites very far from the coal nozzle, normally more than 2÷4 m from the nozzle in the central fireball. Then, because the O_2 content of the carrier gas must be low, the ignition and flame stabilization is poor with such a jet burner. This causes a narrow operation range for boilers, and, for low-load operation, it is necessary to use oil or gas for flame stabilization and for safe boiler operation [16]. On the other hand in Greece, due to the quality of the Greek lignite (high ash and sulphur content) significant amounts of air pollutants are generated during the combustion process. To reduce emissions most plants are equipped with effective emission control equipment. The concentration of pollutants in the flue gases is given in Table 1 [17].

Table 1

Pollutant concentration in the flue gases of a lignite-fired power plant in Greece [17]

Pollutant	**Concentration**
Particulates	< 50 mg/Nm^3
SO_2	170÷300 mg/Nm^3
NO_x	190÷200 mg/Nm^3

For the reduction of particulates electrostatic dust precipitators of high efficiency are usually used. Due to the high alkalinity of the lignite deposits the ash produced during combustion is highly alkaline and can be used for the desulphurisation of the flue gases. The mean efficiency of this system is estimated to be around 90%. However, SO_2 emissions are expected to vary in a relatively wide range due to various uncontrollable parameters of the whole system. Finally, low NO_x burners are used for the reduction of NO_x emissions, which depend highly upon the conditions of the combustion process. Regarding the externalities associated with power generation from thermal station fuelled by lignite in Greece, they have been estimated by ExternE project, the main findings of which are concisely the following [17]:

- Impacts on Public Health: The most important contributors to the quantifiable damage calculated for the lignite fuel cycle is the impact of atmospheric pollutants emitted in the operation stage on human health. Despite the considerable limitations hindering a precise implementation of the impact pathway approach, it is considered that the

obtained results give a good approximation of the real health impacts due to incremental ambient air concentrations and of the associated damages. The most detrimental pollutants to human health, which arise from the lignite fuel-cycle are suspended particulates, SO_2, NO_x, ozone, and the secondary produced aerosols (sulphates and nitrates). More precisely, the World Health Organization's air quality standards are 200 μg/m^3 for 1-h average exposure and 40 μg/m^3 for annual average exposure for NO_x and 125 μg/m^3 for 1-h average exposure and 50 μg/m^3 for annual average exposure for SO_x [18]. The total of the mid damage estimates are approximately 19.9 m€/kWh. Chronic mortality effects are by far the most important component.

- Impacts on Occupational Health: The analysis is restricted to occupational accidents because no data are available for occupational diseases. For the power generation stage of the Greek lignite fuel cycle statistical data covering a 5 years time period and concerning all the Greek lignite power plants have been used. The estimated damage cost ranges between 0.1÷0.14 m€/kWh.
- Impacts on Agriculture: Air pollutants affect the terrestrial ecosystems through dry and wet deposition mechanisms. Dry deposition acts directly on crops, mainly through foliar uptake, and is believed to cause the most serious damages. Wet deposition and the resulting soil acidification are not expected to significantly affect the quality of soils because of their high alkalinity and thus the agricultural production, as well as the forests development. SO_2, NO_x and O_3 emissions have the most detrimental impacts on vegetation.
- Impacts on Materials: The estimated damages to materials due to their exposure to atmospheric pollutants (mainly SO_2 and the secondarily produced wet acid deposition), concern mainly the repair costs of the buildings and other material infrastructure. The resulting damage costs due to the impacts of atmospheric pollution on materials have been estimated at 0.27 m€/kWh. It has also been attempted to approximate damages caused on historical monuments and other ancient constructions of specific cultural value because of the particular importance they have for Greece. A first rough estimate of these damage cost category gives a value ranging between 0.0058 and 0.033 m€/kWh.
- Impacts on Water Resources: The power generation stage of the Greek lignite fuel cycle affects also surface water resources mainly because of the withdrawal of water for cooling purposes. The most important impacts on surface water are the water contamination, the temperature rise and the lessening of water resources. A quantification of these impacts is very difficult at the moment because of the lack of data and the incomplete knowledge about the processes involved in the damaging pathways.
- Global Warming: During the last decade a lot of publications support estimations of the damages associated with this phenomenon for different scenarios. In this context the relevant damage costs have been estimated on the basis of the FUND model, assuming different values of discount rate (1, 3%) as well as a sensitivity analysis for various parameters. For a discount rate of 3%, the total external cost amounts approximately to 23.8 m€/kWh.

The total of the damage cost estimates related to the power generation stage of the Greek lignite fuel cycle are summarised as 15.17-200.77 m€/kWh depending the discount

rate used. Likewise, the external costs of damages associated with NO_x is estimated as 9,298 €/Mg [17]. In addition, in Greece the NO_x emission factor of the electricity generated in lignite-fuelled power stations is 1.6 kg/MWh [19]. This figure seems quite moderate compared to the NO_x emission factors of similar lignite or coal fired thermal power plants in other countries [7, 20, 21]. Relating the NO_x emission factor from Greek lignite-fuelled power stations (1.6 kg/MWh) with the external costs of damages associated with NO_x in Greece (9,298 €/Mg), one can calculate the marginal external cost of NO_x generated in lignite-fuelled power stations as 15 €/MWh approximately. Moreover, the entire annual electricity generated from all lignite-fired thermal plants in Greece is given in Table 2 for the most recent years as well as estimations for the future [22, 23]. Therefore, considering all the above, the total external cost of NO_x formed in Greece from lignite-fired thermal plants is being calculated and presented in Table 3 for the same years.

Table 2

Total annual electricity generation from lignite-fired power plant in Greece [22, 23]

Year	GWh/year
2000	34,537
2001	32,042
2002	31,197
2003	31,643
2010	35,686[a]
2015	33,764[a]
2020	36,989[a]
2025	39,486[a]
2030	36,776[a]

[a] estimation

Table 3

Total annual external cost of the NO_x formed in Greece from lignite-fired power plants

Year	M€/year
2000	518.06
2001	480.63
2002	467.96
2003	474.65
2010	535.29
2015	506.46
2020	554.84
2025	592.29
2030	551.64

Since the net power generated cost in this kind power plants is about 25 €/MWh [24], it can be seen that the external cost examined in the present work (ie of one pollutant only) is comparable to the corresponding conventional private cost (about 60% of this).

Concluding remarks

The present work is concerned with the external cost of NO_x formed during electricity generation in lignite-fired thermal power plants in Greece. This cost has been calculated and the obtained results confirm that it is comparable with the corresponding conventional private cost. This finding seems expected since lignite is one of the most polluting fuels

used for electricity generation. This is especially true in Greece because of the inferior quality characterising the Greek lignite deposits and in particular their low heating value. Consequently, measures and initiatives should be adopted to reduce emissions in the Greek energy system. This may be achieved through the use of non-carbon primary energy sources (such as hydro plants and RES) and by improving their total efficiency. The improvement in total efficiency is achieved either by adopting new technologies or with alternative combinations of technologies and fuels (eg combined cycle units). However, a series of political initiatives is required for a reduction of emissions associated with the energy system. For instance, policy regarding energy savings should continue and intensify in near future, as energy intensity in Greece is a serious concern. It exceeds the International Energy Agency Europe average and it is increasing. Significant potential for energy efficiency improvement has been identified. The cost-effectiveness of specific efficiency measures should be taken into account in setting policy priorities. Many specific measures are self-financing and can be taken without relying on subsidies. Measurable targets should be set. The effectiveness of policies should be carefully monitored. As it concerns lignite, it has been mentioned that investments have been made to reduce pollutant emissions from lignite-fired power plants. These efforts need to be continued. Even though the government favours the use of gas in power generation, new lignite-fired power plants may be licensed, provided they use only state-of-the-art technologies. Besides, with regard to security of supply, lignite is an extremely important energy source for Greece and thus the country's government position on the future role of this fuel in electricity generation must be determined and planned very carefully.

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OSZACOWANIE SZKÓD ŚRODOWISKOWYCH SPOWODOWANYCH PRZEZ TLENKI AZOTU POWSTAJĄCE W ELEKTROWNIACH CIEPLNYCH STOSUJĄCYCH LIGNIT JAKO NOŚNIK ENERGII

Abstrakt: Duża część energii elektrycznej produkowanej na świecie pochodzi z elektrowni opalanych węglem brunatnym. Jednak elektrownie te wytwarzają zanieczyszczenia, m.in. tlenki azotu (NO_x), które są szkodliwe dla ekosystemów. Jednym z powszechnie akceptowanych parametrów, służącym do porównywania różnych technologii produkcji energii elektrycznej i ich oddziaływania na środowisko, jest koszt zewnętrzny, tj. wartość pieniężna szkody spowodowanej produkcją energii elektrycznej. W tej pracy oszacowano zewnętrzne koszty NO_x powstające podczas produkcji energii elektrycznej w opalanej węglem brunatnym elektrowni cieplnej w Grecji. Koszt ten został obliczony aż do roku 2030, a uzyskane wyniki potwierdzają, że jest on porównywalny do odpowiednich konwencjonalnych kosztów własnych. To spostrzeżenie jest bardzo ważne, ponieważ węgiel jest głównym źródłem energii zarówno w Grecji, jak i w wielu innych krajach.

Słowa kluczowe: tlenki azotu, koszty zewnętrzne, wytwarzanie energii elektrycznej, węgiel brunatny, Grecja

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KINETICS OF CARBON DIOXIDE ABSORPTION INTO AQUEOUS MDEA SOLUTIONS

KINETYKA ABSORPCJI CO_2 W WODNYCH ROZTWORACH MDEA

Abstract: The CO_2 absorption rate in aqueous methyldiethanolamine solutions was measured using a stirred cell with a flat gas-liquid interface. The measurements were performed in the temperature range of (293.15 to 333.15) K and amine concentration range of (10 to 20) mass %. Measurements were based on a batch isothermal absorption of the gas. The kinetic experiments were conducted under pseudo-first-order regime. The calculated initial absorption rates enabled to estimate the forward, second order reaction rate constant of CO_2 reaction with MDEA in aqueous solution.

Keywords: CO_2 removal, absorption, methyldiethanolamine, stirred cell

Absorption by aqueous alkanolamine solutions is the dominant industrial process for separation of acid gases such as CO_2 and H_2S, from gas mixtures in natural gas processing, petroleum refining, coal gasification and ammonia manufacturing industries. Another possible application of amine-based technologies is CO_2 separation from the large quantities of flue gases from industrial sources such as fossil-fuel power plants. Since CO_2 is regarded as a greenhouse gas, potentially contributing to global warming, there has been considerable interest in developing effective technologies for its capture, recently. The chemical absorption of CO_2 is generally recognized as the most efficient post-combustion CO_2 separation technology at present. The idea of carbon dioxide sequestration which includes its capture and storage in underground rock formations has progressed steadily over the past ten years. It is claimed that this solution could play an important role in solving the problem of increasing greenhouse gas emissions. As opposed to other capture options which include pre-combustion method and oxy-fuel processes, the post-combustion technology can be fairly installed in the existing power plants while other options involve innovative ways of power generation technology. The amines that have been proved to be of principal commercial interest are monoethanolamine (MEA), diethanolamine (DEA) and methyldiethanolamine (MDEA) [1]. In the last decade interest in using

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methyldiethanolamine, which is a tertiary amine, increased significantly. The main advantages of MDEA as opposed to more traditionally used primary and secondary amines are its relatively high capacity, small enthalpy of reaction with acid gases and a low vapour pressure. Since, the CO_2 reaction rate with MDEA is slow, the addition of small amounts of fast reacting amines is necessary to apply this process in flue gas treatment.

Many studies were performed on the kinetics of the reaction of CO_2 with aqueous MDEA [2-5]. A comprehensive overview on that subject is provided by Vaidya and Kenig [6]. There is an agreement on the reaction mechanism which implies that tertiary amines do not react directly with CO_2. In an aqueous solution, MDEA catalyzes the CO_2 hydration reaction according to the mechanism proposed by Donaldson and Nguyen [7]. However, there are still many discrepancies in the literature concerning the interpretation of the kinetic data.

This causes the relatively high difference in the forward rate coefficient of the MDEA-catalyzed reaction which is ranging from 1.44 $m^3\,kmol\,s^{-1}$ [2] to 5.15 $m^3\,kmol\,s^{-1}$ [3] at 293 K. These discrepancies may be attributed to the different experimental techniques used and the inconsistency of the physical data such as CO_2 solubility and diffusivity applied for interpretation of the absorption rate data. Moreover, there is a disagreement on the effect of the reaction of hydroxide with CO_2 on the measured rate of CO_2 absorption into aqueous MDEA solutions [6].

In the present paper, additional data on the kinetics of the reaction between CO_2 and aqueous MDEA solutions are reported. The CO_2 absorption rate in aqueous MDEA solutions was measured using a stirred cell with a flat gas-liquid interface. The measurements were performed in the temperature range of (293.15 to 333.15) K and amine concentration range of (10 to 20)% mass MDEA. The obtained results were applied to determine the initial CO_2 absorption rate. A simplified film model, which assumes that the reaction between CO_2 and MDEA is irreversible, has been applied for interpreting the experimental data and estimation of the reaction rate coefficient.

Theory

In accordance with the convention used in the amine literature, MDEA is represented as $R_1R_2R_3N$, where $R_1 = R_2 = CH_2CH_2OH$ and $R_3 = CH_3$. When CO_2 is absorbed in aqueous MDEA solutions, the following reactions may occur in the liquid phase [3]:

$$CO_2 + R_1R_2R_3N + H_2O \xleftrightarrow{k_2, K_1} R_1R_2R_3NH^+ + HCO_3^- \quad (1)$$

$$CO_2 + OH^- \xleftrightarrow{k_{OH}, K_2} HCO_3^- \quad (2)$$

$$HCO_3^- + OH^- \xleftrightarrow{K_3} CO_3^{2-} + H_2O \quad (3)$$

$$R_1R_2R_3NH^+ + OH^- \xleftrightarrow{K_4} R_1R_2R_3N + H_2O \quad (4)$$

$$2H_2O \xleftrightarrow{K_5} OH^- + H_3O^+ \quad (5)$$

where: k_2, k_{OH} - forward second order rate constants, K_i - equilibrium constant of reaction „i".

Reactions (1)-(2) are taking place with the finite rates which are described by the forward second order rate constants k_2 i k_{OH} and equilibrium constants K_1 and K_2. Reactions

(3)-(5) are instantaneous with respect to mass transfer as they involve only a proton transfer. Kinetics of direct reaction of CO_2 with OH^- is firmly established [8], however its influence on the absorption rate should be considered vary carefully as the reaction of CO_2 with OH may have a significant contribution to the observed reaction rate, especially at very low partial pressure [3].

Versteeg at al [9] concluded that in a large number of studies of CO_2-MDEA system with an absorption technique, the influence of the OH^- reaction with CO_2 is overestimated due to the presence of other negative charged ions like HCO_3^- and CO_3^{2-}. Littel et al [10] and Moniuk et al [11] claim that the effect of this reaction is negligible due to the low concentration of the hydroxide ions in the solution. According to the numerical simulation of Rinker et al [3] and Glasscock and Rochelle [12] only at low CO_2 concentrations, corresponding to low CO_2 partial pressure, the hydroxide reaction has the largest effect and must be taken into account in predicting the second order reaction rate constant k_2. As the partial pressure is increasing, the hydroxide becomes depleted in the boundary layer and MDEA has a major contribution to the absorption rate. Shi and Zhong [13] demonstrated that in order to obtain the true kinetics of reaction (1), the rigorous mathematical model should be applied for interpreting the absorption rate data. They claim that a better prediction of the CO_2 absorption rate is achieved while applying a model according to a more realistic kinetic mechanism where all chemical reactions are taking into account and treated as reversible, especially at high temperatures.

In the present work, the simplified kinetic model was applied which assumes that the main reaction (1) of CO_2 with MDEA is irreversible and the contribution of reaction (2) on the mass transfer rate is negligible. The conditions for the absorption of CO_2 in MDEA solutions were selected in such a way as to ensure that the absorption occurs in the fast pseudo-first order reaction regime. After these assumptions, the total rate of CO_2 reaction in an aqueous solution of MDEA may be expressed as:

$$r_{ov} = k_2[\mathrm{MDEA}][\mathrm{CO_2}] = k_{ov}[\mathrm{CO_2}] \tag{6}$$

The overall reaction kinetic constant k_{ov} is defined as:

$$k_{ov} = k_2[\mathrm{MDEA}]_0 \tag{7}$$

where $[MDEA]_0$ states for the initial MDEA concentration in the solution.

Experimental

Apparatus

The measurements were performed in the heat flow reaction calorimeter (Chemical Process Analyser, ChemiSens AB, Sweden) which is a fully automated and computer-controlled stirred reactor vessel with possibility of an on-line measurement of thermal power developed by the process. The reactor is a cylindrical, double walled glass vessel with an effective volume of 250 cm^3. It is capped, top and bottom, with stainless steel 316. The reactor lid holds the shaft seal and the necessary armature for charging and sampling both from gas and liquid phases. The schematic diagram of an experimental set-up is shown in Figure 1.

The reactor was equipped with four stainless steel baffles and an impeller stirrer. While in use it is submerged in the thermostating liquid bath. A Peltier element mounted inside the

bottom of the reactor serves as an efficient heating and cooling device and keeps temperature constant to ±0.1 K. The absolute pressure was measured by a pressure transducer mounted on the reactor flange. A separate tube on the top flange allows either to evacuate the cell or to introduce a gas into the reactor. The measurement accuracy of the digital pressure transducer was 0.1% of the full range of (0÷2000) kPa. The gas-liquid interface, A, was flat and could be geometrically estimated.

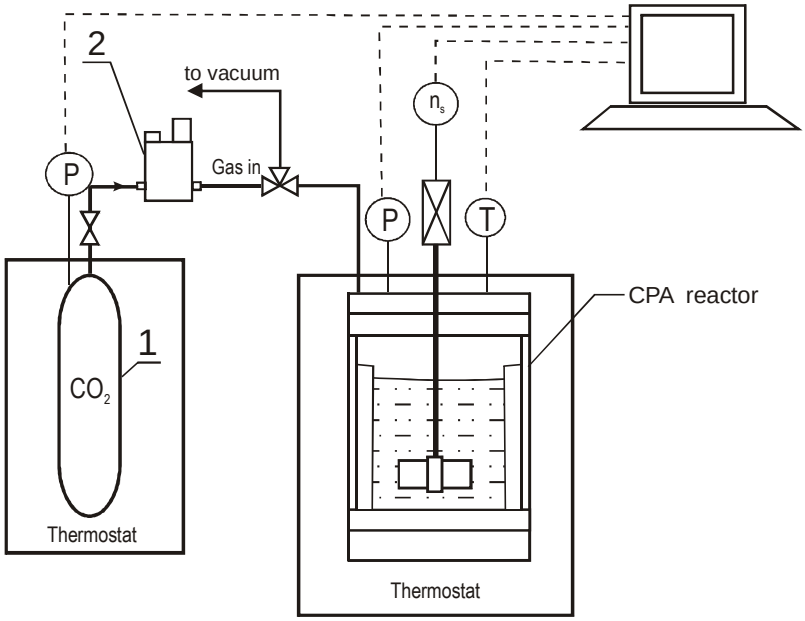

Fig. 1. Experimental set-up: 1 - storage tank; 2 - downstream pressure regulator; ns - stirring speed transducer; T - temperature sensors; P - pressure transducers

Procedure

A series of experiments was conducted at temperature range of (293÷333 K). The initial MDEA concentration in the aqueous solution was varied in the range of 0.83÷1.7 kmol/m^3 which corresponds to amine weight fraction from 10 to 20 mass %. In each experiment, the reactor was charged with 100 cm^3 of the alkanolamine solution. After the reactor was filled with the desired solution, the liquid was degassed and heated to the set temperature under stirring conditions. When the thermal equilibrium was established eg the temperature in the reactor became constant to ±0.01 K, the stirring was stopped for a moment. Pure gas from the CO_2 reservoir was then introduced during a very short time in the upper part of the cell. The resulting initial pressure in the range of (25÷110) kPa was set by the back pressure regulator (Brooks, 5866). Then the absorption process was initiated by switching on the stirrer at the desired stirring speed. The decrease in the system pressure due to absorption of the gas was monitored by the pressure transducer and the "p_{CO_2} vs t" data were recorded as a function of time until the gas-liquid equilibrium state was reached.

The initial "p_{CO_2} vs t" data from t = 0 to 60 s were plotted and the absorption rate was calculated from the value of the slope ($-dp_{CO_2}/dt$). This method based on the fall-in-pressure technique was used previously by other investigators [5, 10, 14] and enabled a simple and straightforward estimation of the absorption rates. The main advantage of the present experimental technique is that no analysis of the liquid phase is required and the recorded pressure decrease versus time was the only parameter necessary for the evaluation of absorption kinetics.

The CO_2 partial pressure in the reactor, p_A, was calculated according to the total pressure in the system P measured by the pressure transducer, corrected for solution vapour pressure by use of Raoults's law. In these calculations, the amine vapour pressure was neglected, as it was very small as compared with the solvent vapour pressure.

The experiments were carried out at three temperatures: 293.15, 313.15 and 333.15 K and with partial pressure of CO_2 in the range of (25÷110) kPa. The chemicals employed, CO_2 (L'Air Liquide, 99.995 vol % pure) and MDEA (Fluka, 99 mass % pure) were used without any further purification. Alkanolamine aqueous solutions were prepared from the distilled, deionized water. Experiments were performed with initial CO_2 loadings of the solutions equal to zero.

The solution volume was maintained at (100±0.1) cm^3 in each experiment. The respective mass of the solutions was determined within ±0.01 g. In the range of stirring speed of 100÷180 min^{-1}, the absorption rate was independent of the stirring speed indicating a pseudo-first-order regime. Thus, in the present work all experimental runs were performed at a constant stirring speed of 120 min^{-1}. Mixing of the liquid under such a stirring speed did not affect the smoothness of the gas liquid interface which could be easily estimated.

Results and discussion

In order to determine the kinetic parameters in the stirred cell using the pressure drop technique, it is essential that the absorption process takes place in the fast reaction regime, without depletion of the amine at the gas-liquid interface [15]. The necessary condition for the fast pseudo-first-order reaction regime is:

$$3 < Ha << E_i \tag{8}$$

where Hatta number is based on reaction (1) and is given by:

$$Ha = \frac{\sqrt{k_{ov} D_{CO_2}}}{k_L} \tag{9}$$

E_i is the enhancement factor for an irreversible instantaneous reaction and defined as follows:

$$E_i = 1 + \frac{D_{MDEA}[MDEA]}{\nu_{MDEA} D_{CO_2}[CO_2]_i} \tag{10}$$

where ν_{MDEA} is the stoichiometric coefficient of amine in reaction (1).

In the case of the present experimental technique, for a given temperature and amine concentration, the Hatta numbers and enhancement factors may by varied by changing the liquid mass transfer coefficient, k_L and CO_2 partial pressure, respectively.

The general expression for CO_2 absorption rate has the following form:

$$N_{CO_2} = E_A k_L \left([CO_2]_i - [CO_2]\right) \tag{11}$$

where N_{CO_2} is expressed in [kmol·m^{-2}·s^{-1}], k_L [m/s] is the mass transfer coefficient of CO_2 in the liquid phase, $[CO_2]_i$ and $[CO_2]$ [kmol/m^3] are the interfacial and bulk CO_2 concentrations, respectively. The enhancement factor E_A describes the influence of a chemical reaction on the mass transfer rate. The enhancement factor is defined as the ratio of the absorption rate of a gas in the liquid in the presence of a chemical reaction to the absorption rate in the absence of a reaction at identical concentration differences of the absorbing gas between the interface and the liquid bulk. When condition (8) is satisfied, the enhancement factor E_A is equal to the Ha number:

$$E_A = Ha \tag{12}$$

The bulk CO_2 concentration at the initial state of the batch absorption run is equal to zero as the aqueous MDEA solutions used in the absorption experiments were not initially loaded with CO_2. Thus, the specific rate of mass transfer of CO_2 becomes:

$$N_{CO_2} = \sqrt{k_{ov} D_{CO_2}}\,[CO_2]_i \tag{13}$$

As can be seen from eq. (13), in the pseudo-first-order chemical absorption regime, the absorption rate is independent of the liquid side mass transfer coefficient k_L and hence it should not depend on the stirring speed. Under the present experimental conditions, no change in the absorption rate was observed while varying the stirring speed in the range of 100÷180 rpm. Hence, it was preliminary assumed that the CO_2 absorption takes place under fast pseudo-first-order regime.

The interfacial concentration of CO_2 can be obtained from Henry's law:

$$[CO_2]_i = \frac{p_{CO_2}}{He} \tag{14}$$

where p_{CO_2} [Pa] is the CO_2 partial pressure and He [Pa·m^3/mol] is the Henry's law constant of CO_2 in aqueous MDEA.

After taking into account the eq. (14), eq. (13) is further transformed to:

$$N_{CO_2} = \frac{\sqrt{k_{ov} D_{CO_2}}}{He} p_{CO_2} \tag{15}$$

The eq. (15) is the basis for the interpretation of the measured absorption rate data. In the fast pseudo-first-order regime, it is possible to determine the overall reaction kinetic rate constant, k_{ov} knowing He and D_{CO_2} for CO_2 in the amine.

The CO_2 absorption rate can be calculated from the experimental data by the mass balance in the reactor gas phase according to:

$$-\frac{V_G}{R \cdot T \cdot A}\frac{dp_{CO_2}}{dt} = N_{CO_2} \tag{16}$$

where V_G [m^3] is the gas phase volume in the reactor, T [K] is the temperature, R [J·mol^{-1}· K^{-1}] is a gas constant and A [m^2] is the gas-liquid interfacial area. In eq. (16), the ideal gas equation was applied.

For the calculation of the overall reaction kinetic rate constant and interpretation of the absorption rate data, many physicochemical properties of the system under consideration are required. The viscosities of MDEA aqueous solutions and Henry's constants for CO_2 were obtained with the correlation proposed by Glasscock [16]. The diffusion coefficient of MDEA was estimated according to the correlation proposed by Pani et al [17]. The diffusion coefficient of CO_2 in aqueous MDEA is calculated according to the modified Stokes-Einstein relation proposed by Versteeg and van Swaaij [18]. The densities of MDEA aqueous solutions were obtained from Rinker at al [19]. The computed physicochemical properties are summarized in Table 1.

Table 1

Physicochemical properties of CO_2-MDEA-H_2O system

T [K]	**$\eta \times 10^3$ [Pa·s] [16]**	
	10 mass %	**20 mass %**
293	1.459	2.19
313	0.931	1.37
333	0.608	0.824
	$D_{MDEA} \times 10^9$[m²/s] [17]	
293	0.564	0.443
313	0.896	0.7098
333	1.43	1.19
	$D_{CO2} \times 10^9$ [m²/s] [18]	
293	1.27	0.923
313	2.03	1.48
313	3.29	5.28
	He [MPa m³/kmol] [16]	
293	2.71	2.91
313	4.43	4.65
333	6.08	6.21
	ρ [kg/m³] [19]	
293	1008.1	1017.3
313	1000.7	1009.3
333	993.3	1001.3

The value of physical mass transfer coefficient, k_L attainable in the experimental apparatus is an important parameter in identifying chemical absorption regime. The mass transfer characteristics were investigated by measuring physical absorption rates for CO_2 in water and N_2O in aqueous MDEA solutions under various operating conditions. The results obtained were correlated as:

$$Sh = 0.3929 Re^{0.6632} Sc^{0.33} \tag{17}$$

where Sh, Re and Sc are, respectively the Sherwood, Reynolds and Schmidt dimensionless numbers defined as:

$$Sh = \frac{k_L d_s}{D_{CO_2}} \tag{18}$$

$$Re = \frac{n_s d_s^2 \rho}{\eta} \tag{19}$$

$$Sc = \frac{\eta}{\rho D_{CO_2}} \tag{20}$$

where d_s and n_s are the dimension and the speed of the stirrer, respectively.

From eq. (15) the overall pseudo-first-order constant k_{ov} can be estimated from measured CO_2 absorption rates, assuming that the condition (8) is met. It should be noted however, that one of the most important drawback of this method is that a priori information is required on the values of the liquid side mass transfer coefficient k_L and a rate constant k_{ov}. Otherwise, neither the lower limit of Ha > 3 cannot be estimated nor the fulfilling of condition (8).

The CO_2 absorption rates in MDEA aqueous solutions at various temperatures and initial amine concentrations are presented in Table 2.

Table 2

CO_2 absorption rates into aqueous solutions of MDEA (n_s = 120 min^{-1})

Temperature	**$[MDEA]_0$**	**$k_L \times 10^5$**	**p_{CO_2}**	**$N_{CO_2} \times 10^6$**
[K]	**[kmol/m^3]**	**[m/s]**	**[kPa]**	**[kmol/(m^2 s)]**
293	0.845	2.02	108.3	2.32
			97.0	2.22
			93.0	2.06
			86.0	1.87
			67.5	1.53
			42.5	0.99
			33.8	0.93
			34.9	1.05
293	1.706	1.43	67.4	1.62
			59.0	1.55
			52.3	1.38
			46.8	1.23
313	0.840	3.19	77.1	2.66
			58.2	2.21
			55.6	1.93
			43.9	1.68
			36.4	1.39
313	1.694	2.28	88.0	3.61
			57.8	2.19
			37.8	1.60
			35.9	1.66
			34.7	1.40
333	0.834	5.07	51.1	2.68
			46.2	2.24
			42.7	2.11
			33.9	1.77
333	1.681	3.91	52.3	3.11
			48.2	2.96
			43.7	2.49
			26.0	1.82

For measurements performed at a given temperature and MDEA concentration, the Hatta number is constant at the given stirring speed. Changing the CO_2 partial pressure in the reactor within the applied range of (25÷110) kPa, changes the infinite enhancement factor E_i which consequently can change the absorption regime. If the reaction takes place

under fast reaction regime under certain conditions, the CO_2 absorption rate N_{CO_2} vs p_{CO_2} should yield the straight line with the slope related to k_{ov}. Thus, plotting experimental N_{CO_2} as a function of p_{CO_2} in the fast reaction regime, should give a straight line passing through the origin at a given temperature and amine concentration and would enable calculation of the combined parameter $\sqrt{k_{ov}D_{CO_2}}/He$. Such plots at 313 K for two amine concentrations are shown in Figure 2. From the slope of the fitted line for 10 mass % MDEA, $\sqrt{k_{ov}D_{CO_2}}/He$ at 313 K was found to be equal to $3.61\cdot10^{-5}$ [kmol m^{-2} s^{-1} MPa^{-1}]. Using the values of D_{CO_2} and He given in Table 1, the second order rate constant k_2 of 15.02 $m^3/(kmol\cdot s)$ was subtracted from this combined parameter. The overall and second order kinetic rate constants were calculated using the described methodology for 10 and 20 mass % MDEA at each temperature. Table 3 shows the computed results of combined parameter and k_2.

Table 3

Experimental results of k_2 determination

Temperature	**$[MDEA]_0$**	**$\sqrt{k_{ov}D_{CO_2}}/He$**	**k_{ov}**	**k_2**	**$k_{2,\ average}$**
[K]	**[kmol/m^3]**	**[kmol m^{-2} s^{-1} MPa^{-1}]**	**[1/s]**	**[m^3/(kmol s)]**	**[m^3/(kmol s)]**
293	0.85	2.21×10^{-5}	2.83	3.35	3.43
293	1.71	2.55×10^{-5}	5.98	3.51	
313	0.84	3.61×10^{-5}	12.61	15.02	14.67
313	1.69	4.09×10^{-5}	24.27	14.33	
333	0.83	5.06×10^{-5}	31.84	38.20	38.11
333	1.68	6.03×10^{-5}	63.89	38.02	

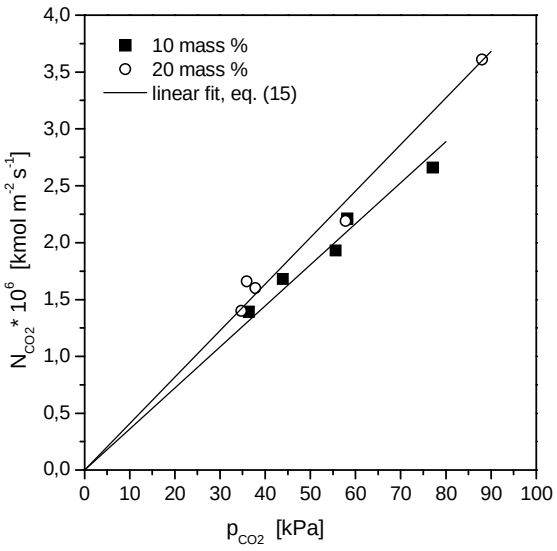

Fig. 2. The absorption rate as a function of CO_2 partial pressure at 313 K - determination of k_{ov}

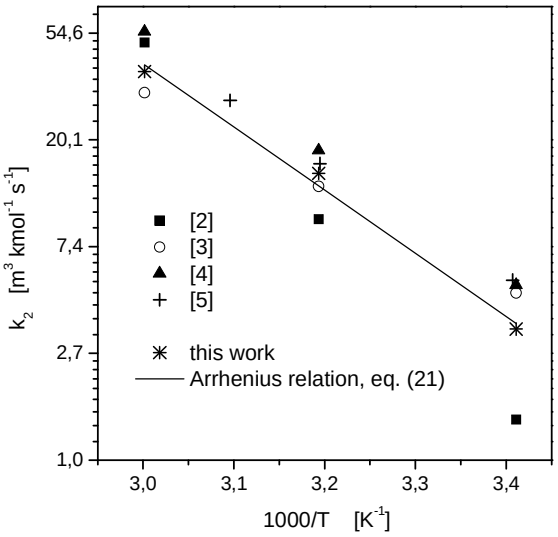

Fig. 3. Comparison of the second-order rate constant for CO_2-MDEA reaction determined in this work with those reported in the literature

Once the values of k_2 were obtained, the values of Ha and E_i were calculated for all the experiments. The Hatta numbers vary from the lowest value of 3.1 (for 10 mass % MDEA at 293 K) to the highest one of 10.4 (for 20 mass % MDEA at 333 K). The instantaneous enhancement factors E_i strongly depend on the applied pressure and vary from 11 to 110 corresponding to the lowest pressure. Enhancement factors for instantaneous reaction are a few times higher than the values of Hatta numbers. The condition given by eq. (8) was found to be satisfied for all the experiments. Thus, the preliminary assumption of fast pseudo-first-order regime was justified in the present study and eq. (15) is valid for interpretation of the measured absorption rate. However, it is noticeable that the Hatta number for 10 mass % MDEA at 293 K is very close to the lower limit of that number above which the reaction can be treated as a fast pseudo-first-order reaction.

The estimates of k_2 are plotted in Figure 3 for comparison with selected literature values. The values of k_2 obtained in this work are in good agreement with those derived by Rinker at al [3] over the whole temperature range. As shown in Figure 3, the k_2 of Jamal et al [4] and Benamour et al [5] are slightly higher than the present ones. The results of Haimour et al. [2] are significantly smaller than other literature values and those determined in this work for T < 313 K. The observed discrepancies may come from the different absorption apparatus and the assumptions involved while interpreting the observed overall kinetic rate constant.

The estimates of k_2 are fitted by the following Arrhenius equation:

$$k_2[\mathrm{m^3 kmol^{-1} s^{-1}}] = 2.07\times10^9 \exp(-5912.7/T) \qquad (21)$$

The activation energy, as determined from eq. (21) has been found to be 49.16 kJ/mol.

Conclusions

The CO_2 absorption rate in aqueous methyldiethanolamine solutions was measured using a stirred cell with a flat gas-liquid interface. The apparatus was operating batchwise with respect to both the gas and liquid phases. Data were obtained over the temperature range 293÷333 K and for MDEA concentrations ranging from 10 to 20 mass %.

In the present work, the simplified kinetic model was applied which assumes that the main reaction (1) of CO_2 with MDEA is irreversible and the contribution of CO_2 reaction with OH^- ions is negligible on the rate of mass transfer. The conditions for the absorption of CO_2 in MDEA solutions were selected in such a way as to ensure that absorption occurs in the fast pseudo-first order reaction regime. The experimental absorption data obtained in this study were interpreted to give an overall rate constant. Based on the assumptions made, the second order rate constants of CO_2 reaction with MDEA were determined. New data for the k_2 were found to be in a good agreement with published values. The activation energy for the second order rate constant was determined to be 49.16 kJ/mol.

Notation

A	gas-liquid interfacial area [m^2]
c	concentration [$mol \cdot m^{-3}$]
D_i	diffusion coefficient of species i in liquid phase [$m^2 \cdot s^{-1}$]
d_s	turbine diameter [m]
E_i	infinite enhancement factor
E_A	enhancement factor
Ha	Hatta number
He	Henry's law constant for CO_2 [$Pa \cdot m^3 \cdot mol^{-1}$]
k_L	liquid side mass transfer coefficient of dissolved CO_2 [$m \cdot s^{-1}$]
k_{ov}	the overall rate constant [s^{-1}]
k_2	second-order rate constant of reaction (1) [$m^3 \cdot kmol^{-1} \cdot s^{-1}$]
K	equilibrium constant
n_s	stirring speed [s^{-1}]
p	partial pressure [Pa]
N_{CO_2}	absorption rate of CO_2 [$kmol \cdot m^{-2} \cdot s^{-1}$]
Re	Reynolds number
r	chemical reaction rate [$kmol \cdot m^{-3} \cdot s^{-1}$]
Sc	Schmidt number
Sh	Sherwood number
t	time [s]
T	temperature [K]
R	universal gas constant 8.314 J/(mol·K)
V	volume [m^3]
	Greek letters
ρ	solution density [$kg \cdot m^{-3}$]
η	solution viscosity [Pa·s]
	Subscripts
i	gas-liquid interface

0	initial state
CO_2	carbon dioxide
G	gas phase
L	liquid phase
MDEA	methyldiethanoloamine

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KINETYKA ABSORPCJI CO_2 W WODNYCH ROZTWORACH MDEA

Wydział Inżynierii Procesowej i Ochrony Środowiska, Politechnika Łódzka

Abstrakt: Metody absorpcyjne usuwania CO_2 z wielu mieszanin gazowych mają szerokie zastosowanie przemysłowe. W niniejszej pracy wykonano pomiary szybkości absorpcji CO_2 w wodnych roztworach metylodietanoloaminy w reaktorze zbiornikowym z mieszadłem z płaską powierzchnią kontaktu. Pomiary wykonano w zakresie temperatur (293,15÷333,15) K i stężeń (10÷20)% mas. MDEA. Absorpcję realizowano w warunkach okresowych w stosunku do fazy ciekłej i gazowej. Stwierdzono, że w warunkach doświadczenia absorpcja przebiegała w reżimie szybkiej reakcji chemicznej pseudopierwszego rzędu. Na podstawie zmierzonej szybkości procesu w chwili początkowej wyznaczono stałe szybkości drugorzędowej reakcji CO_2 w roztworze MDEA. Otrzymane wartości stałej szybkości reakcji skorelowano za pomocą równania Arrheniusa.

Słowa kluczowe: usuwanie CO_2, absorpcja, metylodietanoloamina, reaktor z mieszadłem

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Ewa JASTRZĘBSKA[1]

DEAMINATION OF ALANINE AND ASPARTIC ACID IN SOIL CONTAMINATED WITH PLANT PROTECTION CHEMICALS UNDER LABORATORY CONDITIONS

DEAMINACJA ALANINY I KWASU ASPARAGINOWEGO W GLEBIE ZANIECZYSZCZONEJ ŚRODKAMI OCHRONY ROŚLIN W WARUNKACH LABORATORYJNYCH

Abstract: The objective of this study was to determine the effect of soil contamination with fungicides, Unix 75 WG (cyprodinil) and Riza 250 EW (tebuconazole), and insecticides, Nomolt 150 SC (teflubenzuron) and Dursban 480 EC (chlorpyrifos), on the deamination of DL-alanine and DL-aspartic acid in brown soil with the granulometric composition of loamy sand (pH_{KCl} 6.6). A laboratory experiment was carried out in glass beakers (100 cm^3) containing 50 g samples of soil mixed with one of the studied amino acids as the source of organic nitrogen (0.250 mg $N \cdot kg^{-1}$ soil) and an insecticide/fungicide dose of 0, 0.5, 5.0, 50 and 500 $mg \cdot kg^{-1}$ d.m. soil on active ingredient basis. Soil moisture was brought to the level of 60% capillary water capacity with the use of demineralized water, and the samples were incubated at 25°C. The $N\text{-}NH_4$ and $N\text{-}NO_3$ content of soil was determined colorimetrically at 12-hour intervals (0, 12, 24, 36 and 48 h). The results of the experiment indicate that fungicides and insecticides affected the deamination of organic nitrogen sources, and the direction and force of those reactions was determined by the type and dose of the crop protection chemical and the date of analysis. Tebuconazole and chlorpyrifos had more adverse effects on amino acid mineralization than cyprodinil and teflubenzuron.

Keywords: ammonification, insecticides, fungicides, soil

The nitrogen cycle originates with microbiological processes in the soil. In addition to the microbiological fixation of atmospheric nitrogen, those processes are also inclusive of nitrification, denitrification and immobilization of nitrogen. The process of mineralization of amino acids, referred to as ammonification, is a part of the nitrogen cycle.

More than 90% of nitrogen in soil occurs in organic forms [1]. According to McLain and Martens [2], 90÷98% of the total nitrogen content of soil accounts for proteins secreted by plant roots as well as their decomposition products, namely peptides and amino acids. Amino acids have a 20% to 55% share of the soil's organic matter [1]. Amino acid mineralization in soil is one of the key processes that supply plants with nitrogen [3]. Only 6% of amino acids in soil are absorbed by plant roots as a source of essential nitrogen [4],

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which is why the mineralization of amino acids is an important process from the agricultural point of view. Proteins and amino acids are of special significance for agricultural ecosystems as a vital source of $N\text{-}NH_4$ [5].

The mineralization of amino acids in soil is a relatively rapid process. In a study by Jones [6], the half-life of amino acids ranged from 1 to 12 h. The rate of amino acid mineralization is determined by the quality and properties of soil, including its pH [7] and ecological status [6]. The rate of this process is also affected by the chemical and structural properties of amino acids [2] which determine amino acid sorption by the soil's solid phase [6]. The fate of $N\text{-}NH_4$ released in the ammonification process differs due to the complexity of the nitrogen cycle that comprises a variety of nitrogen transforming processes, many of which take place simultaneously. Ammonium ions produced in the mineralization of the soil's organic matter are oxidized into nitrate ions in the nitrification process, they are absorbed by plant roots and immobilized, ie assimilated, by soil-dwelling microbes [8].

The dynamics of nitrogen mineralization is affected by environmental pollution. Agricultural ecosystems are contaminated with crop protection chemicals, including fungicides and insecticides, which, as demonstrated by various studies, may disturb the soil balance, thus affecting its biological life [9, 10]. The objective of this study was to determine the effect of two fungicides, Unix 75 WG and Riza 250 EW (active ingredients - cyprodinil and tebuconazole), and two insecticides, Nomolt 150 SC and Dursban 480 EC (active ingredients - teflubenzuron and chlorpyrifos), on the deamination of DL-alanine and DL-aspartic acid.

Materials and methods

A laboratory experiment was established on brown soil with the granulometric composition of loamy sand. Samples were collected from the humus horizon (1÷15 cm). The properties and the granulometric composition of soil are given in Table 1. Two fungicides and two insecticides were used in the experiment. The first substance was Unix 75 WG, a commercially available fungicide supplied by Syngenta Crop Protection, with a 75% content of its active ingredient, cyprodinil (a compound of the anilinopyrimidine group). The second tested fungicide was Riza 250 EW manufactured by CHEMINOVA A/S P.O., Denmark. The content of its active ingredient, tebuconazole (a compound of the triazole conazole group), was 250 g per liter of the product. The applied insecticides were: Nomolt 150 SC, supplied by BASF Agro B.V., containing teflubenzuron (a benzoyl urea derivative) in the amount of 150 g per liter, and Dursban 480 EC, manufactured by Dow AgroSciences, with the content of its active ingredient, chlorpyrifos (a phosphoorganic compound), of 480 g per liter of the product.

Table 1

Physicochemical properties of soil used in the experiment

Granulometric composition			**pH_{KCl}**	**Hydrolytic acidity**	**Exchangeable bases**	**C_{org}**
soil grains diameter [mm]				**[mmol(+) ·kg^{-1}]**		**[g·kg^{-1}]**
2÷0.05	0.05÷0.002	<0.002		10.50	146.00	8.5
78	15	7	6.6			

The experiment was carried out in three replications. 100 cm^3 glass beakers were filled with 50 g samples of air-dried soil passed through a 2 mm sieve. Every sample was

thoroughly mixed with an organic nitrogen source and contaminated with one of the studied crop protection products. Soil moisture was brought to the level of 60% capillary water capacity with the use of demineralized water. The experimental variables were: a) type of crop protection chemical - fungicides: Unix 75 WG (cyprodinil), Riza 250 EW (tebuconazole), and insecticides: Nomolt 150 SC (teflubenzuron) and Dursban 480 EC (chlorpyrifos), b) fungicide and insecticide dose in mg·kg^{-1} d.m. soil on active ingredient basis: 0, 0.5, 5.0, 50 and 500, c) source of organic nitrogen: DL-aspartic acid and DL-alanine, d) nitrogen dose in mg N·kg^{-1} soil: 0 and 250. The N-NH_4 and N-NO_3 content of soil was determined at 12-hour intervals (0, 12, 24, 36, 48) according to the method proposed by Wyszkowska et al [11]. N-NH_4 and N-NO_3 were extracted from soil using a 1% aqueous solution of K_2SO_4. The soil/extractant ratio was 1:5. The soil and the extractant were shaken for 30 min, and the content of ammonium ions and nitrate(V) ions in the filtrate was determined in two replications. The N-NH_4 content was determined colorimetrically using Nessler's reagent, and extinction was measured at a wavelength λ = 410 nm. N-NO_3 concentrations were determined colometrically using phenyldisulfonic acid, and extinction was measured spectrophotometrically (UV VIS lambda, Perkin Elmer) at a wavelength λ = 410 nm. The rate of amino acid mineralization was expressed in terms of % ammonified nitrogen using the formula given by Wyszkowska [12]:

$$N = \frac{N_1 - N_0}{D} \cdot 100\%$$

The results were processed statistically using Duncan's multiple range test in the Statistica application [13]. Regression equations and coefficients of determination were computed between the quantity of ammonified nitrogen and the dose of the analyzed crop protection chemical and between the quantity of ammonified nitrogen and the date of analysis.

Results and discussion

In the conducted experiment, the deamination of alanine and aspartic acid was significantly modified by both the type and the dose of the applied crop protection chemical. Time was also an important determinant of process dynamics. In the group of the tested fungicides and insecticides, tebuconazole and chlorpyrifos had a more pronounced effect on the ammonification of alanine and aspartic acid than cyprodinil and teflubenzuron (Fig. 1). The highest doses of the fungicide - cyprodinil (50 and 500 mg·kg^{-1} d.m. soil) had the most inhibiting effect on alanine ammonification after 48 hours of the experiment (at 17 and 22%, respectively), and on aspartic acid ammonification after both 36 and 48 hours. The effect of lower cyprodinil doses was determined by the date of analysis and it was not always significant.

At the beginning of the experiment (12 and 48 hours), lower doses of the second fungicide containing tebuconazole (0.5 and 5 mg·kg^{-1} d.m. soil) significantly stimulated the mineralization of both examined amino acids. After 48 hours, the studied process was significantly inhibited. Higher doses (50 and 500 mg·kg^{-1} d.m. soil) of the fungicide significantly retarded the mineralization of organic nitrogen compounds throughout the entire experiment. After 48 hours, the above doses inhibited the ammonification process by 23 and 44% (alanine) and 14 and 72% (aspartic acid), respectively, in comparison with the

control treatment. It should also be noted that contamination with tebuconazole in the amount of 500 mg·kg^{-1} d.m. soil inhibited alanine decomposition nearly entirely in the first 24 hours, while the rate of aspartic acid mineralization did not exceed 20% even after 48 hours of the experiment.

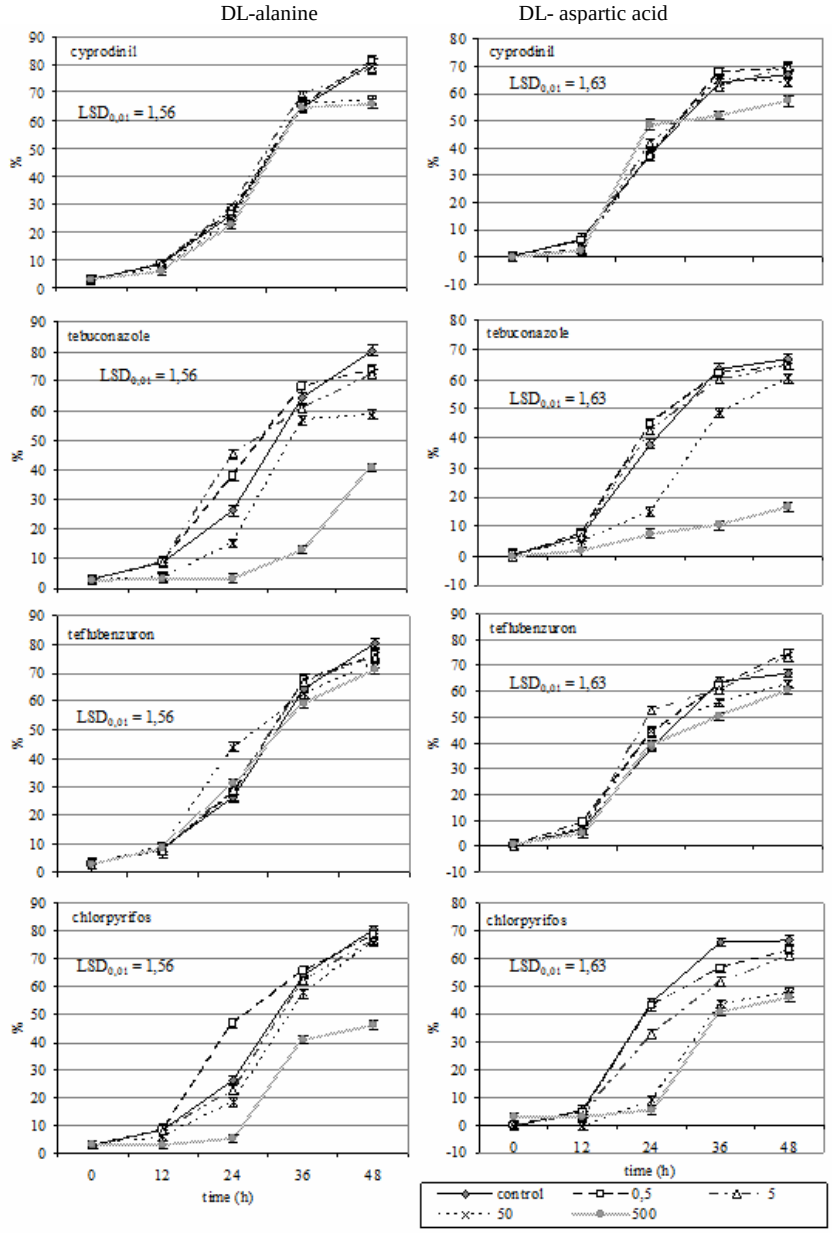
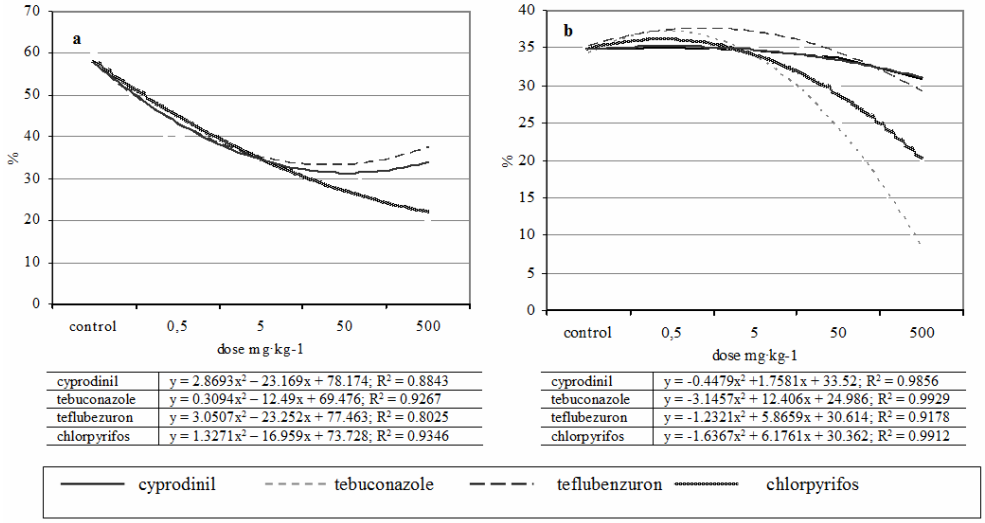


Fig. 1. The effect of soil contamination with crop protection chemicals on the ammonification of DL-alanine and DL-aspartic acid in terms of % ammonified nitrogen

In the group of the analyzed insecticides, teflubenzuron demonstrated a weaker effect on the ammonification process. In comparison with tebuconazole, none of the applied teflubenzuron doses produced an equally powerful effect. Alanine and aspartic acid mineralization was significantly slowed down after 36 and 48 hours in soil samples strongly contaminated with teflubenzuron (50 and 500 mg·kg^{-1} d.m. soil). The second insecticide, chlorpyrifos, had a negative effect on aspartic acid ammonification regardless of the applied dose. Chlorpyrifos also significantly inhibited the mineralization of alanine at doses higher than 0.5 mg·kg^{-1} d.m. soil.

Regardless of the time factor, the degree of soil contamination with the investigated crop protection products had a significant effect on the ammonification of alanine and aspartic acid (Fig. 2). The above was validated by very high coefficients of determination for tebuconazole (R^2 = 0.9929) and chlorpyrifos (R^2 = 0.9912), ie crop protection chemicals that exerted a particularly strong effect on the ammonification process. The process of alanine and aspartic acid deamination progressed with time irresepctive of the level of soil contamination with fungicides and insecticides (Fig. 3). As demonstrated by mean values, deamination was most significantly inhibited by cyprodinil, followed by chlorpyrifos, regardless of the type of the examined amino acid.

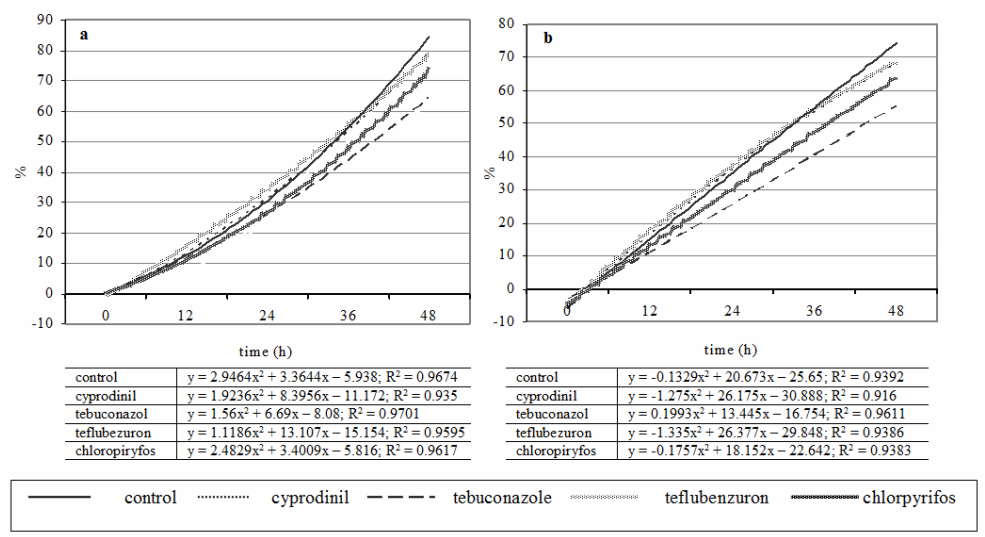


cyprodinil	$y = 2.8693x^2 - 23.169x + 78.174$; $R^2 = 0.8843$
tebuconazole	$y = 0.3094x^2 - 12.49x + 69.476$; $R^2 = 0.9267$
teflubezuron	$y = 3.0507x^2 - 23.252x + 77.463$; $R^2 = 0.8025$
chlorpyrifos	$y = 1.3271x^2 - 16.959x + 73.728$; $R^2 = 0.9346$

cyprodinil	$y = -0.4479x^2 + 1.7581x + 33.52$; $R^2 = 0.9856$
tebuconazole	$y = -3.1457x^2 + 12.406x + 24.986$; $R^2 = 0.9929$
teflubezuron	$y = -1.2321x^2 + 5.8659x + 30.614$; $R^2 = 0.9178$
chlorpyrifos	$y = -1.6367x^2 + 6.1761x + 30.362$; $R^2 = 0.9912$

Fig. 2. The effect of dose of crop protection chemicals on the ammonification of DL-alanine (a) and DL-aspartic acid (b) in terms of % ammonified nitrogen

The effect of crop protection products on the ammonification process remains poorly documented. Pandey and Singh [14] investigated the effect of the recommended doses of chlorpyrifos and quinalfos on the deamination of arginine. The analyzed process was stimulated by chlorpyrifos, and it was inhibited by quinalfos. In a study of Kucharski et al [15], the herbicide Mocarz 75 WG had a negative effect on the mineralization of DL-alanine, L-arginine, L-aspartic acid and urea.



	a	b
control	$y = 2.9464x^2 + 3.3644x - 5.938$; $R^2 = 0.9674$	$y = -0.1329x^2 + 20.673x - 25.65$; $R^2 = 0.9392$
cyprodinil	$y = 1.9236x^2 + 8.3956x - 11.172$; $R^2 = 0.935$	$y = -1.275x^2 + 26.175x - 30.888$; $R^2 = 0.916$
tebuconazol	$y = 1.56x^2 + 6.69x - 8.08$; $R^2 = 0.9701$	$y = 0.1993x^2 + 13.445x - 16.754$; $R^2 = 0.9611$
teflubezuron	$y = 1.1186x^2 + 13.107x - 15.154$; $R^2 = 0.9595$	$y = -1.335x^2 + 26.377x - 29.848$; $R^2 = 0.9386$
chloropiryfos	$y = 2.4829x^2 + 3.4009x - 5.816$; $R^2 = 0.9617$	$y = -0.1757x^2 + 18.152x - 22.642$; $R^2 = 0.9383$

Fig. 3. The effect of time of on the ammonification of DL-alanine (a) and DL-aspartic acid (b) in terms of % ammonified nitrogen

The effect of plant protection products on soil-dwelling microbes receives wider coverage in published studies. Ammonification is a microbiological process, therefore the inhibitory effect of the analyzed products on amino acid mineralization most probably results from the toxic influence of fungicides and insecticides on ammonifying bacteria. In the discussed experiment, low levels of soil contamination with crop protection chemicals often enhanced the decomposition of alanine and aspartic acid. Similar conclusions were formulated by Lupwayi et al [10] who did not observe an adverse effect of fungicides and insecticides on the taxonomic and functional structure of soil-dwelling microbes. In the author's previous study [16], teflubenzuron stimulated the proliferation of oligotrophic bacteria, while chlorpyrifos increased copiotrophic bacterial counts.

In this experiment, plant protection products had a varied effect on the ammonification process. The above could be attributed to differences in the chemical structure, properties and toxicity of the applied substances. As demonstrated by other authors [17], contaminating doses of insecticides and fungicides may have an adverse effect on soil-dwelling microbes by reducing their biomass and inhibiting biochemical processes in soil. These observations were validated by Das and Mukherejee [18] who noted a stimulating effect of soil applied-insecticides on bacteria of the genera *Bacillus*, *Proteus*, *Corynebacterium* and *Streptomyces* and an inhibitory effect of selected insecticides on microbes of the genera *Pseudomonas*, *Staphylococcus*, *Nocardia* and *Micromonospora*. Lakshmi et al [19] reported on the chlorpyrifos-decomposing ability of the following bacteria: *Pseudomonas fluorescens*, *Bacillus melitensis*, *Bacillus subtilis* and *Pseudomonas aeroginosa*. Bacteria of the genera *Bacillus* and *Pseudomonas* are ammonifiers, and the same active substance may have a varied effect on bacteria, subject to environmental conditions which differed across the discussed studies. In this experiment, chlorpyrifos was one of the most potent plant protection products inhibiting the ammonification process.

The results of this experiment indicate that the ammonification process was affected by the type of amino acid, as the analyzed amino acids differed in chemical structure. Both tested compounds contain one amino group, but aspartic acid has higher molecular mass and a higher number of carbon atoms per molecule. Alanine is a non-polar amino acid, whereas aspartic acid is a polar amino acid with a negatively charged side chain. The properties of the studied amino acids determine the rate of their displacement in soil [4], sorption rates [6] and susceptibility to microbial decomposition.

Conclusions

1. In the presented laboratory experiment, the ammonification of DL-alanine and DL-aspartic acid in brown soil contaminated with crop protection chemicals was affected by the type and dose of the applied product and the date of analysis. The course of the ammonification process varied also subject to the examined amino acid.
2. Tebuconazole and chlorpyrifos had a more inhibiting effect on amino acid mineralization than cyprodinil and teflubenzuron.
3. At high levels of soil contamination (50 and 500 $mg \cdot kg^{-1}$ d.m. soil) with tebuconazole and chlorpyrifos, the ammonification of DL-alanine and DL-aspartic acid was inhibited regardless of the date of analysis. After 48 hours of the experiment, the rate of DL-aspartic acid mineralization in soil contaminated with tebuconazole (500 $mg \cdot kg^{-1}$ d.m. soil) did not exceed 20%.

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DEAMINACJA ALANINY I KWASU ASPARAGINOWEGO W GLEBIE ZANIECZYSZCZONEJ ŚRODKAMI OCHRONY ROŚLIN W WARUNKACH LABORATORYJNYCH

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Abstrakt: Celem podjętych badań było określenie wpływu zanieczyszczenia gleby fungicydami Unix 75 WG (cyprodinil) i Riza 250 EW (tebukonazol) oraz insektycydami: Nomolt 150 SC (teflubenzuron) i Dursban 480 EC (chloropiryfos) na deaminację DL-alaniny i kwasu DL-asparaginowego w glebie brunatnej o składzie granulometrycznym piasku gliniastego (pH_{KCl} 6,6). Doświadczenie laboratoryjne przeprowadzono w zlewkach szklanych (100 cm^3), w których umieszczono po 50 g gleby wymieszanej z jednym z aminokwasów jako źródłem azotu organicznego (0, 250 mg $N \cdot kg^{-1}$gleby) oraz odpowiednią dawką insektycydu, w przeliczeniu na substancję czynną: 0; 0,5; 5,0; 50; 500 $mg \cdot kg^{-1}$ s.m. gleby. Następnie wilgotność gleby doprowadzono do 60% kapilarnej pojemności wodnej za pomocą wody demineralizowanej i inkubowano w temperaturze 25ºC. Zawartość $N\text{-}NH_4$ i $N\text{-}NO_3$ w glebie oznaczono kolorymetrycznie w odstępach 12-godzinnych (0, 12, 24 36 i 48 h). W wyniku badań stwierdzono, że fungicydy i insektycydy wpływały na przebieg deaminacji organicznych źródeł azotu, a kierunek i siła ich oddziaływania były uwarunkowane rodzajem środka ochrony roślin, jego dawką i terminem badań. Tebukonazol i chloropiryfos wywierały większy negatywny wpływ na przebieg procesu mineralizacji aminokwasów w porównaniu z cyprodinilem i teflubenzuronem.

Słowa kluczowe: amonifikacja, insektycydy, fungicydy, gleba

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THE ROLE OF INDICATOR MALACOFAUNA IN POLLUTION ASSESSMENT OF INLAND WATERS EXPOSED TO ANTHROPOPRESSURE: THE CASE OF THE KIELCE LAKE

ROLA MALAKOFAUNY WSKAŹNIKOWEJ W OCENIE ZANIECZYSZCZENIA WÓD ŚRÓDLĄDOWYCH PODLEGAJĄCYCH ANTROPOPRESJI NA PRZYKŁADZIE ZALEWU KIELECKIEGO

Abstract: Organisms showing the properties of bioindicators respond to changes in the biotope in a specific way. Those organisms are susceptible to toxic substances or on the contrary they are tolerant to toxins brought into the biotope. Such tolerance means that bioindicators are able to accumulate the pollutants to such extent that is lethal to other living organisms. Using molluscs and their abilities to accumulate heavy metals in amounts higher than those in the environment makes it possible to use this group of organisms as bioindicators of water biotopes. The diversity of types of nutrition and variety of nourishment (deposit-feeding, filtration of suspension and phytophagous properties) allow to indicate differences in concentration of heavy metals despite the origin of species in the same water area. In case of malacofauna preying on bottom deposits it is possible to detect the relation of migration of elements in a water - plant - bottom deposit - malacofauna system. The research was carried out in the Kielce Lagoon - a reservoir formed within the borders of the city of Kielce by closing the Silnica River valley with a weir at 8 km. There are recreational, residential and industrial areas surrounding the reservoir. The trunk road in direction to Lodz being the source of transportation-related pollution is located in the close neighbourhood. The municipal swimming pool from which chlorinated water is discharged to the reservoir is also located nearby. The lagoon is fed by a watercourse collecting the municipal pollutants from the Masłow Village located near the city of Kielce. The water of the Kielce Lagoon is highly polluted because of wide range of organic and inorganic compounds brought into the reservoir. The obtained results of chemical analyses allow to evaluate the cleanliness of the reservoir, form a warning system against danger and show malacofauna response to changes in the biotope.

Keywords: invertebrate malacofauna, bottom deposit, bioindication, detritus

Methods for the assessment of surface water quality, used until recently in Poland, were based primarily on physicochemical research. The scope of microbiological and parasitological research as well as the use of biotests were relatively limited. Rapid

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development of methods based on bioindicators in the world and their application to water environment assessment (a saprophytic system of water classification, known for a century) have created the necessity to conduct similar research in Poland [1].

Polish research is conducted mainly on the basis of bioseston [2], whereas in Western European countries (Belgium, Denmark, France, Ireland, Italy, Luxemburg, Holland, Portugal, Spain, Great Britain) and the USA predominant are methods based on qualitative and quantitative analyses, conducted on invertebrate indicator malacofauna. This research relies on the so-called biotic indexes as well as species diversification ratios. It makes use of the British BMWP (*Biological Monitoring Working Party*) Method, as well as the TBI (*Trent Biotic Index*) and the Chandler system. The multitude of biotic methods poses considerable difficulty as to the choice of those best suited to Polish conditions. The most significant issue here is their adjustment to the country's water characteristics.

An assessment of various systems' usability was carried out in the year 1999 [3]. It was ultimately demonstrated that the most adequate method for Poland is the BMWP as well as modified Margalef's biodiversity index. Thus the Polish biotic system, the BMWP-PL, was developed. Among others, the system includes Phylum: *Mollusca* (molluscs), Class: *Gastropoda* (snails) *Neritidae, Viviparidae, Ancylidae*, as indicating organisms.

The selection of molluscs is conditioned by their bioindication characteristics. These are most frequently organisms of high sensitivity to toxic substances or - vice versa, ie demonstrating high degree of tolerance of toxins introduced into the biotope [4]. This tolerance consists in bioindicators' ability to accumulate pollutants in quantities lethal to other life forms [5, 6]. Using the ability to concentrate heavy metals in quantities exceeding those found in the habitat enables the use of this group of organisms as water biotope bioindicators [7].

Of major importance is the range of feeding methods and the type of food consumed by snails. Feeding on sediments, filtration of the suspension or herbivorosity allow for the identification of differences in heavy metal concentrations in their organisms despite the fact that species occur in the same waterbody.

The sediments originate as a result of sedimentation of allochtonic material from rock and soil erosion, mineral and organic suspensions, industrial and municipal wastes, as well as autochthonic material and non-living remains of plants and animals. They fall to the bottom and provide a feeding basis for detritophagous malacofauna [8]. In the case of malacofauna feeding on bottom sediments, it is possible to observe an element migration pattern in the water - plant - bottom sediment - molluscs system.

Due to its detrimental effect on the biocoenosis, the human being included, accumulation of pollutants in bottom sediments is a major environmental problem. This results from retention of heavy metals and harmful organic compounds (PAHs) in the sediments [9, 10]; consequently, the use of water snails for water biotope monitoring appears to be justified.

The objective of the present study has been to suggest possibilities of using detritophagous malacofauna for the assessment of surface water pollution.

Studied area

Kielce is located in central Poland, in the northern part of the catchments of the Nida river, a left-bank tributary of the Vistula. The study has covered the Kielce Lake -

a reservoir created within city limits by the closure of the Silnica river valley with a weir in its eighth kilometre (Figs 1 and 2). The area around the reservoir as well as the reservoir itself are used as leisure facilities. The area surrounding the reservoir has been developed for residential and business purposes. In direct vicinity, there is an expressway which is a source of transport pollution; adjacent is the municipal swimming pool which discharges chlorinated water into the reservoir. The reservoir also receives municipal waste from satellite towns around the city [11].

Fig. 1. The studied reservoir: the Kielce Lake (Photo M.A. Jóźwiak)

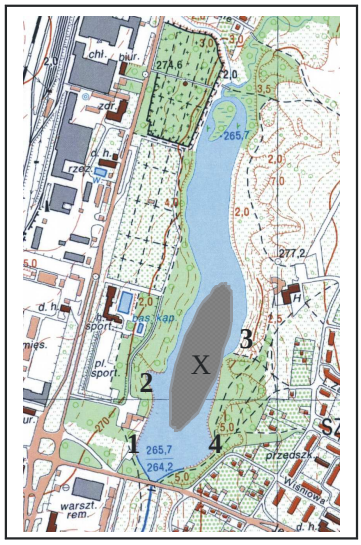

Fig. 2. Arrangement of stands around Kielecki Bay (x - reservoir section on which an aerator moves)

Important for the reservoir's water level is the river which flows into it. It is a mountain-type river, with high gradients in its longitudinal profile and high flow

variability. Its catchment covers an area of 46.68 km^2, and its total length reaches 18.6 km, including 17.0 km within city limits.

Due to continuing expansion of terrain with waterproof surfacing (roadways, concrete pavements, or cycling paths) as well as expansion of drainage and hydrotechnical networks, an imbalance in the natural proportion between infiltration and surface wash appears, exerting heavy anthropogenic influence on the chemical composition of water in the reservoir.

Interannual river flows in the segment leading up to the studied reservoir maintain high variability, characterised by initial increase (0.440 $m^3 \cdot s^{-1}$), followed by a decrease in the river mouth profile (0.369 $m^3 \cdot s^{-1}$). This speeds up the introduction into the reservoir of sediments carried by the river current. Despite their small volume, the interannual flows of the reservoir (V = 170 000 m^3) are almost three times as high as those in the supplying river. This results from additional anthropogenic supply, as well as very high proportion of surface wash which amounts to 85÷90% during heavy rainfall. The reservoir also receives storm drain water, as well as municipal and industrial water discharges. The reservoir location and supply point to the fact that the transported material, particularly in winter and spring seasons, is washed into the reservoir, considerably affecting its ecology.

Studied material and methods

In the studied area, four measurement sites were located. The study was carried out as field work between May and September in the years 2008 and 2009. It covered a chemical analysis of reservoir water (alkaline metals, heavy metals, pH, BOD_5, biogenic compounds), and in the year 2009, additionally, the chemism of bottom sediments and indicator malacofauna: snail shells and soft tissues.

The alkaline metal (Na, K) content was determined by means of the emission flame photometry method with the use of the Sherwood 420 photometer, its parameters adjusted appropriately. For the determination of the content of biogenic compounds, the UV-VIS spectrophotometer with internal calibration and MERCK tests were used. Determination of BOD_5 was carried out with the use of Aqua Lytic Oxi Direct. For the study of the bottom sediment a sampler was used: a core probe which enabled the collection of soft sediment samples with undisturbed structure of the sediment surface layer from the depth of 15 cm.

Molluscs were collected along a 10 m segment at four selected measurement sites with the application of a 30x30 cm landing net. The collected molluscs were sorted and taxonomically classified. The material for the analysis is the European physa *Physella acuta* (Draparnaud 1805) - Figure 3, a common species found worldwide [12, 13]. It demonstrates considerable resistance to pollution which causes it to occupy natural and industrial reservoirs [12]. It prefers waters with exuberant vegetation, and is found mainly on rush macrophytes [14]. It colonises industrial reservoirs with high water temperature. It is the only snail species found at the water discharge zone of the Rybnik Power Station [15].

The factors which make reservoir colonisation more difficult to this species are rapid currents and excess of magnesium ions (>50 $mg \cdot dm^{-3}$). The European physa does not display any food preferences. It feeds mainly on detritus (70% of consumed food) as well as green algae and diatoms [16]. This organism has a one-year life cycle, with two seasonal generations. Specimens which appear in spring die out before winter; the following generation which appears at the turn of September dies out in the early summer of the next

vegetative season [15]. Hence mollusc collection in two catches is justified: early summer (post-winter specimens) and late summer (specimens of full vegetative season).

At the catch point, ten sweeps of coast-rooted plants were performed. Samples of shells together with soft tissues, after removal of impurities, were dried at the temperature of 60ºC. Upon drying, the samples were milled, pounded in an agate mortar and sieved in order to obtain grain diameter fraction <100 μm.

Chemical analysis included a study of both samples of water and of tissues with mollusc shells. Out of representative and homogenous material samples, three 2 g samples were taken for mineralisation and exposed to microwave-radiation-assisted mineralisation with concentrated nitric(V) acid with the use of the Anton-Paar PE Multiwave 3000 microwave mineraliser. For the determination of heavy metals in water samples as well as extraction solutions obtained as a result of microwave mineralisation of bottom sediments, the AAS method was used, with the application of the AAS-SavantAA Sigma spectrophotometer or the AAS-Savant Zeman spectrophotometer, depending on concentration levels in the analysed solutions. Result correctness was checked by means of the spiking recovery method, and the obtained accuracy amounted to ±4%.

Fig. 3. European physa (*Physella acuta*) (Draparnaud 1805)

Results and discussion

At each selected measurement site, chemical analysis of water was conducted in two yearly study cycles (Fig. 4). The first water measurement cycles of 2008 and 2009 were conducted in June; the second - following the end of the vegetative period in September. Mean analyte values for each study site are shown in Table 1.

A combined result analysis showing biogenic compound concentrations in the studied reservoir points to increasing contents of NO_3^-, NH_4^+ and K^+ ions in 2009 as compared with the same study period in 2008.

Concentrations of NO_3^- and Na^+ demonstrate various values (increase at measurement sites 2 and 3; decrease at sites 1 and 4). The reservoir waters, due to biogene concentration (including nitrate(V) nitrogen, may be counted among non-class waters (four sites).

A significant increase of ammonium nitrogen (study sites 1, 3, and 4) has been found, which would suggest an increase in ammonification process intensity and poor aerenchymatic potential of the reservoir.

Table 1

Mean values of analyte concentration in water samples taken at four sites in the years 2008 and 2009

Indicator	Unit	2008				2009			
		1	2	3	4	1	2	3	4
NO_3^-	[mg·dm^{-3}]	22.000	3.690	22.400	2.800	14.020↓	31.150↑	27.25↑	1.620↓
SO_4^{2-}	[mg·dm^{-3}]	58.600	26.800	52.400	29.200	18.715↓	53.000↑	57.000↑	35.200↑
NH_4^+	[mg·dm^{-3}]	0.035	0.123	0.035	0.086	1.520↑	0.111↓	0.070↑	0.540↑
Na^+	[mg·dm^{-3}]	32.925	26.775	33.750	25.650	6.450↓	47.200↑	45.600↑	15.650↓
K^+	[mg·dm^{-3}]	6.675	3.825	6.025	3.625	7.250↑	8.300↑	8.250↑	5.550↑

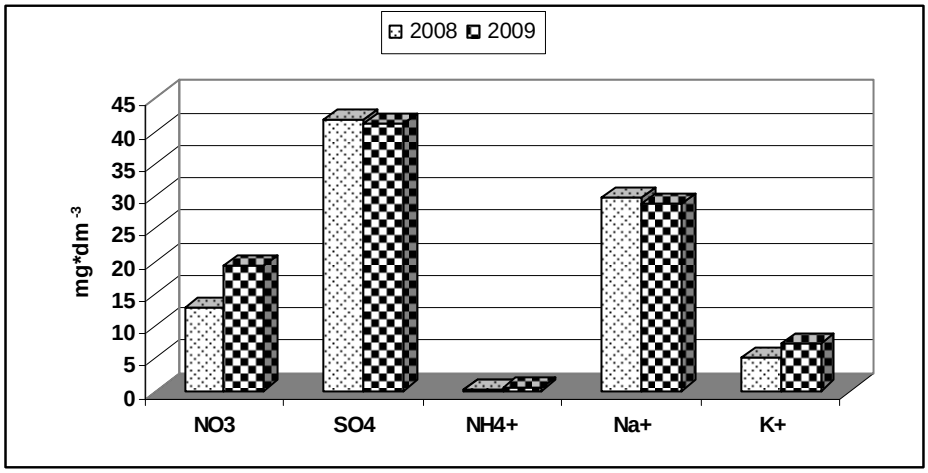

Fig. 4. Mean values of analyte concentration in water samples taken from the Kielce Lake between May and September 2008 and 2009

Growing values of biogenic analyte concentration point to eutrophic tendencies in the studied ecosystem. This process may be assisted by municipal wastewater discharges, surface wash carried by the river current as well as transport of the material washed off the roads and pavements in spring and winter. Significant is also an increase in sedimentation of terrigenic material, which occurs due to intensification of denudation processes in the catchment [17].

When describing the ecochemical status of the reservoir and characterising medium- and long-term risk of unfavourable change in biotope conditions, it is advisable to apply an ecochemical index of water status, such as for example the ANCaq - Acid Neutralizing Capacity [11]. This index is defined as the difference between alkali and strong acids in water solutions and calculated according to the formula:

$$ANCaq\ [\mu Eq \cdot dm^{-3}] = K^+ + Na^+ + 2Mg^{2+} + 2Ca^{2+} - NO_3^- - Cl^- - 2SO_4^{2-}$$

The ANCaq is a superior tool for characterising water acidity because, unlike the pH, it is independent of CO_2 partial pressure.

Natural acidic waters are dominated by organic acids, while waters acidified by anthropogenic activity - by sulphate(VI) ions and aluminium. Originating probably from chemical decomposition of bottom sediments, Ca, Mg and Na ions can balance SO_4^{2-} concentrations. Due to these reactions, ANCaq indexes maintain their positive values.

For the studied reservoir, a comparative record sheet of pH values, measured at selected measurement sites, has been developed (Table 2).

Table 2

Mean pH and ANCaq values in the studied reservoir in the years 2008 and 2009

Measuring point	pH	ANCaq	pH	ANCaq
	2008		2009	
1	7,42	39,83	7,87	37,48
2	7,38	113,3	7,96	98,72
3	7,32	53,41	7,57	48,09
4	7,09	123,40	7,27	115,05

The obtained results point to a pH increase in 2009, as compared with 2008, by further values: measurement site 2 - by 0.58, measurement site 1 - by 0.45, measurement site 3 - by 0.25, measurement site 4 - by 0.18.

Heavy anthropopressure which manifests itself in the waterbody's increasing pollution does not pose an obstacle for the colonisation of this habitat by a representative of the *Physidae* family, the *Pulmonata* order, the European physa (*Physella acuta*).

Table 3

Comparison of Zn, Cd and Pb content in water, bottom sediments and molluscs

Measuring point/month	Water			Bottom sediments			Molluscs		
	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb
	$\mu g \cdot dm^{-3}$								
August									
1	0.9	0.1	3.6	4.1	2.0	3505.0	0.02	0.93	7.2
2	1.0	0.1	4.5	4.8	1.7	877.0	14.1	0.8	69.7
3	2.6	0.2	2.2	2.5	3.2	592.0	63.7	1.1	36.1
4	0.9	0.2	2.1	2.6	2.6	17.1	27.3	2.1	9.1
September									
1	0.03	0.1	2.2	3.5	12.3	15300.0	0.03	0.1	7.2
2	0.03	0.2	1.8	2.9	0.6	925.0	17.9	75.2	11.3
3	0.06	0.2	5.5	2.6	0.8	859.0	108.0	34.8	9.1
4	0.04	0.1	3.0	3.8	9.0	14395.0	36.5	86.1	16.0

Research by Serafinski et al [17] has proved that intensity of the expansion of this mollusc species in Upper Silesia reservoirs is related to the speed of industrialisation, and was at its most intensive in the years 1971-1990. The European physa's low sensitivity to pollution is an advantage in the colonisation of anthropogenic water biocenosis habitats [18]. This mollusc demonstrates an ability to concentrate heavy metals in quantities exceeding those in the habitat [7]. Feeding on sediments, suspension filtration as well as herbivorosity of the European physa make it possible to formulate patterns of element

migration in the water - bottom sediment - malacofauna system. The above factors have been the reason for this species selection as a bioindicator organism. A comparative record sheet of heavy metal concentrations in water, bottom sediments, mollusc shells and tissues has been presented in Table 3.

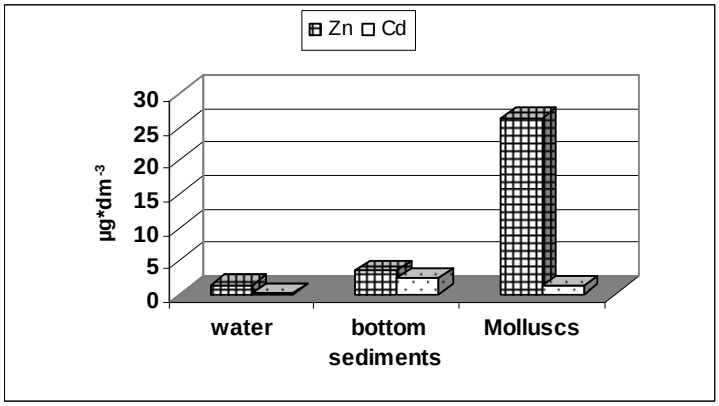

Fig. 5. Heavy metals content in studied samples in June 2009

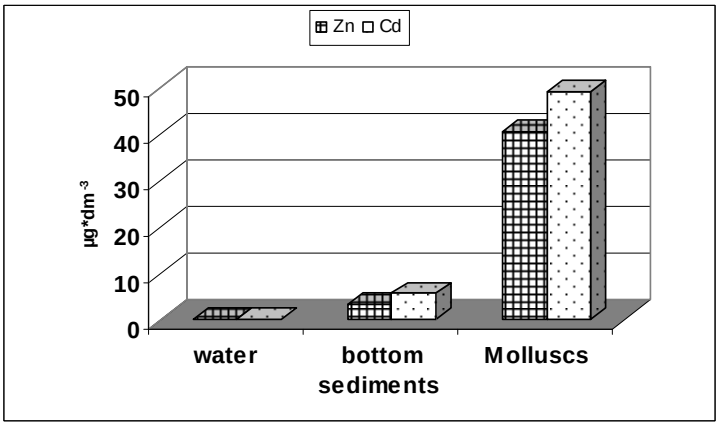

Fig. 6. Heavy metal content in studied samples in September 2009

The problem of heavy metal accumulation in snail shells and soft tissues has been subject to analysis by several authors [18-24]. Yet these studies have always differentiated the results by showing discrepancies in element accumulation levels in mollusc tissues and shells. In these analyses, authors either point to metal concentration in shells several times exceeding that in mollusc tissues [25], or vice versa - they show lower values in shells than in tissues [21].

Piotrowski [26] points to two metals, Pb and Cd, which are firmly bonded with carbonates, particularly with aragonite, to form shells. Zn, however, as follows from the

reaseach by Van-Balognah et al [27] on *Lyminea stagnalis* accumulates in the soft tissues of the body. Therefore it seems justified to treat a mollusc's organism as a whole.

The present study has not split the results into two groups (shells and soft tissues) as it has been assumed that metals accumulated in shells originate from metabolic food routes, and consequently their temporary presence in tissues is a fact.

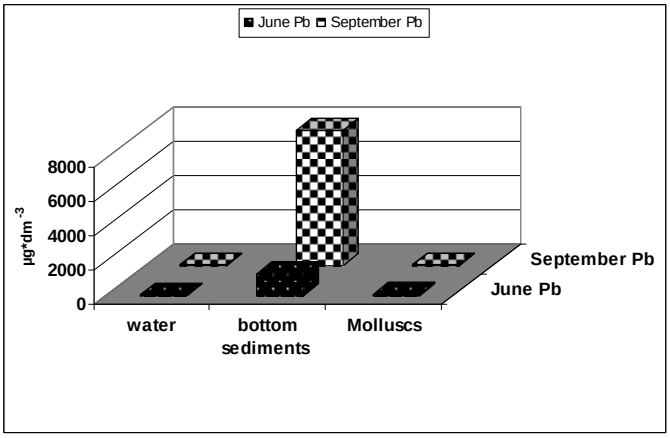

Fig. 7. Lead content in studied samples in June and September 2009

The objectives of the present study have been to formulate patterns of metal accumulation in the water - bottom sediment (detritus) - molluscs system, as well as demonstrating the extent to which the selected mollusc species fulfils bioindicator criteria. The analysis has shown a growth tendency for Zn (Figs 5 and 6), the metal's concentration increasing in the following order: water < sediment < mollusc; and for Pb: Water < mollusc < sediment.

In the case of Cd, a discrepancy has been observed depending on the seasons in which the study was conducted (Table 3). For the second quarter of the year, it takes the form: mollusc < water < sediment; for the third quarter of the year: water < sediment < mollusc.

Out of the three studied heavy metals, the highest concentrations found were those for Pb (Fig. 7) which accumulates in bottom sediments (maximum values in the third quarter of the year at 7869.7 $\mu g \cdot dm^{-3}$); next, Zn and Cd accumulate in mollusc bodies (maximum Zn values of 162.4 $\mu g \cdot dm^{-3}$ - third quarter; maximum Cd values of 49.05 $\mu g \cdot dm^{-3}$ - third quarter).

A comparison of Zn concentration in water, bottom sediments and mollusc bodies in both study periods points to the highest concentration of this element in mollusc bodies with respect to the other two systems (water and sediment).

Conclusion

1. The study conducted in the years 2008-2009 at the Kielce Lake points to the possibility of using detritophagous malacofauna for the assessment of the degree of reservoir pollution.

2. The presence of a *Physella acuta* population in a water reservoir exposed to heavy anthropopressure allows for this species to be counted among hydrobioindicators.
3. The study of heavy metal distribution in the Kielce Lake, exposed to heavy anthropopressure, enables the conclusion that heavy metal distribution varies for different elements. Pb accumulates primarily in bottom sediments, while Zn and Cd - in living organisms (*Physella acuta*).

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ROLA MALAKOFAUNY WSKAŹNIKOWEJ W OCENIE ZANIECZYSZCZENIA WÓD ŚRÓDLĄDOWYCH PODLEGAJĄCYCH ANTROPOPRESJI NA PRZYKŁADZIE ZALEWU KIELECKIEGO

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Abstrakt: Organizmy wykazujące cechy bioindykatorów w charakterystyczny dla siebie sposób reagują na zmiany w biotopie. Są to najczęściej organizmy o dużej wrażliwości na substancje toksyczne lub odwrotnie, tzn. wykazujące wysoki stopień tolerancji względem wprowadzanych do biotopu toksyn. Tolerancja ta polega na zdolności bioindykatorów do kumulowania zanieczyszczeń w ilościach, które dla innych form życia stanowią wartości letalne. Wykorzystanie mięczaków i ich zdolności do koncentracji metali ciężkich w ilościach wyższych niż w środowisku stwarza możliwość użycia tej grupy organizmów jako bioindykatorów biotopów wodnych. Różnorodność sposobów odżywiania i rodzaj pobieranego pokarmu (żerowanie na osadach, filtracja zawiesiny, roślinożerność) pozwalają na wskazanie różnic w koncentracji metali ciężkich mimo pochodzenia gatunków z tego samego akwenu. W przypadku malakofauny żerującej na osadach dennych możliwe jest uchwycenie zależności migracji pierwiastków w układzie: woda - roślina - osad denny - malakofauna. Badaniami objęto Zalew Kielecki, zbiornik utworzony w granicach miasta Kielce poprzez zamknięcie doliny rzeki Silnicy jazem, na jej 8 km. Tereny wokół zbiornika zagospodarowane są zarówno na cele rekreacyjne, mieszkaniowe, jak i gospodarcze. Dodatkowo w bezpośrednim sąsiedztwie przebiega droga krajowa w kierunku Łodzi, będąca źródłem zanieczyszczeń komunikacyjnych, oraz zlokalizowany jest basen miejski, z którego wprowadzana jest do zbiornika woda chlorowana. Zalew zasilany jest ciekiem wodnym stanowiącym odbiornik zanieczyszczeń komunalnych z miejscowości Masłów, położonej w pobliżu Kielc. Ilość oraz różnorodność związków zarówno organicznych, jak i nieorganicznych, dostających się do zbiornika, powoduje, że wody zalewu są bardzo zanieczyszczone. Uzyskane wyniki analiz chemicznych oraz biologicznych pozwalają na ocenę stanu czystości zbiornika, stwarzają system ostrzegania przed zagrożeniem oraz pokazują reakcje malakofauny na zmiany zachodzące w biotopie.

Słowa kluczowe: malakofauna bezkręgowa, osad denny, bioindykacja, detrytus

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Józef ANTONOWICZ[1*] and Jan TROJANOWSKI[1]

ACCUMULATION CAPACITY OF CADMIUM AND MANGANESE IN SURFACE WATER MICROLAYER OF ESUTARINE LAKE

WZBOGACANIE MIKROWARSTWY POWIERZCHNIOWEJ W KADM I MANGAN W ESTUARIOWYM JEZIORZE

Abstract: In order to investigate the effect of salinity level on the concentration of cadmium and manganese in the surface microlayer, several samples were collected from an estuarine lake Gardno in a quarterly cycle over the period of four years. Three testing stations were established in locations typical of this lake. In the estuarine lake Gardno, one can distinguish three areas with various levels of salinity, with a sampling site for each of these areas. The volume of water taken as samples varied, depending on the techniques used. During the experiment, surface microlayer samples of ca 0.1 and 0.25 mm were obtained. The subsurface water was obtained at the depth of 15 cm. The collected samples were analysed with an atomic absorption spectrometer in order to determine the concentration of cadmium and manganese. Cadmium accumulation in microlayers of surface waters in Lake Gardno was dependent on the concentration of this metal in the deep waters and varied with water salinity. In turn, counts of heterotrophic bacteria had a significant effect on the concentration of manganese. Concentrations of both metals in analyzed surface water microlayers were higher than in the subsurface layer.

Keywords: surface microlayer, heavy metals, estuarine lake, seasonal changes

The surface water microlayer is a thin layer, found at the interface of water and the atmosphere. This layer represents a tiny section of the aqueous environment, forming a unique ecotone at the boundary of this exchange zone. This surface microlayer in water reservoirs is a special chemical and physical environment, completely different from subsurface water [1, 2]. The surface microlayer plays a significant role in gas exchange and transport of different substances from the column of water to the atmosphere and *vice versa*. Dissolved substances, particles and microorganisms are transported to this unique zone by simple diffusion, lifted eg by convection movements from bottom deposits and subsurface water. At the same time the surface microlayer is supplied by precipitation with dust and aerosols. The above mentioned processes lead to the accumulation of chemical substances and microorganisms in the boundary microlayer [3].

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The aim of the study was to investigate the accumulation capacity of the surface water microlayers of estuarine lake in relation to manganese and cadmium in three parts of lake characterized different scale dilution of brackish water in freshwater. The second aim was to analyze the seasonal changes in the concentrations of investigated metals in the microlayers and in subsurface water.

Material and methods

Object of study was an estuarine lake Gardno which is characterized by a total area of 2,468 ha, shallow mean depth 1.3 m and maximum depth 2.6 m and salty water. The lake is located at a latitude of 53°39.4' and longitude of 17°07.1' [4]. Three research stations were located on the lake. The first station was established near the Lupava river confluence (Fig. 1), where water of the lake indicates inland characteristics, the second station was located in the middle part, and the third near the canal that links the lake with the Baltic Sea. On the third post highest cumulation of chloride anions was observed. This is due to the fact, that winds transfer sea water into the lake, where it mixes with the lake's waters.

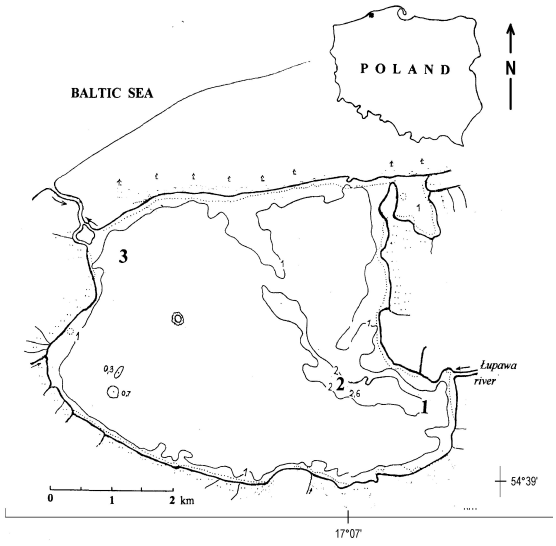

Fig. 1. Lake Gardno with marked sampling positions

Samples of surface water were collected period 4 years from 1998 to 2002 in quarterly cycle using three methods:

a) the glass plate method (SM) - was used [5] to collect the surface water microlayer, with a thickness of approx. 100 µm ± 20 µm. The plate was submerged and after the surface stabilized it was taken out with a speed of ca 15 $cm \cdot s^{-1}$;
b) the Garrett's mesh technique (GM) - was applied [6] when sampling the surface layer with a thickness of approx. 240 µm ± 50 µm. The mesh was submerged like the glass plate and after taking it out, the water sample was put in a container with the help of a wiper;

c) subsurface water (PW) was collected at a depth of 20 cm from the water table, and it was taken as a comparison sample for the surface microlayer samples.

Determinations of heavy metals in samples and statistical analysis

Water samples after being delivered to the laboratory were mineralized using HNO_3 trace pur. In mineralized water samples contents of analyzed metals were determined using a Carl-Zeiss Jena AAS3 atomic absorption spectrophotometer. Manganese was analyzed by the flame technique [7], while cadmium using an EA3 electromagnetic atomizer and an automatic sample injector [8].

Statistical analysis of results was conducted with the use of a Statistica software package. When comparing means a one-way analysis of variance (ANOVA) with Tukey's t-test was used [9].

In order to compare both investigated environments, ie water from the surface microlayer and subsurface water, enrichment factors (EF) were used, calculated from the following formula: $EF = C_M/C_P$ where: C_M - concentration of the analyzed component in respective surface microlayer; C_P - concentration of the same component in subsurface water [1, 10].

Results and discussion

Lake Gardno, which is connected with the Baltic Sea, is periodically supplied with sea water. Due to this, the lake has three areas with different salinity. The first one (station no. 1) is dominated by fresh water and is characterized by a low concentration of Cl^- ions. In the central area (station no. 2), the concentration of those ions was intermediate. The highest concentration was observed in the third area (station no. 3), which is under a direct influence of sea water supply.

Table 1

Concentrations of cadmium [$\mu g \cdot dm^{-3}$] in surface microlayers and subsurface waters of Lake Gardno; arithmetic mean (X) minimum (MIN) maximum (MAX) standard deviation (SD) enrichment factor (EF), analysis of variance (ANOVA) with Tukey's t-test was used to compare stations (** $p < 0.01$, * $p < 0.05$)

	X	**R**	**SD**	**CV [%]**	**EF**
1SM	2.75	11.93	3.51	127.4	3.11
1GM	3.25	10.42	2.88	88.4	4.15
1PW	**0.82**	**0.89**	**0.28**	**33.4**	
2SM	1.65	1.67	0.47	28.7	6.82
2GM	1.35	1.88	0.48	35.7	4.52
2PW	**0.42**	**0.88**	**0.27**	**63.3**	
3SM	2.13	1.65	0.55	26.1	4.73
3GM	2.16	3.46	1.12	51.6	5.49
3PW	**0.68**	**0.78**	**0.30**	**45.1**	

ANOVA	df = 33, F = 6.30, p < 0.01	**
Tukey Test	* (2PW – 1PW) ; ** (2PW – 3PW)	

Table 1 presents average concentrations of cadmium in the three areas of Lake Gardno, while Table 2 gives manganese concentrations. On the basis of provided data, one can observe that both elements were more concentrated in the surface microlayer than in the subsurface water (Tables 1 and 2). This is consistent with results reported by [11-16] and it is true for both fresh and sea water reservoirs. Probably this is due to the absorbing and combining ability of chemical substances and microorganisms contained in the surface microlayer.

Table 2

Concentrations of manganese [$\mu g \cdot dm^{-3}$] in the surface microlayers and subsurface waters of Lake Gardno; arithmetic mean (X) minimum (MIN) maximum (MAX) standard deviation (SD) enrichment factor (EF), analysis of variance (ANOVA) with Tukey's t-test was used to compare stations (** $p < 0.01$)

	X	**R**	**SD**	**CV [%]**	**EF**
1SM	101.94	291.52	77.95	76.5	2.41
1GM	151.90	282.70	92.13	60.7	3.61
1PW	**82.77**	**231.94**	**79.25**	**95.8**	
2SM	144.67	331.25	88.30	61.0	6.20
2GM	198.50	887.76	233.61	117.7	7.25
2PW	**76.33**	**254.13**	**72.88**	**95.5**	
3SM	329.41	1119.85	296.70	90.1	1.86
3GM	298.86	883.83	244.67	81.9	1.71
3PW	**174.10**	**219.85**	**59.51**	**34.2**	

ANOVA (PW)	df = 33, F = 7.11, p < 0.01	**
Tukey Test	3PW – 1PW and 3PW – 2PW	**

When analyzing the concentration of cadmium and manganese in the surface microlayer samples from the three samplings sites, it ought to be mentioned that the concentration of the latter element was the lowest for both the surface microlayer and subsurface water layer in the central area of the lake (station no. 2). In the case of this metal a correlation between its concentration in the subsurface and in the surface microlayer becomes significant. This suggests that in the investigated lake cadmium migrates to the surface microlayer from subsurface water, to which it probably gets from the bottom sediments. This kind of correlation was not observed in the case of manganese. The concentration of this metal was higher in areas of elevated salinity. In the subsurface water, the concentration of manganese was, similarly as for cadmium, lowest in the central part of the lake (station no. 2).

Using the calculated enrichment factors (Tables 1 and 2) we may determine the cumulative abilities of the investigated surface microlayer for cadmium and manganese. The obtained averages of cadmium enrichment were rising simultaneously with the ratio of chlorine ions (station 1). This is clearly evident in the GM microlayer, for which the EF ratio increases from 4.15 (station 1) to 5.49 (station 3). The literature concerning studies on the microlayer indicates that the sea water surface microlayer enrichment ratios for most metals are higher than those observed in fresh water reservoirs [17]. Thus it may be inferred that variation in salinity influenced the concentration of cadmium, especially in the GM microlayer. The high enrichment factor for cadmium in the SM microlayer, recorded in station number 2, correlates with a high ratio of manganese in this area. Both microlayers

analyzed in terms of manganese enrichment have the highest factor in the central area of the lake (SM = 6.20 and GM = 7.25), while the lowest in waters under the direct influence of the Baltic Sea (station no. 3).

Probably the key factor that influences the concentration of cadmium and manganese in the surface microlayer is the level of salinity and the presence of various microorganisms, exhibiting different preferences for salinity. The lower manganese enrichment in the SM layer in comparison with the GM layer may have been caused by the effect of sea water and the behaviour of microorganisms. This influence is clearly visible in the GM microlayer. Enrichment ratios (EF) in this microlayer correlate to the number of heterotrophic bacteria (CFU) (Fig. 2). In testing site no. 2, exceptionally high rates of microlayer enrichment were observed for manganese and CFU (EF_{GM} = 7.25 and EF_{GM} = 5.82, respectively), even though the concentration of manganese in the subsurface water is lower than that in stations nos. 1 and 3. In addition, despite the fact that the population of heterotrophic bacteria in this lake is the smallest in this area, the EF in case of the number of heterotrophic bacteria is high here. In this case manganese, as an element essential for the existence of these organisms, is likely to stimulate their population growth in the surface microlayer of Gardno. Probably the reason for the high concentration ratio of heterotrophic bacteria in sampling site no. 1 is the domination of particular strains, that are characteristic for fresh water reservoirs. These bacteria have different metabolisms and thus the diagrams for CFE and magnesium are not consistent in case of testing site no. 1. Sources in available literature [18-20] describe the relation of these microorganisms with heavy metals.

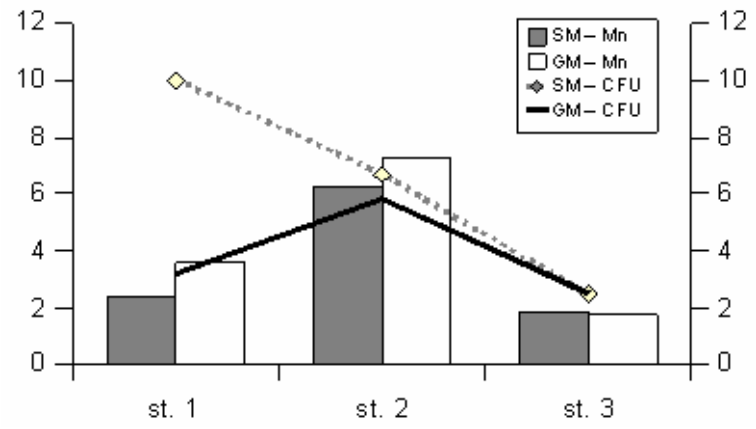

Fig. 2. Enrichment factors in heterotrophic bacteria [21] and manganese in SM and GM microlayers in lake Gardno for research stations 1-3

Seasonal dynamics of cadmium concentration in the analysed water layers of Lake Gardno varied for different testing stations. In stations nos. 1 and 3 the minimum value of the factor for the SM microlayer was observed during summer, while in station no. 2 in autumn (Fig. 3). No considerable seasonal differences were observed in the concentration of this metal in testing stations nos. 2 and 3. In contrast, the above mentioned concentration in station no. 1 was three times higher in autumn than in summer and spring. Seasonal changes in cadmium concentration in the GM microlayer were similar to the ones in the SM

microlayer. Only in sampling site no. 1 no summer-minimum was observed. The concentration of this metal in the GM microlayer in summer was similar to that in spring. In the PW layer slight seasonal differences in cadmium concentration were observed.

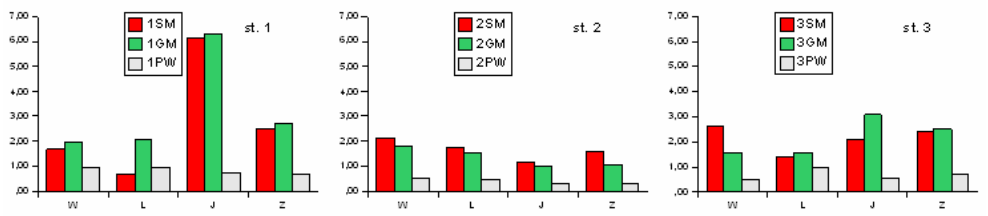

Fig. 3. Seasonal changes in concentrations of cadmium [µg·dm^{-3}] in analysed water layers (SM, GM and PW) of Lake Gardno at sampling stations nos. 1, 2 and 3 in seasonal cycle: W - spring, L - summer, J - autumn, Z - winter

The dynamics of seasonal changes in manganese concentration in the analyzed water layers is shown in Figure 4. Changes in both microlayers were similar for stations nos. 1 and 2. The highest concentration was observed during winter, while in the GM microlayer in station no. 1 the ratio was high also during autumn. In turn, in station no. 3 the highest value of this factor was recorded in spring and winter. In the PW layer seasonal changes of manganese concentration were different in all sampling sites.

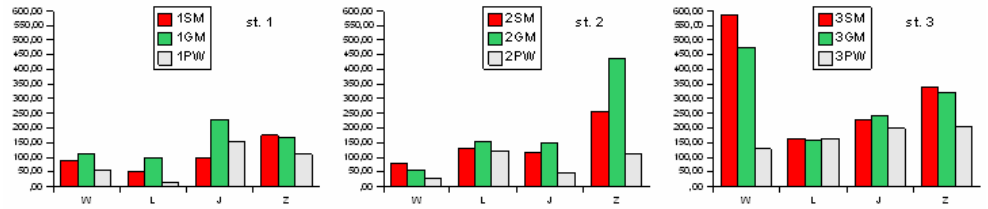

Fig. 4. Seasonal changes in concentrations of manganese [µg·dm^{-3}] in analysed water layers (SM, GM and PW) of Lake Gardno at sampling stations nos. 1, 2 and 3 in seasonal cycle: W - spring, L - summer, J - autumn, Z - winter

Conclusion

Cadmium and manganese show the ability to accumulate in the surface microlayer of Lake Gardno at a much higher ratio than in the subsurface water. Cadmium concentration in surface microlayers is mainly related to its concentration in the subsurface waters. In the case of cadmium, the ability to enrich the microlayer was affected by mixing of sea water with the fresh water. In the case of manganese, the factor stimulating enrichment was not only the sea water, but also heterotrophic bacteria. The accumulation of analysed heavy metals in the surface microlayers seems to exhibit seasonal dynamics and it was generally comparable in the analyzed water layers.

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WZBOGACANIE MIKROWARSTWY POWIERZCHNIOWEJ W KADM I MANGAN W ESTUARIOWYM JEZIORZE

Zakład Chemii Środowiskowej, Akademia Pomorska w Słupsku

Abstrakt: Badając wpływ zasolenia na koncentrację kadmu i manganu w mikrowarstwie powierzchniowej wody, próbki tej mikrowarstwy pobierano z estuariowego jeziora Gardno w cyklu kwartalnym przez okres 4 lat. Na jeziorze usytuowano trzy stanowiska badawcze w jego reprezentatywnych punktach. Jezioro Gardno jest zbiornikiem estuariowym i można w nim wyróżnić trzy zasadnicze strefy o różnym zasoleniu. W zależności od stosowanej techniki pobierania próbek wody zbierano mikrowarstwę powierzchniową wody o grubości około 0,1 mm i około 0,25 mm. Wodę podpowierzchniową pobierano na głębokości 15 cm od lustra wody. W pozyskanych próbkach wyznaczono stężenie kadmu i manganu metodami spektrometrii atomowej. Kumulacja kadmu w mikowarstwach powierzchniowej wody jeziora Gardno była zależna od stężenia tego metalu w toni wodnej i zmieniała się wraz z zasoleniem wody. Natomiast na koncentrację manganu duży wpływ miała liczebność bakterii heterotroficznych. Stężenia obydwu metali w badanych mikrowarstwach powierzchniowych wody były większe niż w warstwie podpowierzchniowej.

Słowa kluczowe: mikrowarstwa powierzchniowa, metale ciężkie, jezioro estuariowe, zmiany sezonowe

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EFFECT OF EFFECTIVE MICROORGANISMS (EM) ON NUTRIENT CONTENTS IN SUBSTRATE AND DEVELOPMENT AND YIELDING OF ROSE (*Rosa x hybrida*) AND GERBERA (*Gerbera jamesonii*)

WPŁYW EFEKTYWNYCH MIKROORGANIZMÓW (EM) NA ZAWARTOŚĆ SKŁADNIKÓW POKARMOWYCH W PODŁOŻU ORAZ ROZWÓJ I PLONOWANIE RÓŻY (*Rosa x hybrida*) I GERBERY (*Gerbera jamesonii*)

Abstract: The aim of conducted studies was to assess the effect of the application of Effective Microorganisms (EM) on changes in contents of available and readily soluble forms of nutrients in the peat substrate as well as growth, development and yielding of rose (*Rosa x hybrida)* and gerbera (*Gerbera jamesonii*) grown on the substrate. Effective Microorganisms were applied before the vegetation season in the form inoculum at 3 g preparation·5 dm^{-3} substrate and in foliar application as plant spraying (repeated three times, with an aqueous solution at a concentration of 0.1%). In the conducted studies the effect of EM, applied both to the roots and as foliar application, was found on changes in contents of available nutrients in the substrate, at the simultaneous substrate acidification, in relation to the control combination. The significantly highest yield of flowers in case of both examined species was recorded at the application of the EM inoculum to the roots. This had a positive effect on the number or formed shoots and the diameter of flowers (in case of roses) and the number of formed inflorescences (in case of gerberas). Foliar application of Effective Microorganisms had a positive effect on the diameter of flowers in roses and the number of formed inflorescences and the number of leaves in case of gerberas. Results of conducted analyses indicate that Effective Microorganisms may be useful in the cultivation of roses and gerberas, due to their positive and at the same time significant effect on growth and yielding of these plants.

Keywords: Effective Microorganisms, rose, gerbera, development, yielding, nutrient content, substrate

Several popular species of ornamental plants, such as eg rose, gerbera or pelargonium, are grown in soilless culture systems, ie in substrates being completely isolated from the soil medium. The substrate used most commonly in container culture of these species is peat

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substrate. In the course of plant vegetation the organic matter it contains is mineralized, as a result of which process nutrients are released to the root medium of plants, at the simultaneous deterioration of air and water relations. Mineralization of organic matter is performed by soil microorganisms. Thanks to the intensification and targeting of microbiological processes taking place in the root medium, among other things as a result of enhanced nutrient availability, it is feasible to improve quantitative and qualitative yielding of plants.

One of the microbiological preparations used for this purpose is Effective Microorganisms (EM), being a commercial mixture of photosynthesizing bacteria, Actinomycetes, lactic acid bacteria, yeasts and fermenting fungi (*Apergillus* and *Penicilium*) [1-3]. The microbiological composition of the EM concentrate (population size in 1 cm^3 given in brackets): *Streptomyces albus* (10^5), *Propionibacterium freudenreichil* (10^5), *Streptococcus lactis* (10^5), *Aspergillus oryzae* (10^5), *Mucor hiemalis* (10^5), *Saccharomyces cerevisiae* (10^5) and *Candida utilis* (10^5) [4]. Moreover, EM also contain an unspecified amount of *Lactobacillus* sp., *Rhodopseudomonas* sp. and *Streptomyces griseus*. Effective Microorganisms have a positive effect on the decomposition of organic matter, limiting putrefaction, increasing nitrogen content in the root medium of plants (the role of nitrifying bacteria), phosphorus (the role of Actinomycetes), improving soil fertility and as a result contributing to growth and development of root systems of plants [3, 5, 6]. To date several studies have been conducted on the application of EM in growing of different plant species [7-13]. The use of EM, apart from the improvement of plant yielding, also results in increased counts of beneficial soil microorganisms and enhanced resistance of plants to pathogens [12, 13].

The aim of the presented studies was to assess the effect of the application of Effective Microorganisms on changes in contents of available forms of nutrients in the peat substrate, as well as growth, development and yielding of roses and gerberas grown to produce cut flowers.

Material and methods

The vegetation and laboratory experiments were conducted at the Experimental Station of the Department of the Faculty of Horticulture, the Poznan University of Life Sciences. Analyses were conducted on the effect of Effective Microorganisms (EM) on changes in contents of available nutrient forms in the peat substrate (macro- and microelements as well as sodium, substrate pH and salinity), as well as growth, development and yielding of roses (*Rosa x hybrida)* and gerberas (*Gerbera jamesonii*) grown for cut flowers.

In the experiments highmoor peat was used, coming from deposits in Latvia (Hartmann), with the following chemical composition (in $mg \cdot dm^{-3}$): $N\text{-}NH_4$ 28, $N\text{-}NO_3$ 7, P 37, K 11, Ca 107, Mg 21, $S\text{-}SO_4$ 10, Fe 50.2, Zn 1.3, Mn 1.3, Cu 0.4, B 0.43, Na 11, Cl 27, pH 3.86, EC 0.16 $mS \cdot cm^{-1}$ and bulk density of 460 $g \cdot dm^{-3}$. In order to optimize its reaction peat was limed based on the neutralization curve, applying 7.5 g dolomite$\cdot dm^{-3}$. After liming the chemical composition of peat was as follows (in $mg \cdot dm^{-3}$): $N\text{-}NH_4$ 35, $N\text{-}NO_3$ trace amounts, P 20, K 18, Ca 2045, Mg 164, $S\text{-}SO_4$ 25, Fe 19.8, Zn 1.8, Mn 2.7, Cu 0.4, B 0.50, Na 18, Cl 29, pH 6.31, EC 0.49 $mS \cdot cm^{-1}$. At 14 days after liming the following fertilizers and technical salts were applied: ammonium saltpetre (34% N), monobasic potassium phosphate (22.3% P, 28.2% K), magnesium sulfate (9.5% Mg,

12.7% S-SO_4), iron chelate Librel FeDP7 (7% Fe), manganese sulfate ($MnSO_4 \cdot H_2O$, 32.3% Mn), zinc sulfate ($ZnSO_4 \cdot 7H_2O$, 22.0% Zn), copper sulfate ($CuSO_4 \cdot 5H_2O$, 25.6% Cu) and borax ($Na_2B_4O_7 \cdot 10H_2O$, 11.3% B), providing the contents of nutrients conforming to the standard levels, recommended for growing the analyzed plant species, amounting in case of roses to (in $mg \cdot dm^{-3}$): N-NH_4 35, N-NO_3 220, P 220, K 260, Ca 2045, Mg 220, Fe 50, Zn 20, Mn 20, Cu 5, B 0.50, pH 6.0 - 6.5, while in case of gerberas it was (in $mg \cdot dm^{-3}$) N-NH_4 35, N-NO_3 230, P 220, K 250, Ca 2045, Mg 240, Fe 50, Zn 20, Mn 20, Cu 5, B 0.50 and pH 6.0÷6.5, respectively.

The experiments were established in the systematic design in 10 replications. The following application methods of Effective Microorganisms (EM): root and foliar applications (described in the text as EM-substrate and EM-spraying) were used. The control was not treated with EM. In case of the combination in which EM was applied to the substrate, its inoculation at a dose of 3 g preparation per 5 dm^3 substrate was performed 10 days before planting to a permanent location. In turn, plants were sprayed 3 times in the vegetation season in June at 10-day intervals, with spray liquid at a concentration of 0.1%. Both roses and gerberas were growing at a standard stocking of 3 plants$\cdot m^2$. Throughout the vegetation period cultivation measures for investigated species were performed following current recommendations.

During the vegetation period at 3- to 4-day intervals the following biometric measurements of plants were taken: the length of the peduncle, the number of cut flowers, diameters of the flower head, the number of inflorescences, the number of leaves, the number of removed leaves (for gerberas), the number and length of shoots, the number of cut flowers and their diameters (for roses).

Chemical analyses. Towards the end of the vegetation period substrate samples were collected in order to conduct chemical analyses for the contents of soluble nutrients and sodium. One mixed sample comprised 8-10 individual samples collected from each combination. Collected samples were analyzed chemically using the universal method according to Nowosielski [14]. Macroelements (N-NH_4, N-NO_3, P, K, Ca, Mg, S-SO_4), Cl and Na were extracted in 0.03 M CH_3COOH, at a substrate: extraction solution ratio of 1:10. After extraction the following parameters were determined: N-NH_4, N-NO_3 - by microdistillation according to Bremner as modified by Starck, P - by colorimetry with ammonium vanadium molybdate, K, Ca, Na - by photometry, Mg - by absorption atomic spectrometry (AAS, a Carl Zeiss Jena apparatus), S-SO_4 - by nephelometry with $BaCl_2$ and Cl - by nephelometry with $AgNO_3$. Microelements (Fe, Mn, Zn and Cu) were extracted from the substrate using Lindsay's solution, containing in 1 dm^3, 5 g EDTA (ethylenediaminetetraacetic acid), 9 cm^3 25% NH_4OH solution, 4 g citric acid and 2 g $Ca(CH_3COO)_2 \cdot 2H_2O$. Microelements were determined by AAS. Salinity was determined using conductometry, as electrolytic conductivity of the substrate (EC in $mS \cdot cm^{-1}$), while pH was determined by potentiometry (substrate:water = 1:2). Results of investigations were analyzed statistically and means were clustered using the Duncan test at the significance level $\alpha = 0.05$.

Results

Multi-faceted changes were observed in the contents of nutrients and sodium in the radical zone of plants (Table 1). In case of substrate inoculation with EM the assayed

contents of ammonium nitrogen, nitrate nitrogen, phosphorus, potassium and magnesium were markedly lower than in the control combination, at a similar content of sulfate sulfur (in case of gerberas) as well as phosphorus, calcium and magnesium (in case of roses). A reduction of nutrient contents may be the effect of their higher uptake by plants, stimulated by the formation of a higher number of flowers. Root application of Effective Microorganisms in case of both species resulted in a slight acidification of substrate. The highest contents of nitrate nitrogen, phosphorus, potassium, magnesium and sulfur were shown for substrates sampled from under gerbera plants, while those of nitrate nitrogen in case of roses, sprayed with Effective Microorganisms. The application of EM as a microbiological inoculum of the substrate resulted in a slight increase in its salinity.

Table 1

The effect of Effective Microorganisms on contents of ammonium nitrogen, nitrate nitrogen, phosphorus, potassium, calcium, magnesium, sulfate sulfur (in mg·dm^{-3}) as well as pH and salinity (EC) of substrate in growing of gerberas and roses

Combination	N-NH_4 [mg·dm^{-3}]	N-NO_3 [mg·dm^{-3}]	P-PO_4 [mg·dm^{-3}]	K [mg·dm^{-3}]	Ca [mg·dm^{-3}]	Mg [mg·dm^{-3}]	S-SO_4 [mg·dm^{-3}]	pH	EC [mS·cm^{-1}]
Gerbera									
Control	101.5b	150.5b	169.7a	134.4b	1649.2b	328.3a	454.6a	6.27b	0.09a
EM-substrate	52.5a	73.5a	157.2a	76.8a	1665.8b	287.0a	451.5a	5.57a	0.12ab
EM-spraying	31.5a	518.0c	396.8b	236.8c	1095.3a	689.8b	651.5b	5.46a	0.14b
Rose									
Control	35.0a	59.5a	262.5b	184.3a	2166.2b	412.7a	508.4a	5.97a	0.16a
EM-substrate	49.0b	61.3a	212.0a	155.0a	1989.7ab	361.6a	514.4a	5.69a	0.17a
EM-spraying	42.0a	280.0b	163.1a	365.8b	1626.3a	360.8a	487.5a	6.06a	0.36b

Table 2

The effect of Effective Microorganisms on contents of iron, manganese, zinc, copper, chlorides and sodium (in mg·dm^{-3}) in substrate in growing of gerberas and roses

Combination	Fe [mg · dm^{-3}]	Mn [mg · dm^{-3}]	Zn [mg · dm^{-3}]	Cu [mg · dm^{-3}]	Cl [mg · dm^{-3}]	Na [mg · dm^{-3}]
Gerbera						
Control	17.85a	5.52a	21.40a	2.15ab	87.1a	174.3a
EM-substrate	21.37a	7.90a	17.52a	1.88a	112.3a	179.6a
EM-spraying	26.29b	13.45b	23.11b	2.46b	197.9b	427.9b
Rose						
Control	27.03b	15.21b	27.83b	2.39ab	250.4a	273.5a
EM-substrate	36.25b	19.52c	28.92b	2.44b	257.3a	260.1a
EM-spraying	16.90a	6.74a	17.06a	1.92a	248.0a	230.8a

A marked increase was shown for contents of iron, manganese and chlorides in substrates both in the cultivation of gerberas and roses, in case of application of EM to the radical zone (Table 2). This may have resulted from a reduction of substrate pH, with which metal solubility increased, including that of metallic microelements. In case of gerberas the highest contents of iron, manganese, zinc, copper and chlorides were recorded for substrate sampled from under plants sprayed with Effective Microorganisms, while in roses at the application of these microorganisms to the root zone.

The application of Effective Microorganisms in the growing of **roses** for cut flowers had a positive effect on growth and development of plants. In the conducted analyses

a significant effect on the number of shoots was shown for EM supplied to the roots (an increase by 32.9%) (Fig. 1). Significant differences were found towards the end of the vegetation period (August) in terms of the length of formed shoots. The longest shoots were recorded for plants in the control combination (37.7 cm), while significantly shorter (33.5 cm) were observed in case of plants grown in the substrate inoculated with EM. This dependence was confirmed for the mean value from the analyzed combinations. A significant positive effect connected with the application of Effective Microorganisms to the radical zone was shown on quantitative yielding of roses. This measure, similarly as spraying of plants with EM, had a significant effect on an increase in diameters of rose flowers.

A positive effect of spraying plants with EM, as well as EM being supplied to the radical zone was shown on the number of formed inflorescences in **gerberas**, which is confirmed by the number of cut inflorescences in case of plants sprayed with EM (an increase by 31%), and for plants grown in the substrate inoculated with EM (an increase by 62.1%) in comparison with the control combination. The length of peduncles was similar in case of all tested combinations. No effect of the application of Effective Microorganisms was shown on the diameter of flower heads in the inflorescences.

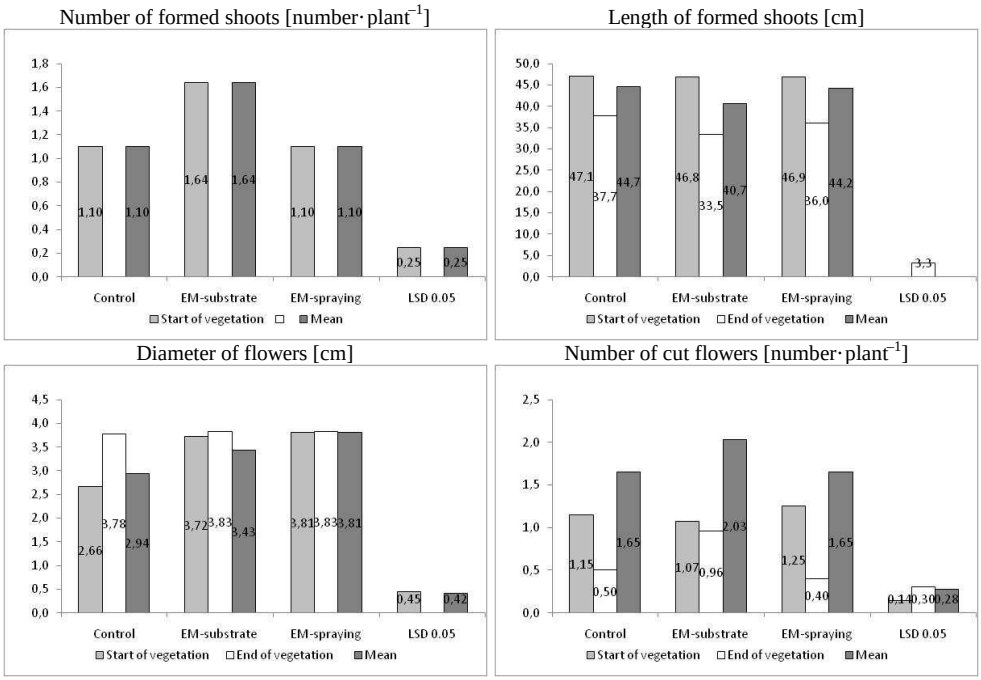

Fig. 1. The effect of Effective Microorganisms on selected biometric parameters of roses; number of formed shoots [number·plant^{-1}]; length of formed shoots [cm]; diameter of flowers [cm]; number of cut flowers [number·plant^{-1}]

Significantly the highest number of leaves was formed by plants sprayed with EM (30.5 leaves·plant^{-1}) in comparison with the control combination (25 leaves·plant^{-1}) and to

plants grown in the substrate inoculated with EM (27.3 leaves·plant^{-1}). Similar trends were recorded in case of the number of leaves removed as a result of cultivation measures.

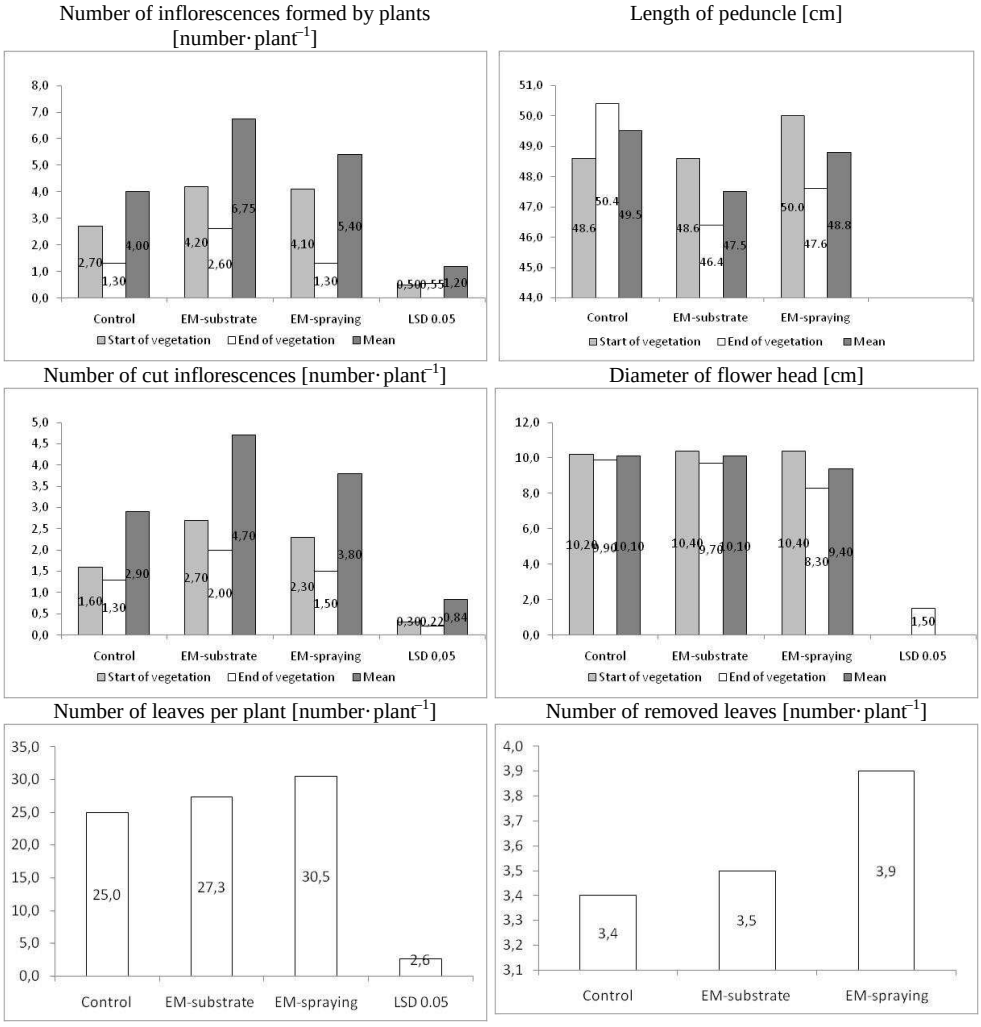

Fig. 2. The effect of Effective Microorganisms on selected biometric parameters of gerberas; number of inflorescences formed by plants [number·plant^{-1}]; length of peduncle [cm]; number of cut inflorescences [number·plant^{-1}]; diameter of flower head [cm]; number of leaves per plant [number·plant^{-1}]; number of removed leaves [number·plant^{-1}]

Discussion

Results of investigations conducted by the authors of this study are positively correlated with literature data. Studies conducted to date indicate an advantageous effect of the application of Effective Microorganisms on plant health, including the protection of

wheat against septoria leaf spot (*Septoria nodorum*) and leaf-spot (*Drechslera tritici-repentis*), winter rape against brown rust (*Puccinia recondita*) [13] and triticale against leaf diseases [15].

Numerous authors have confirmed the positive effect of EM on growth and yielding in different plant species. Effective Microorganisms have an advantageous effect on the uptake of nutrients by mung bean, they improve its yielding and the development of the root systems [16]. The application of a mixture of EM and molasses in growing of onion contributed to yielding of this vegetable increased by 29% [4]. In case of pea the increase in yields amounted to 31%, while in sweat corn it was 23%. Moreover, the effect of Effective Microorganisms was shown on yielding of cotton [11] and maize [17].

Studies also confirmed the positive effect of the application of Effective Microorganisms in case of orchard plants, as under their influence yielding of plum was improved [18]. Moreover, a positive effect was shown on growth, development and yielding of apple trees [12]. Trees treated with the above - mentioned microbiological preparation formed more shoots, which were markedly longer and thicker than in case of the control combination. The application of EM had a positive effect on leaf area in apple trees and chlorophyll content in leaves. Application of EM significantly modified nutrient content in leaves, improving nutrient status of plants in terms of nitrogen, phosphorus, potassium, iron, manganese and zinc. Similar changes in nutrient contents in leaves as a result of the application of Effective Microorganisms were also recorded in cotton growing [11].

Conclusions

1. A significant effect was found for the application of Effective Microorganisms, both in the radical zone and foliar applications, on changes in contents of available nutrients in peat substrate.
2. A trend was observed for substrate pH to decrease in case of the combinations with applied Effective Microorganisms in relation to the control combination.
3. Significantly the highest yield of flowers in case of both analyzed species was recorded at the application of Effective Microorganisms inoculum to the radical zone. This method of EM application had a positive effect also on the number of formed shoots and the diameter of flowers (in case of roses) and the number of formed inflorescences (in case of gerberas).
4. Spraying of plants with EM had a positive effect on the diameter of flowers in roses and on the number of formed inflorescences and leaves in case of gerberas.
5. Results of conducted analyses indicate that Effective Microorganisms may be useful in the cultivation of roses and gerberas due to their positive and at the same time significant effect on growth and yielding of these plants.

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WPŁYW EFEKTYWNYCH MIKROORGANIZMÓW (EM) NA ZAWARTOŚĆ SKŁADNIKÓW POKARMOWYCH W PODŁOŻU ORAZ ROZWÓJ I PLONOWANIE RÓŻY (*Rosa x hybrida)* I GERBERY (G*erbera jamesonii)*

[1]Katedra Metod Ochrony Roślin
[2]Katedra Nawożenia Roślin Ogrodniczych
Uniwersytet Przyrodniczy w Poznaniu

Abstrakt: Celem przeprowadzonych badań była ocena wpływu stosowania Efektywnych Mikroorganizmów (EM) na zmiany zawartości dostępnych i łatwo rozpuszczalnych form składników pokarmowych w substracie torfowym, a także wzrost, rozwój i plonowanie uprawianych w nim róży (*Rosa x hybrida)* i gerbery (*Gerbera jamesonii*). Efektywne Mikroorganizmy zastosowano przedwegetacyjnie w formie szczepionki w dawce 3 g preparatu·5 dm^{-3} podłoża oraz dolistnie w formie opryskiwania roślin (3-krotnie roztworem wodnym o stężeniu 0,1%). W przeprowadzonych badaniach stwierdzono wpływ EM, aplikowanych zarówno w formie dokorzeniowej, jak i dolistnej na zmiany zawartości dostępnych form składników pokarmowych w podłożu, przy jednoczesnym zakwaszeniu podłoża, w stosunku do kombinacji kontrolnej. Istotnie największy plon kwiatów, w przypadku obydwu badanych gatunków, uzyskano przy dokorzeniowym stosowaniu szczepionki EM. Wpływała ona

pozytywnie na ilość wytwarzanych pędów oraz średnicę kwiatów (w przypadku róży) oraz liczbę wytworzonych kwiatostanów (w przypadku gerbery). Aplikowanie dolistne Efektywnych Mikroorganizmów oddziaływało pozytywnie na średnicę kwiatów róży oraz liczbę wytworzonych kwiatostanów oraz liści w przypadku gerbery. Wyniki przeprowadzonych badań wskazują, iż Efektywne Mikroorganizmy mogą być przydatne w uprawie róży i gerbery ze względu na ich pozytywny i zarazem duży wpływ na wzrost i plonowanie tych roślin.

Słowa kluczowe: Efektywne Mikroorganizmy, róża, gerbera, rozwój, plonowanie, zawartość składników pokarmowych, podłoże

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ESTIMATION OF INTRINSIC AND EXTRINSIC ENVIRONMENT FACTORS OF AGE-RELATED TOOTH COLOUR CHANGES

OCENA CZYNNIKÓW WEWNĘTRZNYCH I ZEWNĘTRZNYCH POWODUJĄCYCH ZMIANY BARWY ZĘBÓW ZWIĄZANE Z WIEKIEM

Abstract: Age-related colour changes of biological objects are the results of changes in structure and properties which may reflect the influence of the extrinsic and intrinsic environment. The method able to discriminate these two factors was not published yet. Non-erupted teeth which are isolated from the environment of the oral cavity (intrinsic factors) have not yet been explored too. A device for research the dentist's chair-side measurements of vital erupted teeth as well as extracted impacted teeth (immediately after extraction) was built from commercially supplied components (fiber optic spectrometer) coupled with specific custom-made parts and a specific software driver. The measurement method related tooth for total colour changes in system CIELAB 1976 was evaluated to be inadequate. For more precise method of the ΔE_{CMC}(l:c) were theoretical trichromatic coordinates of standard tooth and the ratio of extrinsic and intrinsic factors for vital erupted and impacted teeth modelled by multivariate 3D-mathematical regression models. The rate of complex discolouration caused by the total factors decreases over the life of humans. The rate of colour changes caused by intrinsic factors is nearly constant over the life-time. Age estimation of the vital erupted teeth 21 (inversion exponencial function of $CMC_{(2:1)}$) will be only approximate (s.d. 6.2 years). More convenient for approximate age estimation are the impacted teeth immediately after extraction (inversion linear function of $CMC_{(2:1)}$), significant correlation with the known real age *p*-value < 0.001, (s.d. 3.1 years). Correlation between the subjects age and the yellowness of b* values of skulls is significant (r^2 = 0.80). The similar correlation between the subjects age and yellowness of b* values of impacted teeth (r^2 = 0.79) suggests a presumably similar mechanism of colour changes in bone and impacted teeth. These teeth are relatively available biological samples and can be obtained without any difficult medical or ethical issue.

Keywords: age-related colour changes of teeth, intrinsic and extrinsic factors, 3D mathematical regression models, estimation of real age

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The colour of teeth is determined by combined effects of intrinsic and extrinsic colourations. Age related colour changes have proved useful in clinical dental practice, concerned with prosthetic treatment (especially traumatogenic). Intrinsic tooth colour is associated with the light scattering and absorption properties of the enamel and dentine. Extrinsic colour is associated with the absorption of materials at the surface of enamel and in particular the pellicle coating which ultimately causes extrinsic stains etc. [1, 2].

Many colour scales has been developed for this purpose Berns [3]. Most frequently used system CIELAB was designed in 1976 year. In this three-dimensional colour space the colour co-ordinates values L*, a*, b* are calculated from the X,Y,Z tristimulus values for both the reference and specimen as follows:

$$L^* = 116\left(Y/Y_n\right)^{1/3} - 16 \tag{1}$$

$$a^* = 500\left[\left(X/X_n\right)^{1/3} - \left(Y/Y_n\right)^{1/3}\right] \tag{2}$$

$$b^* = 200\left[\left(Y/Y_n\right)^{1/3} - \left(Z/Z_n\right)^{1/3}\right] \tag{3}$$

where X/X_n, Y/Y_n, $Z/Z_n > 0.008856$
or:

$$L^* = 903.3\left(Y/Y_n\right) \tag{4}$$

$$a^* = 3893.5\ \left[\left(X/X_n\right) - \left(Y/Y_n\right)\right] \tag{5}$$

$$b^* = 1557.4\left[\left(Y/Y_n\right) - \left(Z/Z_n\right)\right] \tag{6}$$

where X/X_n, Y/Y_n, $Z/Z_n \leq 0.008856$.

Tristimulus X_n, Y_n, Z_n values are of the iluminant/observer combination.

Evaluation of total colour changes E^*_{CIELAB} (shortened symbol E*) are simple, but too much imprecise.

$$E^* = \left[\left(a^*\right)^2 + \left(b^*\right)^2 + \left(L^*\right)^2\right]^{1/2} \tag{7}$$

The L^* value is lightness (black 0, reflecting 100), the a^* value is redness ($+a^*$) or greenness ($-a^*$), and the b^* value is yellowness ($+b^*$) or blueness ($-b^*$). The a^* and b^* co-ordinate values near zero correspond to neutral colours (white, greys) and their values increase in for more saturated or intense colours.

CIELAB colour space has been modified to a more precise CMC system and standardised in International Standard ISO 105-J03:1995(E): Calculation of colour differences [4]. Evaluation of total colour changes ΔE_{CMC} (l:c), (shortes symbol $CMC_{(l:c)}$) is calculated

$$CMC_{(l:c)} = \left[\left(\frac{\Delta L^*}{l \cdot S_L}\right)^2 + \left(\frac{\Delta C^*}{c \cdot S_C}\right)^2 + \left(\frac{\Delta H^*}{S_H}\right)^2\right]^{\frac{1}{2}} \tag{8}$$

The three separate components of the colour differences ΔE_{CMC}(l:c), (shortened symbol $CMC_{(l:c)}$) lightness ΔL^*, chroma ΔC^*, and hue ΔH^* are weighted by weighting factors S_L, Sc, S_H calculated from the chromatic coordinates of a standard L^*_0, a^*_0, b^*_0. Other weighting factors (1:c) may be numerically optimised to suit the desired purposes. The calculation CMC of colour differences was used in several studies published recently. These

articles usually compare teeth and selected filling or prosthetic materials and use $CMC_{(l:c)}$ calculation just as an indicator of perceptibility or acceptability. The purposes of this study were to evaluate the $CMC_{(1:1)}$, $CMC_{(2:1)}$ and $CMC_{(3:2)}$ formulas to identify which of them provides the best indicator for acceptability of small colour differences in the esthetic dental restorative materials. Trichromatic coordinates of third standards are defined from these materials [5-7].

The third standard is used for the calculation of weighting factors. These values were not yet determined for human teeth (theoretical standard tooth) and so another aim of this study was to set them and consider the benefits of using the spectral parameter (eg the colour change compared with a standardised tooth instead of the white standard and establishing weighting factors, the rest is used for dark signal). $CMC_{(l:c)}$ values had not yet been used as a spectral parameter for age-related correlations of teeth. Age-related colour changes of impacted teeth which are isolated from the environment of the oral cavity have not yet been explored. These teeth are not affected by the ambient conditions, the change in colour is only caused by physiological processes inside the human body and so they are ideal for determination of the ratio of intrinsic and extrinsic factors that cause the change in tooth colour. The concept of a custom-made instrument, new use of the CMC system for calculating colour differences and 3-D mathematical models of the ratio of extrinsic and intrinsic factors for vital and impacted teeth and age estimation using inverse functions of colour-age relationship has been demonstrated and are described in the following text.

Materials and methods

A special instrument was built from commercially supplied components. The designed device consisted of an Avantes S 2000 fibre optics spectrometer coupled with an XE 2000 xenon light source and an FCR 7 UV 200-2-ME reflectance probe, which contained six 200 μm optical fibres for the light source around one 200 μm sampling fibre (AVANTES, Eerbeek, Netherlands). These components were completed with custom-made parts, for example a special holder for the measurement of extracted teeth or spectrometric standards or special plastic probe shield for chair-side measurements, which was designed for frequent resterilisations. The same geometric configuration, 45/8, was found optimal for all types of determinations (chair-side measurements, extracted teeth). A special software-driver (called VIS) was supplied with the spectrometer for Object oriented Design Lab view $TM^{5.0}$. This unique software was programmed using VIS to control the spectrometer, the xenon light source, collect data and calculate trichromatic coordinates X, Y, Z, colour co-ordinates L*, a*, b*, E* and $CMC_{(l:c)}$ values.

The spectrometer Avantes S 2000 was Tzerny-Thurner design spectrophotometer with CCD detector with 2048 diodes. Just 1902 pixels are used for data collection, the rest is used for electric dark signal correction function. The output of the VIS is Spectral Output table containing 1902 values of subject reflectance. The spectrometer was calibrated with white and black standards before each measurement. It was programmed to switch off the light for black standard calibration. The following spectrometer parameters were set: integration time 100 ms, delay before flashes 0 ms, averaged samples 10. It was necessary to perform one cycle of ten flashes to stabilize the xenon light source before every measurement.

The following groups of patients were collected with the agreement of the ethical committee of Central Army Hospital in Prague, CZ (according to the Helsinki Declaration):

The group for the determination of Total factors of vital erupted teeth discolourations consisted of 69 probands (35 males and 34 females), 20-75 years old. All of them were non-smokers, in good overall health and their oral hygiene was satisfactory (standard hygiene indices PBI, CPITN, API, DMF > 10). This teeth was was polished with a mechanical brush with DepuralNeo paste (Dental a.s., CZ) and rinsed with deionised water. This tooth was measured in its central area in all subjects.

The group for the determination of Intrinsic factors of extracted impacted teeth discolourations (measured immediately after extraction) consisted of 23 probands (12 males and 11 females), 20-69 years old, non-smokers, in good overall health with satisfactory oral hygiene (standard hygiene indices PBI, CPITN, API, DMF > 10). The tissue and blood residues of these extracted teeth were removed carefully and the coronal part was polished with a mechanical brush with DepuralNeo paste and rinsed with deionised water.

The Extrinsic factors were determined by a differential method using data of extracted impacted teeth (immediately after extraction) and vital erupted teeth of the some probands before surgery. This group of differential measurement was collected over more than five years, because the patient had to have not only a extracted impacted tooth, but also a vital and intact erupted tooth 21.

The SubVISs for calculating X, Y, Z, and L^*, a^*, b^* and CMC were created. The computation of CMC was based on predefined values of the trichromatic coordinates L_0^*, a_0^*, b_0^* of third standard (theoretical standard tooth and weight factors l, c). Another additional module was created for approximate age determination using inverse functions of colour-age relationship [8, 9].

Results and discussion

Parameters of all used mathematic-statistical models mentioned above were obtained by linear and nonlinear regression methods, depending on the model type. Data were fitted using the least squares method (LSM). Since the LSM requires normal distribution, all residual errors were tested for normality. Statistical significance of all parameters was tested using t-test and normal standard deviations were used to quantify uncertainty of parameters.

Figure 1 shows the age dependence of the mean trichromatic coordinates $L^*(21)$, $a^*(21)$, $b^*(21)$ of the vital left central incisors. A 3D-mathematical model of this dependence of the age-related changes trichromatic coordinates of the vital erupted and the extracted impacted teeth were used to determine theoretical values of standard tooth. This model used data to determine the extrapolated values of the trichromatic coordinates for near-zero age.

For all significance tests and for construction of confidence intervals of parameter estimates and confidence intervals of prediction (Figs 2 and 3) at the significance level $\alpha = 0.05$ was used.

Figure 2 shows the age dependence of mean spectral parameters E* of vital erupted teeth 21 as well as extracted impacted teeth (IMP - immediately after extraction).

Figure 4 shows estimated models for total discolouration $CMC_{(2:1)}$ vital erupted teeth (21), extracted impacted teeth (IMP) and differential values (21-IMP) for erupted and impacted teeth after surgery as a function of age based on experimental data (Fig. 4a) and mathematical models of total, extrinsic and intrinsic discolouration derived from spectral

parameters (Fig. 4b). The outer limits are 0.25% quantiles of the data, ie ± 3σ. The relationship is described by the formulas in Table 1.

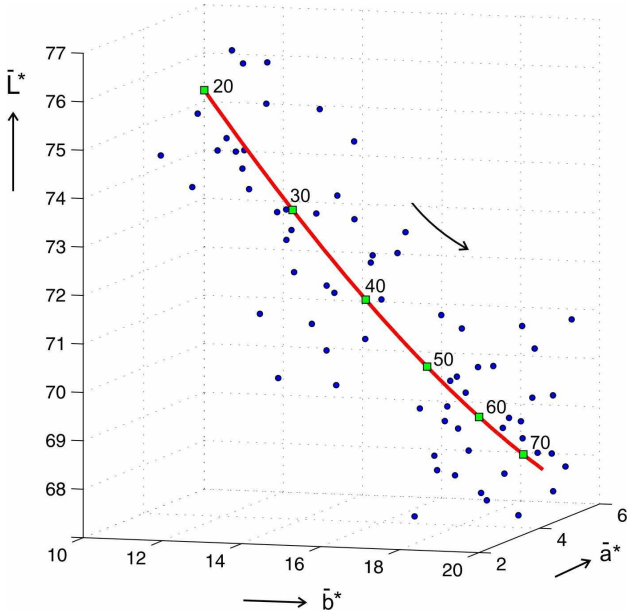

Fig. 1. The age dependence of mean trichromatic coordinates L*(21), a*(21), b*(21) of the vital left central incisors. Method CIELAB (L* - lightness, +a* - redness, +b* - yellowness). *Note: Estimates of the trichromatic model parameters are given in Table 1*

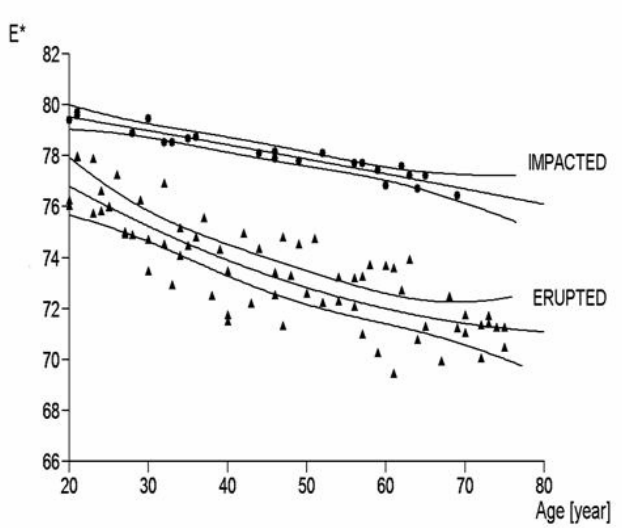

Fig. 2. Age dependence of mean spectral parameters E* of vital erupted teeth (21) as well as extracted impacted teeth (IMP) immediately after extraction

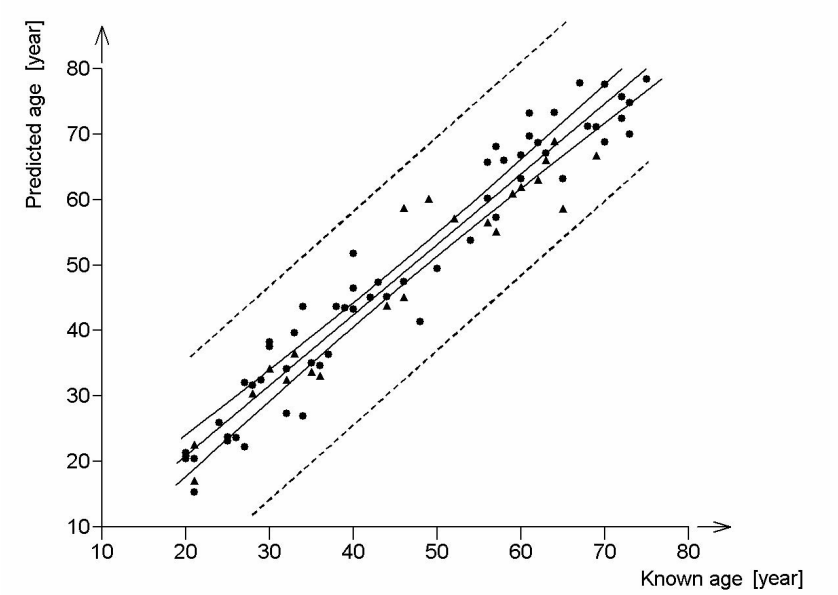

Fig. 3. Mean age estimation from inverse models of spectral parameters of the vital erupted teeth (21), use of inversion exponential function $^{Inv}CMC_{(2:1)}$ and extracted impacted teeth (IMP), use of inversion linear function of $^{Inv}CMC_{(2:1)}$

Table 1

Estimates of parameters and their confidence intervals for the models of trichromatic coordinates and spectral parameters of vital teeth (21), extracted impacted teeth (IMP) and differential values (21-IMP) for vital (21) and impacted teeth (IMP) after surgery

Spectral parameter Y	**Age t [year]**	**Number of teeth MF(M)(F)**	**Parameter estimates of the model Y = A ± C∗exp(-k∗Age) and Y = A ± C∗Age with their confidence intervals (CI)**					
			A	**CI** (A)	**C**	**CI** (C)	**k**	**CI** (k)
L*(21)	20÷75	69 (35) (34)	66.09	64.23	18.26	13.61	–0.03	–0.06
				68.44		22.78		–0.02
a*(21)	20÷75	69 (35) (34)	4.99	3.83	–4.75	–5.90	–0.04	–0.06
				6.11		–3.55		–0.02
b* (21)	20÷75	69 (35) (34)	26.32	25.00	–18.01	–21.53	–0.02	–0.03
				27.62		–14.1		–0.01
E*(21)	20÷75	69 (35) (34)	69.97	68.18	15.21	10.68	–0.04	–0.06
				71.76		19.74		–0.02
CMC(IMP)	20÷69	23 (12) (11)	0.07	0.04	0.08	0.07	0.00)^{x}	---
				0.09		0.09		---
CMC(21)	20÷75	69 (35) (34)	23.90	18.05	–22.22	–25.76	–0.02	–0.03
				26.39		–22.03		–0.01
CMC(21-IMP)	20÷69	46 (24) (22)	13.05	11.99	–13.02	–10.97	–0.03	–0.04
				15.22		–14.98		–0.01

Note:)^{x} ... Very low value of the parameter k allows the use of linear model

Figure 4 shows approximate age estimation, significant correlation with the known real age p-value < 0.001, from inverse models of spectral parameters of the vital erupted teeth

(21), use of inversion exponential function $^{Inv}CMC_{(2:1)}$ and extracted impacted teeth, use of inversion linear function of $^{Inv}CMC_{(2:1)}$.

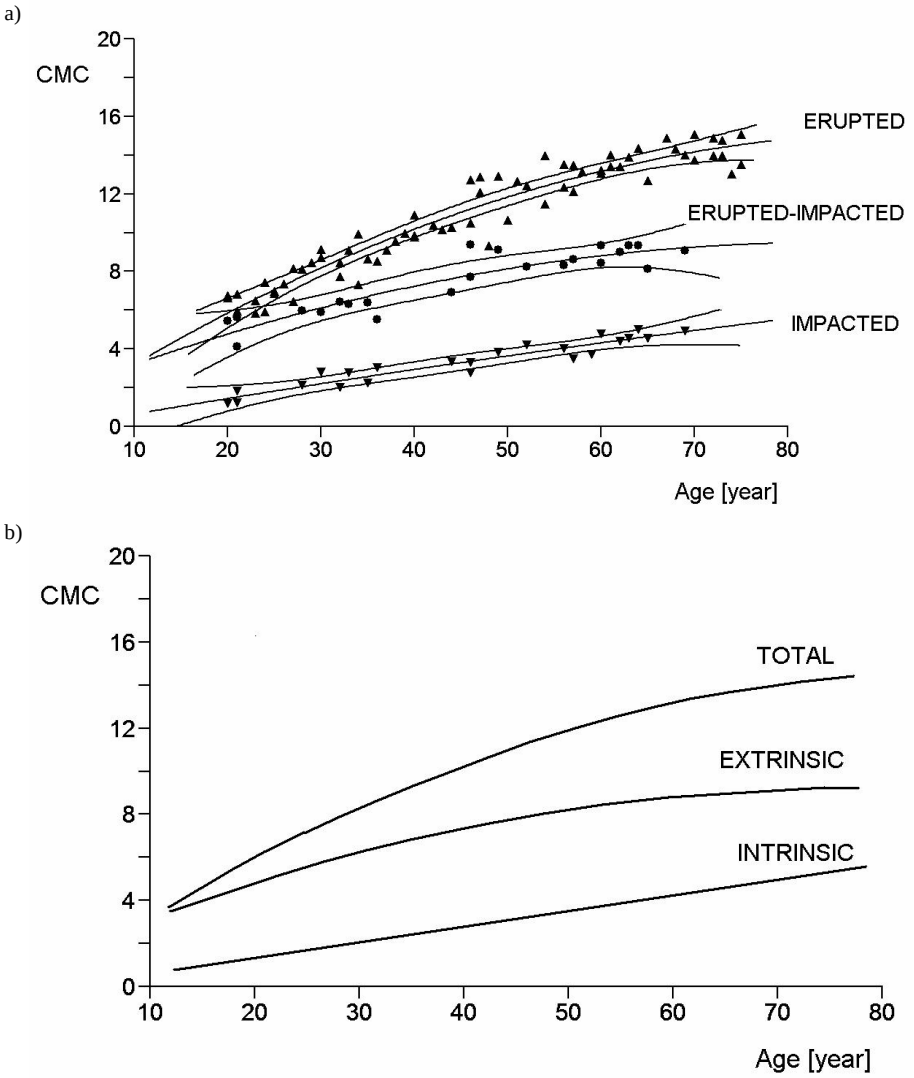

Fig. 4. Estimated models for total discolouration of vital teeth (21), extracted impacted teeth (IMP) immediately after extraction and differences values of vital erupted and extracted impacted teeth (21-IMP) from patients after surgery as a function of age based on experimental data (Fig. 4a) and mathematical models of total, extrinsic and intrinsic factors of discolouration derived from spectral parameters (Fig. 4b). *Note: Estimates of the trichromatic model parameters are given in Table 1*

Table 1 gives age dependence of the mean trichromatic coordinates $L^*(21)$, $a^*(21)$ $b^*(21)$, $E^*(21)$ of the vital left central incisors and estimates of parameters and their

confidence intervals for the mathematic-statistical models of Total factors-vital erupted teeth (21), Intrinsic factors-extracted impacted teeth (IMP), immediately after extraction and of Extrinsic factors-differences values of vital erupted and extracted impacted teeth (21-IMP) of probands after surgery as a function of age based on experimental data.

Table 2 gives of the contributions of intrinsic and extrinsic factors to the total discolouration and rate of colour changes obtained from mathematical models of spectral parameters of vital and extracted impacted teeth as a function of age.

Table 2

Estimates of the contributions of intrinsic and extrinsic factors to the total discolouration and rate of colour changes obtained from mathematical models of spectral parameters of vital teeth (21), extracted impacted teeth (IMP) and differential values (21-IMP) for vital (21) and impacted teeth (IMP) after surgery

Age group	Number of teeth	Discoloration		Discoloration rate [year^{-1}]			
		$w_{(Intrinsic)}$		$s_{(Intrinsic)}$		$s_{(Extrinsic)}$	
[year]	MF	CMC(IMP) / CMC(21)	s.d.	d(CMC(21) / d(Age)	s.d.	d(CMC(21-IMP)) / d(Age)	s.d.
20÷29	20	0.26	0.02	0.08	0.01	0.18	0.03
30÷39	20	0.27	0.03	0.08	0.02	0.14	0.04
40÷49	20	0.29	0,07	0.08	0.02	0.11	0.04
50÷59	20	0.31	0.08	0.07	0,02	0.08	0,06
60÷69	12	0.34)^{x}	—	0.07)^{xx}	—	0.06)^{xx}	—

Note:)^{x} extrapolated values,)^{xx} calculated from extrapolated values

The statistical software package QC-Expert 3.1 (TriloByte Statistical software, CZ) was used to calculate the regression models and to plot the figures. This software is validated with US National Institute for Standards in Technology (NIST).

The vital erupted central left incisor of 69 (35 M, 34 F) probands was measured using the method CIELAB. The averaged values and standard deviations for trichromatic coordinates were L* = 71.3 (s.d 4.7), a* = 3.6 (s.d. 1.3), b* = 15.4 (s.d. 4.8). Hasegawa and Motonomi [10] published trichromatic coordinate values of L* = 73.0 (s.d. 5.0), a* = 3.5 (s.d. 1.5), b* = 16.5 (s.d. 5.0) for the upper incisors (87 humans, age 20-75). Analogous values were determined by Xiao et al [11] (405 young humans) and Tenbosch and Coops [12] (102 extracted teeth). According to these results, the developed method of tooth colour measurement is comparable with the above-mentioned authors.

The trichromatic coordinates of the vital impacted teeth, immediately after extraction 23 (12M, 11F) were measured by the same method. The average values were L* = 77.5 (s.d. 1.9), a* = 2.7 (s.d. 0.5), b* = 8.7 (s.d.1.65). It is very difficult to compare this, because it was measured for the first time. Schafer [13] measured 124 skulls post mortem (0-83 years), which was isolated from the environment too. Trichromatic coordinates values were L* = 72.5 (s.d. 8.22), a* = –7.4 (s.d. 3.22), b* = 16.4 (s.d. 5.52).

The difference between the male and female age-related colour change of the vital central incisor 21 is not conditioned sexually, this difference was not found in the same group in impacted areas (two sample t-test, p-value < 0.05). The divergence in the average

L* value for the male and female group could be merely caused just by a different level of hygiene at a younger age.

The age dependences of the trichromatic coordinates vital erupted teeth *in vivo* are usually measured for use in prosthetic stomatology Gozalo-Dias et al [14] and are expressed by a linear mathematical model. In forensic odontology linear mathematical model was used for age related colour changes of extracted teeth [15].

Experimental age related data of L*, a*, b*, E* discolouration of vital erupted teeth have decreasing rate of colour change and the spectral parameters converge to a constant. However, the total discolouration E* is not suitable for differential method of determination extrinsic factors. A modern weighting method based on $CMC_{(l:c)}$ could not be applied because the trichromatic coordinates of the standard tooth are experimentally unavailable. A 3D-mathematical models of this dependence of the age-related changes trichromatic coordinates of the vital erupted and the extracted impacted teeth was used to determine theoretical values of standard tooth. This model used data to determine the extrapolated values of the trichromatic coordinates for near-zero age.

These values L_0^*, a_0^*, b_0^* rounded to 2 decimal places were defined as the absolute standard for teeth colour. The computation of CMC was based on predefined this values of the trichromatic coordinates theoretical standard tooth and weight factors *l, c*.

The ratio of extrinsic and intrinsic factors for vital and non-erupted teeth was demonstrated by mathematic-statistical models. The curves were calculated by new weighting method to the general spectral parameter $CMC_{(2:1)}$ from the above values of the spectral parameters of vital erupted teeth 21 as well as extracted impacted teeth (immediately after extraction) and their differential determination. The extrinsic factors are more powerful in terms of the colour change over the life of the human being compared with intrinsic factors. This effect decreases with age. The extrinsic/intrinsic ratio is more than three times larger for the youngsters than for seniors. The rate of complex discolouration decreases over the life. The rate of colour change caused by intrinsic factors is nearly constant over time and the extrinsic change decreases with the age. The contributions of intrinsic and extrinsic factors of the total discolouration obtained from the differential values for vital erupted and impacted teeth (immediately after extraction). The rates of colour changes were obtained as the mean first derivatives from the curves of total, extrinsic and intrinsic environmental factors within ten-year intervals.

The inverse model age-related colour-changes $CMC_{(2:1)}$ of teeth can also be used to estimate standard deviations and confidence intervals of the estimated age. Mean errors of age determination as well as two calibration systems CIELAB and CMC system were compared. The mean error of the determined age from 46 people (23 males, 23 females) of the vital erupted teeth 21 was 11.9 years for linear regression model CIELAB and 6.2 years for nonlinear regression model $^{Inv}CMC_{(2:1).}$ In the case of 20 extracted impacted teeth (10 males, 10 females), immediately after extraction, this error for linear regression model CIELAB was 3.1 years. More convenient for approximate age estimation are the vital impacted teeth immediately after extraction (inversion linear function of $^{Inv}CMC_{(2:1)}$). There is only one possible article for comparison [13]. Correlation between the subjects age and the yellowness of b* values of skulls is ($r^2 = 0.80$). The similar correlation between the persons age and yellowness of b* values of impacted teeth ($r^2 = 0.79$) suggests a presumably similar mechanism of colour changes in bone and impacted teeth.

Conclusions

Extracted impacted teeth are relatively available biological samples and can be obtained without any difficult medical or ethical issues. These teeth may become suitable objects for research of the age-dependent and environmental changes in human organism. The new biophysics concept of custom-made instrument with fibre-optic spectrometer, new use of the CMC system for calculating colour differences and 3-D mathematical models described here is suitable even for other biological objects where the estimation of intrinsic and extrinsic environmental factors or properties reflecting biological age is necessary.

Acknowledgement

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OCENA CZYNNIKÓW WEWNĘTRZNYCH I ZEWNĘTRZNYCH POWODUJĄCYCH ZMIANY BARWY ZĘBÓW ZWIĄZANE Z WIEKIEM

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Abstrakt: Zmiany koloru obiektów biologicznych wraz z wiekiem są wynikiem zmian w ich strukturze i właściwościach, które mogą odzwierciedlać wpływ środowiska zewnętrznego i wewnętrznego. Dotychczas nie badano wpływu środowiska jamy ustnej zębów niewyrzniętych. Opisano stanowisko do badania zarówno zębów niewyrzniętych, jak również zębów usuniętych (bezpośrednio po ekstrakcji), zbudowane z elementów dostępnych w handlu (spektrometr światłowodowy), obsługiwane sterownikami programowanymi. Metodę pomiaru całkowitej zmiany barwy zębów w systemie CIELAB 1976 oceniono jako niewystarczającą. W badaniach wykorzystano bardziej precyzyjną metodę ΔE_{CMC}(l:c), wprowadzając współrzędne trójbarwne standardowych zębów oraz stosunek zewnętrznych i wewnętrznych czynników istotnych dla zębów niewyrzniętych i zatrzymanych. Do opisu zmian zastosowano wielowymiarowy model regresji 3D. Szybkość przebarwienia zębów spowodowana przez wiele czynników zmniejsza się w ciągu życia człowieka. Zmiana barwy zębów spowodowana przez czynniki wewnętrzne jest prawie niezmienna przez cały okres życia. Ocena wieku zębów wyrzniętych 21 (odwrotna zależność wykładnicza funkcji $CMC_{(2:1)}$) jest tylko przybliżona (SD = 6,2 roku). Wygodniejsze dla przybliżonej oceny wieku są zęby badane natychmiast po ekstrakcji (odwrotna funkcja liniowa $CMC_{(2:1)}$), dobrze korelująca ze znanymi, prawdziwymi długościami życia $p < 0,001$ (SD = 3,1 roku). Korelacja między wiekiem badanych szczątków oraz zażółceniem wartości b* czaszek jest znacząca ($R^2 = 0,80$). Podobna zależność między wiekiem pacjentów i zażółceniem wartości *b zębów zatrzymanych ($R^2 = 0,79$) sugeruje podobny mechanizm zmiany koloru kości i zębów. Zęby są stosunkowo łatwo dostępną próbką biologiczną i mogą być uzyskane bez trudności natury medycznej lub etycznej.

Słowa kluczowe: zmiany barwy zębów związane z wiekiem, czynniki wewnętrzne i zewnętrzne, model regresji 3D, szacowanie rzeczywistego wieku

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BIOFUMIGACJA JAKO ALTERNATYWNA METODA OCHRONY ROŚLIN

BIOFUMIGATION AS AN ALTERNATIVE METHOD OF CROP PROTECTION

Abstrakt: Zagrożenia zdrowotne związane z powszechnym stosowaniem pestycydów i nawozów sztucznych przyczyniły się do wzrostu zainteresowania alternatywnymi środkami ochrony roślin. Wśród nich coraz większe znaczenie zyskuje biofumigacja. Polega ona na wykorzystaniu naturalnych związków występujących głównie w roślinach z rodziny kapustowatych (*Brassicaceae*) w zwalczaniu szkodników i drobnoustrojów atakujących uprawy rolne. Ponadto korzystnie wpływają one na jakość gleby oraz wielkość plonu. Przedstawiono informacje o substancjach wykorzystywanych w biofumigacji, ich działaniu antybiologicznym oraz opisano próby praktycznego zastosowania tej metody.

Słowa kluczowe: biofumigacja, alternatywne metody ochrony roślin, glukozynolany, izotiocyjanianiy, mirozynaza

Wprowadzenie

Rolnictwo stanowi główne źródło żywności oraz zapewnia miejsca pracy dla znacznej części społeczeństwa. Ze względu na rosnące zapotrzebowanie na żywność, ograniczoną powierzchnię gleby dostępnej pod uprawę oraz jej pogarszającą się jakość niezbędne stało się chemiczne wspomaganie rolnictwa. Przejawem postępującej chemizacji jest stosowanie przemysłowo otrzymywanych środków chemicznych, takich jak pestycydy i nawozy sztuczne, w celu zwiększenia wydajności upraw oraz ochrony roślin przed szkodnikami i chorobami.

Nawozy sztuczne zawierają przede wszystkim ważne dla wzrostu roślin pierwiastki, takie jak azot (azotany, mocznik, sole amonu), fosfor (superfosfaty, polifosforany) oraz potas (azotan, siarczan lub chlorek potasu). Dla zdrowia ludzkiego, szczególnie małych dzieci, niebezpieczne są azotany(V), które gromadzą się w warzywach pochodzących z nawożonych upraw. Azotany(V) są przekształcane w przewodzie pokarmowym do azotanów(III), które po związaniu się z hemoglobiną, transportującą tlen we krwi, powodują

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jej przejście w słabo wiążącą tlen methemoglobinę, co prowadzi do niedotlenienia organizmu, a nawet zagraża życiu.

Większe obawy są jednak związane ze stosowaniem pestycydów. Stanowią one dużą i różnorodną grupę środków chemicznych wykorzystywanych w rolnictwie. Na podstawie danych zawartych w roczniku statystycznym (2008) można stwierdzić, że udział poszczególnych grup pestycydów w ogólnym ich zużyciu w Polsce jest następujący: herbicydy (62,3%), fungicydy (23,3%) oraz insektycydy (4,6%), a ich łączna produkcja wynosiła blisko 41 tys. ton [1]. Związki te umożliwiają ochronę i wzrost wydajności upraw. Należy zauważyć, że niekorzystnym efektem stosowania pestycydów jest ich toksyczne działanie, które nie ogranicza się tylko do szkodników, ale ma wpływ również na pożyteczne organizmy bytujące na danym terenie oraz ludzi - w szczególności osób pracujących przy opryskach. Ponadto pestycydy mogą być przemieszczane przez wiatr z miejsc stosowania na sąsiednie uprawy i tereny. Jest to zjawisko niekorzystne, ponieważ przenikanie tych związków do gleby, wody, powietrza narusza naturalną równowagę i może przyczyniać się do wyginięcia niektórych dziko rosnących gatunków roślin oraz zwierząt. Ze środowiska pestycydy mogą wnikać do pasz i żywności oraz kumulować się w organizmach zwierzęcych. Ich ilość może zostać zwielokrotniona na kolejnych piętrach łańcucha pokarmowego. Przykładowo węglowodory chlorowcoorganiczne (DDT, PCB), obecne w produktach spożywczych, łatwo kumulują się w organizmie ludzkim w tkance tłuszczowej, wątrobie, nerkach, mózgu i sercu, co może być przyczyną zaburzenia funkcjonowania tych organów i prowadzić do związanych z tym chorób. Z tego względu największe obawy dotyczą przenikania pestycydów do żywności. Artykuły spożywcze mogą zostać zanieczyszczone pestycydami na skutek:

- bezpośredniego spryskania warzyw i owoców (związki te przenikają do wszystkich części roślin, niezależnie od sposobu ich stosowania),
- zatrzymania w tkance tłuszczowej zwierząt rzeźnych pestycydów, użytych do zwalczania szkodników bezpośrednio zagrażających zwierzętom lub w wyniku spożycia pestycydów z zanieczyszczoną paszą lub wodą,
- stosowania pestycydów do zapobiegania stratom żywności podczas magazynowania i transportu [2].

Obawy dotyczące bezpieczeństwa stosowania DDT (dichlorodifenylotrichloroetan) spowodowały wycofanie tego środka powszechnie używanego w ochronie roślin w latach 80. XX w. Obecnie związek ten podejrzewany jest o zaburzanie gospodarki hormonalnej, a być może także o wywoływanie chorób nowotworowych. Od 2005 roku obowiązuje również zakaz produkcji i stosowania bromku metylu z wyjątkiem szczególnych sytuacji typu kwarantanna, zgodnie z zaleceniami tzw. Protokołu Montrealskiego, w których są zawarte zalecenia ograniczenia produkcji i wykorzystania związków mających wpływ na niszczenie stratosferycznej warstwy ozonowej [3]. Pestycyd ten był wykorzystywany w rolnictwie do niszczenia obecnych w ziemi nicieni i grzybów, ochrony upraw zbóż, kawy, kakao, tytoniu, a także do zwalczania owadów w spichlerzach, podczas przechowywania oraz transportu. Jednak okazało się, że bromek metylu ma szkodliwe działanie na organizm ludzki. Przy zwiększonym poziomie narażenia powoduje wymioty, drgawki, utratę przytomności, zaburzenia widzenia i mowy, uszkodzenie nerek, a nawet śmierć w wyniku porażenia układu oddechowego [4]. Stwierdzono także jego niekorzystne działanie na uprawy, bowiem obniża siłę kiełkowania nasion, ma wpływ na powstawanie uszkodzeń owoców, warzyw i roślin ozdobnych. W przypadku żywności ujemnie wpływa na jakość

przechowywanych produktów, m.in. prowadzi do zmniejszenia zawartości witamin, obniża wartość wypiekową mąki, zmienia jej smak i zapach. Inny powszechnie stosowany związek - izotiocyjanian metylu - jest półproduktem w otrzymywaniu pestycydów z grupy karbaminianów, ale może też być stosowany samodzielnie. Skutecznie zwalcza wiele szkodników, grzybów, insektów powodujących straty i choroby upraw roślinnych. Jest to związek silnie toksyczny, powodujący u ludzi zaburzenia oddychania, krwotoki i śmierć, dlatego jego stosowanie ze względu na możliwość przedostania się do żywności uznano za wyjątkowo groźny i wycofano go z użytku, niedawno także w Polsce [5].

Pestycydy są na ogół związkami trwałymi, a produkty ich rozpadu czasem mogą być bardziej szkodliwe i trwalsze niż wyjściowy związek, dlatego odchodzi się od stosowania pestycydów trwałych na rzecz związków o krótkim czasie rozpadu, bez tendencji do biokumulacji [2]. Poszukuje się też naturalnych metod ochrony roślin, które byłyby skuteczne, ale i bezpieczne w stosowaniu. Ich rozpowszechnienie powinno przyczynić się do zmniejszenia zużycia syntetycznych pestycydów, a tym samym przyniosłoby korzyści zdrowotne i ekologiczne. Jednym z alternatywnych sposobów ochrony roślin jest biofumigacja.

Biofumigacja

Fumigacja to sposób zwalczania szkodników i patogenów za pomocą trujących substancji stosowanych w postaci gazów i dymów (fumigantów). Ze względu na ochronę środowiska odchodzi się od używania syntetycznych fumigantów na rzecz ich naturalnych odpowiedników. To bardziej ekologiczne podejście nazywane jest biofumigacją. Polega ona na wykorzystaniu związków o działaniu antybiologicznym, naturalnie występujących w roślinach. Substancje te nie tylko niszczą niepożądane grzyby, bakterie i inne szkodniki, ale również korzystnie wpływają na biologiczną jakość gleby, a przede wszystkim są bezpieczne w stosowaniu. W biofumigacji głównie wykorzystywane są zarówno rośliny z rodziny kapustowatych (*Brassicaceae*), do których należą liczne rośliny uprawne: warzywa (kapusta, rzodkiew, rzepa, brukiew, kalafior), rośliny oleiste (rzepak, rzepik, lnianka, gorczyca), jak również rośliny ozdobne (np. lewkonia) i zielarskie (rukiew, tasznik).

Rośliny z rodziny kapustowatych zawdzięczają swoje biobójcze działanie obecności glukozynolanów i enzymu mirozynazy. W nienaruszonej komórce tkanki roślinnej glukozynolany są oddzielone od enzymu, który znajduje się w tzw. komórkach mirozynowych. Gdy komórka roślinna zostanie uszkodzona, mechanicznie lub w wyniku ataku roślinożerców, dochodzi do kontaktu mirozynazy z glukozynolanami. Wówczas uwalniane zostają produkty ich hydrolizy o właściwościach biobójczych, takie jak izotiocyjaninany, nitryle, tiocyjaniany i epitionitryle (rys. 3). W ten naturalny sposób zaatakowane rośliny bronią się przed szkodnikami [6-8].

Termin biofumigacja jest także stosowany w przypadku wykorzystania przeciwko patogenom lotnych związków syntetyzowanych przez pewne mikroorganizmy. Wyniki badań informują o skuteczności niektórych metabolitów wytwarzanych przez grzyba *Muscodor albus* w hamowaniu wzrostu grzybów *Phytophthora erythroseptica, Sclerotinia sclerotiorum, Botrytis cinerea, Penicillium expansum, P. digitatum, Geotrichum citri-aurantii* oraz bakterii *Erwinia carotovora pv. carotovora, Pseudomonas fluorescens, Escherichia coli, Listeria innocu,* mikroorganizmów przyczyniających się do rozkładu

warzyw i owoców podczas przechowywania. Grzyb *M. albus* syntetyzuje alkohole, estry, ketony, kwasy (m.in. kwas izomasłowy i izobutanol), które są toksyczne dla patogenów i dzięki temu może być wykorzystany w ochronie płodów rolnych [9-11]. Wyniki badań prowadzonych w innym ośrodku dowodzą skuteczności *M. albus* w walce z larwami szkodników *Phthorimaea operculella* niszczących uprawy ziemniaków [12].

Wyniki badań dowodzą, że nie tylko rodzina kapustowatych, ale także inne rośliny, np. papryka (*Capsicum annum*), jako przedstawiciel rodziny psiankowatych (*Solanaceae*), wykazuje właściwości biofumiganta. Stosując eksperymentalną mieszankę papryki i odchodów zwierzęcych podczas uprawy pomidorów, zaobserwowano znaczny spadek populacji nicieni *M. incognita* (nawet 98% śmiertelności) [13].

Jednak to właśnie rośliny z rodziny kapustowatych stanowią główny obiekt zainteresowania naukowców poszukujących naturalnych pestycydów na potrzeby biofumigacji. Wykorzystanie glukozynolanów w ochronie roślin ma bowiem wiele wymiernych zalet:

- bezpieczeństwo stosowania,
- dostępność i mały koszt stosowania biofumigantów,
- przypadkowe przedostanie się produktów hydrolizy glukozynolanów do żywności, w przeciwieństwie do syntetycznych pestycydów, nie tylko nie stanowi zagrożenia, ale dzięki prozdrowotnym właściwościom mogą one pozytywnie oddziaływać na zdrowie człowieka,
- możliwość biodegradacji przy jednoczesnym wykorzystaniu jako źródła łatwo przyswajalnego węgla i azotu dla upraw,
- do korzyści środowiskowych można też zaliczyć zmniejszenie emisji CO_2 do atmosfery w wyniku ograniczenia produkcji syntetycznych pestycydów.

Biofumiganty nie są jedynym typem naturalnych pestycydów. Obecnie podejmuje się próby wykorzystania innych związków roślinnych i mikrobiologicznych w zwalczaniu szkodników o znaczeniu rolniczym. Antyfidanty to substancje częściowo lub całkowicie hamujące żerowanie owadów. Jako związki naturalne pochodzenia roślinnego są biodegradowalne, a ponadto są aktywne tylko wobec wąskiej grupy owadów. Oddziałują na ich narządy smaku, powodując zaprzestanie żerowania na chronionych roślinach i w efekcie śmierć głodową [14]. Jako przykłady antyfidantów można wymienić: demisynę, solaninę oraz tomatynę, glikozydy wyizolowane z roślin z rodziny psiankowatych. Stosunkowo uniwersalnym antyfidantem jest acetoanilid dimetylotriazenowy, chroniący między innymi kapustę przed gąsienicami bielinka rzepnika (*Pieris rapae*).

Związki pochodzenia naturalnego, zwalczające insekty, są określane mianem bioinsektycydów. Owadobójcze działanie mogą wywoływać pojedyncze związki zawarte w roślinach, jednak najczęściej są to kompozycje związków, takich jak: alkaloidy, niebiałkowe aminokwasy, steroidy, fenole, flawonoidy, glikozydy, chinony, terpenoidy i garbniki. Zazwyczaj działanie bioinsektycydów jest wolniejsze niż syntetycznych pestycydów, a cena znacznie wyższa. Ponadto niektóre z nich mogą wywoływać reakcje alergiczne lub toksyczne u zwierząt i ludzi [15].

Odmiennym sposobem biologicznej ochrony roślin jest zastosowanie insektycydów pochodzenia drobnoustrojowego. W Hiszpanii używa się preparatu sporządzonego z grzyba *Paecilomyces fumosoroseus* do zwalczania jaj, larw i dorosłych mączników, występujących np. na pomidorach w szklarniach. W ochronie roślin wykorzystuje się także Gram-dodatnią bakterię *Bacillus thuringiensis,* powszechnie występującą w glebie. Jest ona uważana za

bezpieczną dla ludzi, zwierząt i mikroorganizmów niespecyficznych. Dzięki wąskiemu spektrum działania *Bacillus thuringiensis* nie zagraża pożytecznym drobnoustrojom, a czasem nawet wspomaga ich działanie. Wykorzystywana jest do zwalczania głównie szkodników żywiących się liściami, m.in. nicieni, bakterii *Helicoverpa zea*. Niektóre szczepy (*Bt. israelensis*) działają też przeciwko komarom i meszkom [16, 17]. Bakteria *Bacillus thuringiensis* syntetyzuje białko, tzw. toksynę krystaliczną Bt, która w środowisku zasadowym przewodu pokarmowego owada łączy się ze specyficznymi receptorami i powoduje powstawanie porów w błonie, co prowadzi do śmierci szkodnika [18]. Bardzo skuteczny insektycyd uzyskano z toksyn bakterii *Photorhabdus luminiscens*, o działaniu podobnym jak preparaty na bazie Bt. Mogą być one stosowane wymiennie w celu uniknięcia tworzenia się odpornych odmian szkodników.

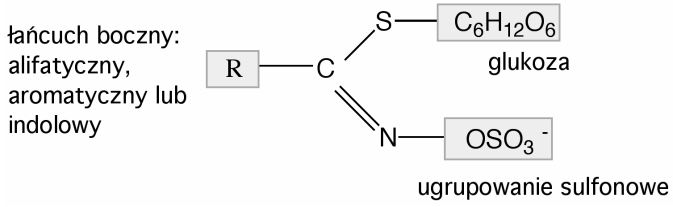
Preparaty zawierające komórki *Bacillus thuringiensis* mogą być stosowane w formie płynnej lub jako granulat. Niestety ich działanie jest krótkotrwałe, po aplikacji bakterie utrzymują się tylko około tygodnia na powierzchni roślin. Ponadto, aby bakterie *Bacillus thuringiensis* mogły działać efektywnie, ich komórki muszą zostać zjedzone przez szkodniki, a to ogranicza zakres działania tylko do tych szkodników, które żywią się liśćmi [19]. Problemy te udało się rozwiązać, wprowadzając do roślin bakteryjne geny odpowiedzialne za syntezę toksyny Bt. Zastosowanie genetycznej modyfikacji roślin umożliwia powstawanie białek szkodliwych dla pasożytów wewnątrz rośliny. Dotychczas odkryto ponad 100 genów kodujących toksyczne białka, co pozwala na specyficzne wykorzystywanie ich w zwalczaniu tylko konkretnych gatunków szkodników. Jednak genetycznie modyfikowane rośliny wywołują wiele kontrowersji dotyczących bezpieczeństwa ich stosowania.

Takich zastrzeżeń nie budzi wykorzystanie roślin z rodziny *Brassicaceae* jako naturalnych biofumigantów. Nie ma tu ryzyka wprowadzenia obcych genów do środowiska i powstawania niekontrolowanych mutacji, a to właśnie jest źródłem obaw związanych ze stosowaniem organizmów modyfikowanych genetycznie. Uprawianie kapusty naprzemiennie z innymi warzywami było już dawniej stosowane w celu poprawy jakości gleby i zwiększenia wydajności zbiorów. Aspekty ekologiczne i bezpieczeństwo stosowania powodują, że biofumigacja ma szansę stać się technologią preferowaną w zrównoważonym rolnictwie.

Aby w pełni zrozumieć zjawisko biofumigacji, należy dokładnie poznać wszystkie czynniki biorące udział w tym procesie, m.in. glukozynolany jako związki wyjściowe, mirozynazę, dzięki której możliwa jest hydroliza i powstające substancje o właściwościach antybiologicznych.

Glukozynolany

Glukozynolany (rys. 1) to związki organiczne zawierające grupę β–D-tioglukozową, sulfonowe ugrupowanie oksymowe oraz łańcuch boczny (alifatyczny, aromatyczny lub indolowy). Zaliczane są do drugorzędowych metabolitów roślin. Ich biosynteza obejmuje elongację łańcucha aminokwasowego, konwersję do tiohydrooksymu i ewentualne dalsze modyfikacje. Glukozynolany alifatyczne powstają z pochodnych metioniny, aromatyczne z pochodnych fenyloalaniny lub tyrozyny, indolowe z pochodnych tryptofanu.



Rys. 1. Ogólny wzór glukozynolanów

Fig. 1. General structure of glucosinolates

Dotychczas zidentyfikowano ok. 200 różnych glukozynolanów w wielu gatunkach roślin, głównie z rodziny *Brassicaceae*, *Capparaceae* i *Caricaceae* [20]. Związki te występują w różnych ilościach zarówno w korzeniach, liściach, pędach, jak i nasionach tych roślin. Przykładowo korzenie kapusty polnej (*B. rapa*) i rzepaku (*B. napus*) zawierają więcej i bardziej różnorodną gamę glukozynolanów niż pędy [8]. Wyniki podobnych badań potwierdzają taką zależność, wskazując na 4-5 razy większą ilość tych związków w korzeniach m.in. gorczycy (*Sinapsis alba*), kapusty czarnej (*B. nigra*) i białej (*B. rapa*) [21, 22]. Występowanie poszczególnych glukozynolanów i ich stężenie zależy także od stadium rozwoju rośliny. W nieskiełkowanych ziarnach poziom glukozynolanów waha się od 93 μmol/g s.m. (w brokułach) do 120 μmol/g s.m. (w czerwonej kapuście), podczas gdy 3-4-dniowe kiełki zawierają od 46 μmol/g s.m. (biała kapusta) do 142 μmol/g s.m. (czerwona kapusta) [23]. Dowiedziono także, że w młodych liściach rzodkiewnika (*Arabidopsis thaliana)* przeważają alifatyczne glukozynolany, które stanowią ok. 80% całkowitej ilości tych związków, jednak z upływem czasu ich liczba maleje na rzecz indolowych pochodnych. Ponadto w wewnętrznych liściach znajduje się więcej glukozynolanów niż w zewnętrznych [24]. Ilość i rodzaj tych związków zależy jednak przede wszystkim od gatunku rośliny. Najczęściej występującymi glukozynolanami w roślinach z rodziny *Brassicaceae* są synigryna, glukoiberyna, glukobrassycyna (odpowiednio w 63, 30 i 23 odmianach na 153 badanych odmian). Na podstawie wyników badań przeprowadzonych w Hiszpanii można stwierdzić, że jarmuż zawiera glukozynolany w ilości 11÷52,8 μmol/g s.m. Są to głównie glukozynolany alifatyczne i stanowią one ponad 60% całkowitej zawartości tej grupy związków. Natomiast w kapuście białej zawartość tych związków waha się na poziomie 10,9÷27 μmol/g s.m., a w największej ilości występuje glukobrassycyna i glukoiberyna [25, 26]. Na ilość glukozynolanów ma wpływ także pora zbioru i klimat regionu, w którym zlokalizowane są uprawy. W kapuście ogrodowej (*Brassica oleracea*) zbieranej wiosną znajduje się więcej tych związków (22 μmol/g s.m.) niż w tej ze zbiorów jesiennych (13 μmol/g s.m.) [25]. W czasie innych badań zawartość glukozynolanów w kapuście białej oznaczono na poziomie 3,3÷7,7 μmol/g s.m. w większych ilościach ze zbiorów jesiennych niż wiosennych. Różnice w ilości tych substancji mogą wynikać z różnic klimatycznych regionów, na których uprawiana jest kapusta, i czasem ekspozycji słonecznej, która sprzyja syntezie większych ilości aktywnych związków w roślinie [27]. W tabeli 1 zestawiono informacje o najczęściej występujących glukozynolanach i produktach ich hydrolizy.

Oprócz warunków uprawy roślin, ważne są także sposób i warunki ich dalszej obróbki. Transport i przechowywanie roślin z rodziny kapustowatych, nawet w niskiej temperaturze, mogą prowadzić do straty znacznych ilości glukozynolanów. Stwierdzono, że w trakcie

przychowywania kapusty *Brassica oleracea* przez 7 dni w temp. 1ºC (co odpowiada warunkom magazynowym), a następnie kolejne 3 dni w temp. 15ºC (warunki sklepowe) ubytek glukozynolanów był rzędu 70÷80% w stosunku do zawartości początkowej, zmniejszyła się również zawartość innych cennych składników odżywczych, m.in. flawonoidów [28]. Natomiast wyniki innych badań wykazały, że dwudniowe przechowywanie warzyw w temperaturze –22ºC nie ma wyraźnego wpływu na ilość glukozynolanów [29]. Ich zawartość może się zmieniać także w zależności od składu atmosfery, w której przechowywane są warzywa. W przypadku brokułów zaobserwowano spadek ilości glukozynolanów o 15% w stosunku do ilości początkowej podczas przechowywania (7 dni, 10ºC) w atmosferze zawierającej 20% CO_2. Natomiast w powietrzu lub atmosferze zawierającej 0,5% tlenu i 20% CO_2 zanotowano wzrost odpowiednio o 42 i 21% w porównaniu do zawartości glukozynolanów w świeżych warzywach [30]. Informacje te mogą być ważne przy przygotowaniu roślin do zastosowania ich jako biofumigantów.

Tabela 1

Glukozynolany i produkty ich hydrolizy najczęściej występujące w roślinach z rodziny kapustowatych

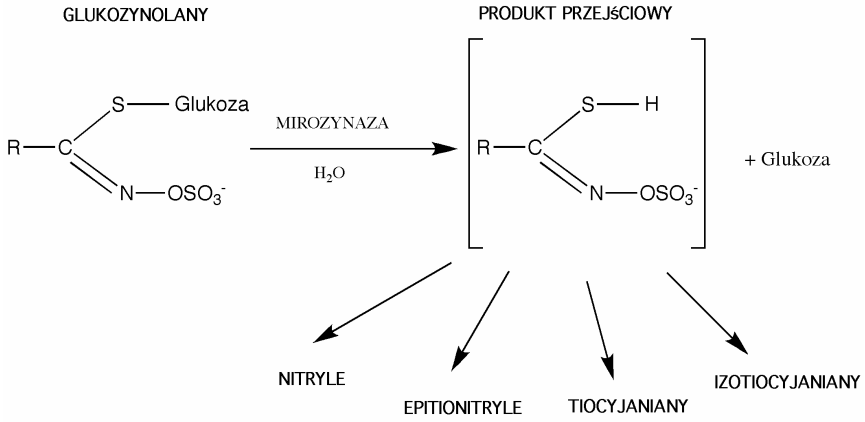
Table 1

Glucosinolates and products of their hydrolysis frequently occuring in plants of *Brassica* family

Budowa łańcucha bocznego	**Nazwa systematyczna glukozynolanu**	**Nazwa zwyczajowa glukozynolanu**	**Główne produkty degradacji**
alifatyczne	3-metylosulfinylopropylo GLS	glukoiberyna	izotiocyjanian 3-metylo-sulfinylopropylu
	2-propenylo GLS	synigryna	izotiocyjanian allilu
	4-metylosulfinylobutylo GLS	glukorafanina	izotiocyjanian 4-metylo-sulfinylobutylu
	3-butenylo GLS	glukonapina	izotiocyjanian 3-butenylu
	4-pentenylo GLS	glukobrassikonapina	izotiocyjanian 4-pentenylu
	4-metylotiobutylo GLS	glukoerucyna	izotiocyjanian 4-metylotiobutylu
indolowe	3-indolometylo GLS	glukobrassycyna	indolo-3-karbinol, askorbigen, indolo-3-acetonitryl
	1-metoksy-3-indolometylo GLS	neoglukobrasycyna	1-metoksy-3-indoilometyl
aromatyczne	2-fenyloetylo GLS	glukonasturcyna	izotiocyjanian 2-fenyloetylu
	benzylo GLS	glukotropaeolina	izotiocyjanian benzylu
	4-hydroksybenzylo GLS	sinalbina	izotiocyjanian p-hydroksybenzylu

Glukozynolany nie wykazują właściwości biobójczych, dopiero w wyniku enzymatycznej hydrolizy powstają związki o takiej aktywności: tiocyjaniany, nitryle, epitionitryle i najważniejsze z nich izotiocyjaniany (rys. 2) [31, 32].

Na skuteczność działania izotiocyjanianów ma wpływ ich budowa; im większa lotność związku, tym większa aktywność antybiologiczna. Ważny jest także rodzaj zwalczanych mikroorganizmów, a nawet faza ich wzrostu [32, 33].



Rys. 2. Produkty enzymatycznej hydrolizy glukozynolanów

Fig. 2. Products of enzymatic hydrolysis of glucosinolates

Aktywność biobójcza izotiocyjanianów jest porównywalna ze skutecznością syntetycznych pestycydów, takich jak bromek metylu, oraz niektórych antybiotyków (gentamycyna) [8, 34]. O antybiologicznym działaniu produktów hydrolizy glukozynolanów decyduje to, z jakiej części stosowanej rośliny pochodzą te związki. Produkty hydrolizy glukozynolanów obecnych w korzeniach mają znacznie skuteczniejsze działanie biobójcze niż związki pochodzące z pędów. Stosowanie preparatu uzyskanego z korzeni kapusty i rzepaku wywołało zahamowanie wzrostu prawie całej populacji grzybów *R. fragariae* (96% kolonii), podczas gdy preparat z pędów tych roślin spowodował śmierć tylko 16% kolonii [8]. Wykazano także, że im starsze korzenie, tym skuteczniejsze jest działanie biobójcze uwalnianych z nich izotiocyjanianów. Z kolei młode liście *B. rapa* zawierają więcej glukozynolanów (75,9 μmol/g s.m.) niż stare liście i dlatego są rzadziej atakowane przez szkodniki [35, 36]. W gorczyczniku (*Barbarea vulgaris)* najwięcej glukozynolanów znajduje się w nasionach (40÷90 μmol/g s.m., w zależności od odmiany), a najmniej w kwiatach (10 μmol/g s.m). Wyniki badań pokazują, że w Europie dominują odmiany tej rośliny bogate w glukobarbarynę (glukozynolan 2-hydroksy-2-fenyloetylu), która stanowi aż 94% całkowitej zawartości glukozynolanów. Możliwe produkty jej hydrolizy to izotiocyjaniany lub mało szkodliwe tiony. Rzadziej występują odmiany zawierające dużo glukonasturcyny (82% całkowitej zawartości glukozynolanów), której produkty hydrolizy (izotiocyjanian 2-fenyloetylowy) są bardziej toksyczne dla szkodników [37].

Proces enzymatycznej degradacji glukozynolanów do izotiocyjanianów zachodzi szybciej w obecności wody, przy podwyższonej temperaturze gleby i przy silnym uszkodzeniu komórek [33]. Z całych komórek roślinnych przechodzi do ziemi tylko 1% izotiocyjanianów, które powstały z glukozynolanów zawartych w roślinie, natomiast po uszkodzeniu (zamrażanie i rozmrażanie) aż 26% izotiocyjanianów [38]. Największa ich ilość jest uwalniana po 30 min od wprowadzenia utartych roślin do gleby, a obecność izotiocyjanianów można wykryć nawet 12 dni później. Do izotiocyjanianów zostało przekształconych odpowiednio 14 i 53% glukozynolanów z rzepaku i gorczycy w czasie 30 min. Ponadto indolowe glukozynolany, które nie są hydrolizowane do izotiocyjanianów, pozostają w ziemi przez dłuższy czas [39].

Spośród produktów enzymatycznej degradacji glukozynolanów największe znaczenie w biofumigacji mają izotiocyjaniany. To one wykazują najskuteczniejsze działanie biobójcze, dlatego tak ważna jest wiedza na temat warunków hydrolizy i czynników wpływających na ich powstawanie.

Mirozynaza

Hydroliza glukozynolanów jest możliwa dzięki enzymowi o zwyczajowej nazwie mirozynaza, należącemu do β-tioglukozydaz (EC 3.2.3.1). Mirozynaza katalizuje hydrolizę wiązań tioglukozydowych, w wyniku czego powstaje niestabilny produkt przejściowy - tiohydroksym *o*-sulfonowy, który w zależności od środowiska reakcji może zostać przekształcony do izotiocyjanianów, tiocyjanianów, nitryli lub epinitryli [40].

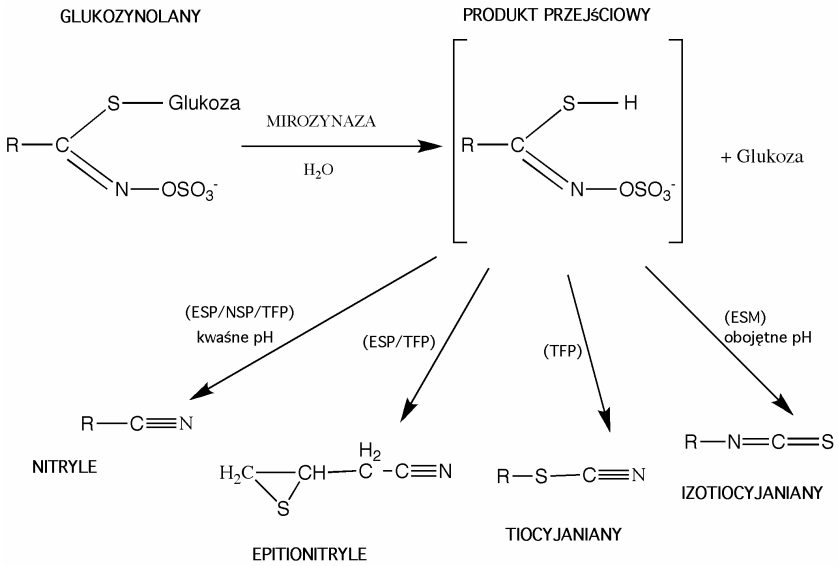
Warunki hydrolizy enzymatycznej mają wpływ na rodzaj i właściwości produktu reakcji. Przykładowo glukozynolany zawierające alifatyczny łańcuch boczny w środowisku o pH 7 po procesie hydrolizy z udziałem mirozynazy ulegają przekształceniu do odpowiednich izotiocyjanianów, natomiast w kwaśnym środowisku (o pH 3÷5) lub w obecności jonów żelaza Fe(II) powstają mniej szkodliwe nitryle [31]. Na aktywność mirozynazy ma także wpływ obecność innych substancji, np. kwas askorbinowy może działać jak kofaktor, ułatwiając odszczepienie molekuły glukozy [41-44].

Kinetyka działania i aktywność mirozynazy różni się także w zależności od gatunku i części rośliny, z której enzym pochodzi. Przykładowo, w rzodkiewniku pospolitym (*A. thaliana*) znajduje się kilka rodzajów mirozynazy. Geny odpowiedzialne za syntezę tych enzymów mogą być odmiennie uruchamiane w tej samej roślinie: w korzeniu ulegają ekspresji geny *TGG4, TGG5*, a w części naziemnej geny *TGG1, TGG2* [35].

Na kierunek reakcji hydrolizy glukozynolanów wpływ mają także dodatkowe czynniki białkowe (rys. 3). Należą do nich: białko nitrylospecyficzne NSP, białko epitiospecyficzne ESP, modyfikujące białko epitiospecyficzne ESM oraz białko tworzące tiocyjaniany TFP. Obecność białka epitiospecyficznego ESP sprzyja powstawaniu nitryli i epinitryli z glukozynolanów [45, 46]. W roślinach, w których powstają wyłącznie izotiocyjaniany, nie zachodzi ekspresja genów odpowiedzialnych za syntezę białek ESP. Jest to cecha gatunkowa roślin, np. niektóre gatunki rzodkiewnika *Arabidopsis* (ekotyp Landsberg *erecta*) syntetyzują białko ESP, a w innych roślinach, które są zdolne do wytwarzania alkenowych glukozynolanów hydroliza biegnie raczej do nitryli jako produktów enzymatycznego rozkładu niż izotiocyjanianów [31]. Natomiast epitiospecyficzne białko modyfikujące (*epithiospecifier modifier protein* - ESM), zidentyfikowane m.in. w rzodkiewniku, promuje powstawanie izotiocyjanianów, a nie nitryli jak w przypadku działania ESP. Okazało się, że owad błyszczka ni (*Trichoplusia ni*) preferuje liście roślin pozbawionych ESM ze względu na mniejszą zawartość izotiocyjanianów [47]. Oprócz białek ESP i ESM opisano jeszcze jedno białko, tzw. białko tworzące tiocyjaniany (*thiocyanate-forming protein* - TFP), które także ma wpływ na rodzaj produktów hydrolizy glukozynolanów. Zostało ono zidentyfikowane w nasionach i kwiatach rzeżuchy (*Lepidium sativum*), a powoduje tworzenie się tiocyjanianów z glukotropaeoliny oraz prostych nitryli i epitionitryli z alifatycznych glukozynolanów [38, 48].

Oprócz roślinnej mirozynazy zidentyfikowano także mirozynazę pochodzącą z drobnoustrojów obecnych w glebie oraz bakterii zasiedlających przewód pokarmowy

ludzi i zwierząt. Wyniki badań dowiodły, że zachodzi proces degradacji synigryny do izotiocyjanianu allilu przez mikroflorę jelitową człowieka [41, 49].



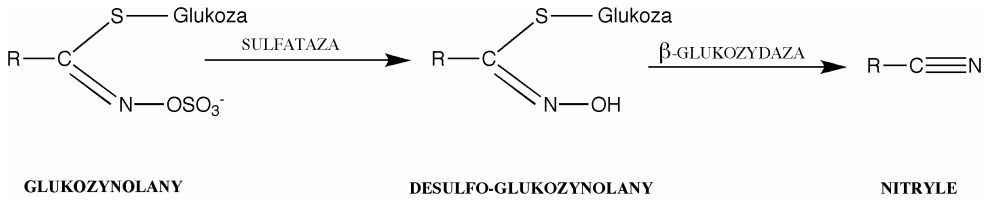
Rys. 3. Schemat przebiegu reakcji hydrolizy glukozynolanów w zależności od obecności czynników białkowych [ESP - białko epitiospecyficzne, NSP - białko nitrylospecyficzne, TFP - białko tworzące tiocyjaniany, ESM - epitiospecyficzne białko modyfikujące]

Fig. 3. The routes of hydrolysis of glucosinolates depending on the presence of specific protein factors [ESP - epithiospecifier protein, NSP - nitrile-specifying protein, TFP - thiocyanate-forming protein, ESM - epithiospecifiermodifier protein]

Bakterie *Pseudomonas borepolis* także mają zdolność degradacji syningryny i glukonapiny do odpowiednich izotiocyjanianów. Związki te działają toksycznie na grzyby *Rhizoctonia solani* i *Sclerotium rolfsii*, patogeny warzyw i owoców, co być może będzie można wykorzystać w ochronie upraw [50]. Szczególnie ważna z punktu widzenia biofumigacji jest aktywność mirozynazy drobnoustrojów zasiedlających glebę. Wyniki badań próbek gleby na zawartość glukonasturcyny mogą być podstawą do stwierdzenia, że mikroorganizmy, dla których ziemia jest naturalnym środowiskiem bytowania, mają zdolność do rozkładu glukozynolanów. Po 44 godzinach w niesterylizowanej próbce gleby stwierdzono obecność tylko śladowych ilości glukonasturcyny, natomiast w sterylnej ziemi taki efekt osiągnięto po 91 godzinach [51]. Potwierdzają to wyniki innych badań, w trakcie których także oznaczono większe stężenie glukozynolanów w sterylnej glebie niż w glebie z naturalną mikroflorą [52]. Kolejne wyniki badań dowodzą zwiększonej aktywności enzymatycznej mirozynazy w glebie, na której uprawiane były rośliny z rodziny kapustowatych, syntetyzujące ten enzym. Wynika z tego, że obecność glukozynolanów, pochodzących z uprawianych warzyw, może stymulować produkcję enzymu przez mikroorganizmy glebowe [33, 53].

Jednak z drugiej strony pewna aktywność enzymatyczna wynikająca z obecności drobnoustrojów może przyczyniać się do obniżenia skuteczności stosowania

biofumigantów. Grzyb *Aspergillus niger* obecny w glebie syntetyzuje enzym sulfatazę (EC 3.1.5.6), który hydrolizuje glukozynolany do desulfoglukozynolanów. Są one następnie przekształcane przez β-glukozydazę (EC 3.2.1.21) także syntetyzowaną przez *A. niger* do nitryli, które wykazują mniejsze właściwości biobójcze (rys. 4) [54].



Rys. 4. Schemat przebiegu reakcji rozkładu glukozynolanów do nitryli przez grzyby *A. niger*

Fig. 4. The reaction of glucosinolate decomposition to nitriles induced by fungi *Aspergillus niger*

Mirozynazę zidentyfikowano także w tkankach szkodników, m.in. w mszycy kapuściance (*Brevicoryne brassica)* i kapustnicy wielożernej (*Lipaphis erisimi*) [20]. Owady te potrafią wykorzystywać glukozynolany zawarte w roślinach, na których żerują, do obrony przed atakami innych szkodników. Mirozynaza obecna w ich organizmach bierze udział w hydrolizie glukozynolanów, a uwalniane izotiocyjaniany działają synergicznie z E-β-farnezenem, feromonem, który sygnalizuje innym mszycom nadchodzące niebezpieczeństwo [55].

Dzięki naturalnej zawartości glukozynolanów i mirozynazy rośliny z rodziny kapustowatych są w stanie skutecznie bronić się przed atakami grzybów, bakterii oraz większością roślinożerców, a mechanizm ten można też wykorzystać w ochronie innych roślin.

Aktywność roślin z rodziny kapustowatych jako biofumigantów

Jak wspomniano, właściwości biobójcze wykazują produkty hydrolizy glukozynolanów, a spośród nich największe znaczenie mają izotiocyjaniany. Ich szeroki zakres aktywności antybiologicznej obejmuje zarówno bakterie, grzyby, jak i owady. Dane literaturowe wskazują na zróżnicowany wpływ tych związków na drobnoustroje. Na przykład, ekstrakt z mielonych nasion rzepaku (*B. napus*) wykazuje działanie hamujące wzrost grzybów *Aphanomyces euteiches,* ale i lekko stymulujące wzrost bakterii propionowych [56, 57].

Toksyczne działanie izotiocyjanianów w stosunku do patogenów korzysta z dwóch głównych mechanizmów: hamowania syntezy ATP lub inaktywacji enzymów wewnątrzkomórkowych. U podłoża tych zjawisk leży zdolność izotiocyjanianów do rozrywania mostków disiarczkowych oraz wchodzenia w reakcje z grupami NH_2, występującymi w strukturze białek [34].

Aktywność bakteriobójcza

Wyniki badań wskazują, że izotiocyjaniany działają przeciwko takim Gram-ujemnym bakteriom, jak: *Agrobacterium tumefaciens, Erwinia chrysanthemi, Pseudomonas cichorii, Pseudomonas tomato, Xanthomonas campestris, X. juglandis*. Bakterie te powodują

żółknięcie lub ciemnienie liści, zaburzenia rozprowadzania składników odżywczych w roślinie, gnicie owoców, łodyg i korzeni. W efekcie prowadzi to do śmierci roślin i wpływa na obniżenie wydajności upraw. Co więcej, atakowane są wewnętrzne części roślin, co utrudnia stosowanie tradycyjnych środków ochrony. Najskuteczniejsze w hamowaniu wzrostu wymienionych patogenów okazały się izotiocyjaniany powstające w wyniku hydrolizy glukonasturcyny oraz glukorafaniny. Generalnie, izotiocyjaniany otrzymane z aromatycznych glukozynolanów wykazują silniejsze właściwości bakteriobójcze niż produkty hydrolizy alifatycznych glukozynolanów [34]. Wśród tych ostatnich znaczące właściwości antybakteryjne ma izotiocyjanian allilu, produkt rozpadu rozpowszechnionej w roślinach z rodziny kapustowatych synigryny, który stanowi aż 90% wszystkich lotnych związków obecnych w świeżym chrzanie. Ma on zdolność niszczenia komórek patogenów w każdym stadium rozwoju. Badane bakterie Gram-ujemne, *Salmonella* oraz *E. coli* były bardziej wrażliwe niż Gram-dodatnie pałeczki *Listeria monocytogenes*, natomiast bakterie fermentacji mlekowej (*Lactobacillus sake*) okazały się być odporne na jego działanie. Izotiocyjanian allilu wykazuje skuteczne działanie zarówno w stanie gazowym, jak i ciekłym, co daje możliwość jego wykorzystania również w technologii utrwalania produktów spożywczych [34, 58, 59].

Aktywność grzybobójcza

Niektóre gatunki grzybów powodują choroby roślin, przez co są przyczyną dużych strat w rolnictwie i przechowalnictwie płodów rolnych. Izotiocyjaniany w różnym stopniu hamują rozwój grzybów, co dokumentują dane zawarte w tabeli 2.

Tabela 2

Zakres toksycznego działania produktów hydrolizy glukozynolanów na różne gatunki grzybów

Table 2

Spectrum of toxicity of glucosinolates hydrolysis products towards various species of fungi

Roślina wykorzystana w badaniach	**Glukozynolany, których produkty hydrolizy są toksyczne**	**Badane grzyby**	**Literatura**
gorczyca czarna *(Brassica nigra*), kapusta sitowata (*Brassica juncea*)	synigryna	*Fusarium sambucinum*	[60]
ubiorek gorzki (*Iberis amara*), rokietta siewna (*Eruca sativa*)	glukoiberyna, glukoerucyna	*Fusarium culmorum* *Rhizoctonia solani* *Sclerotinia sclerotorium* *Diaporthe phaseolorum* *Pythium irregulare*	[61]
rokietta siewna (*Eruca sativa*), kapusta sitowata (*Brassica juncea*), ubiorek gorzki (*Iberis amara*)	glukoerucyna, synigryna, glukoiberyna	*Rhizoctonia solani* *Pythium irregulare*	[62]
gorczyca etiopska (*Brassica carinata*), kapusta sitowata (*Brassica juncea*), gorczyca czarna (*Brassica nigra*)	glukotropaeolina	*Fusarium oxysporium*	[63]
kapusta sitowata (*Brassica juncea*)	synigryna	*Pythium irregulare* *Rhizoctonia solani*	[64]
gorczyca biała (*Brassica mirta*)	glukotropaeolina	*Meloidogyne javanica* *Tylenchulus semipenetrans*	[65]
gorczyca etiopska (*Brassica carinata*)	synigryna	*Sclerotinia minot* *Sclerotinia sclerotorium*	[66]

kapusta rzepak (*Brassica napus*)	glukonasturcyna	*Rhizoctonia solani* *Phytophthora erythroseptica* *Pythium ultimum* *Sclerotinia sclerotiorum* *Fusarium sambucinam*	[67]
rokietta siewna (*Eruca sativa*)	glukoerucyna	*Monilinia laxa*	[68]
kapusta polna (*Brassica rapa*) kapusta rzepak (*Brassica napus*)	glukonapina, glukonasturcyna	*Rhizoctonia fragariae* *Pythium ultimum* *Fusarium oxysporium* *Altenaria alternata* *Phytophthora cactorum*	[8]

Ważną obserwacją jest to, że gatunek pożytecznego grzyba obecnego w glebie, *Trichoderma*, jest mniej wrażliwy na działanie izotiocyjanianów niż patogeny, np. *Pythium ultimum, Rhizoctonia solani* czy *Fusarium oxysporium*. Stąd też propozycja, żeby połączyć grzybobójcze działanie izotiocyjanianów z wypieraniem patogennych grzybów przez pożyteczne gatunki mikroorganizmów. Jednak analiza wyników, które uzyskano w efekcie przeprowadzenia doświadczenia polegającego na wspólnym stosowaniu mączki z gorczycy *Brassica carinata* i grzybów *Trichoderma* do ochrony uprawy buraka cukrowego, świadczy, że nie nastąpiła redukcja populacji badanego patogena *P. ultimum*, chociaż znacznie zmniejszyła się liczba zaatakowanych przez niego buraków. Wydaje się więc, że połączenie działania izotiocyjanianów oraz kolonizacji uprawianych roślin przez grzyby *Trichoderma* może dać dobre efekty, ale możliwość wykorzystania takiego podejścia w praktyce rolniczej i ogrodniczej wymaga dalszych badań [69].

Zwalczanie roślinożerców

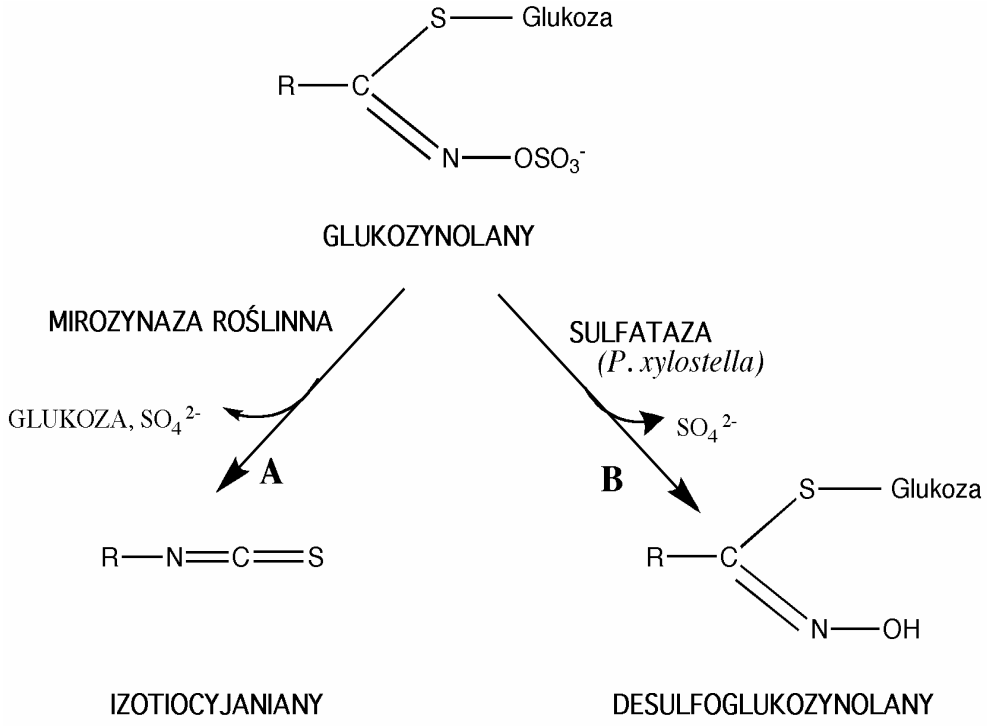
Szkodniki, takie jak nicienie, stonki czy wołki, są pasożytami roślin o znaczeniu gospodarczym, w uprawie których mogą powodować olbrzymie straty. Zwalczanie szkodników za pomocą biofumigacji daje możliwość ograniczenia tych strat, a ponadto chroni konsumentów przed szkodliwym działaniem syntetycznych pestycydów, co jest szczególnie ważne w przypadku roślin jadalnych.

Nicienie *Meloidogyne incognita* są przyczyną sękowacenia korzenia roślin uprawianych w szklarniach, głównie pomidorów i ogórków. Izotiocyjaniany, zwłaszcza produkty hydrolizy synigryny, glukoerucyny i glukotropaeoliny, zastosowane w stężeniu 11÷35 μM, skutecznie hamują rozwój tych szkodników [70]. Zbadano także wpływ omawianych związków na nicienie *Heterodera schachtii*. Glukozynolany: sinalbina, synigryna i glukotropaeolina nie wykazały żadnego efektu letalnego wobec badanych roślinożerców nawet po 96 godzinach ekspozycji. Jednak izotiocyjaniany otrzymane w trakcie hydrolizy tych samych związków spowodowały śmierć prawie wszystkich nicieni już po 24-48 godzinach, nawet dla małych stężeń (0,05÷0,5% w/v) [7]. Dowiedziono także, że aromatyczne izotiocyjaniany są najbardziej toksyczne dla jajeczek chrząszcza opuchlaka truskawkowca (*Vine weevil*), natomiast metylowe izotiocyjaniany w stosunku do larw wołka.

Wyniki wieloletnich badań wykazały, że uprawa truskawek na jednym polu przez 10 lat powoduje zahamowanie ich wzrostu i rozwoju. Uprawiane w ten sposób truskawki wydają o 50% mniej owoców w porównaniu z truskawką uprawianą przez dwa lata na danym polu. Najczęściej do obniżenia wzrostu i plonowania truskawki dochodzi w 4-6 roku uprawy

[71]. Jednak wyniki niedawnych badań pokazały, że naprzemienna uprawa truskawek, brokułów i kapusty warzywnej (*Brassica oleracea*) zwiększyła plon truskawek o 18÷44% w porównaniu ze zwykłą pojedynczą uprawą. Mieszana uprawa nie wpłynęła natomiast na zmniejszenie zachorowalności owoców na werticilozę, wywołaną przez grzyby *Verticillium dahliae* [8].

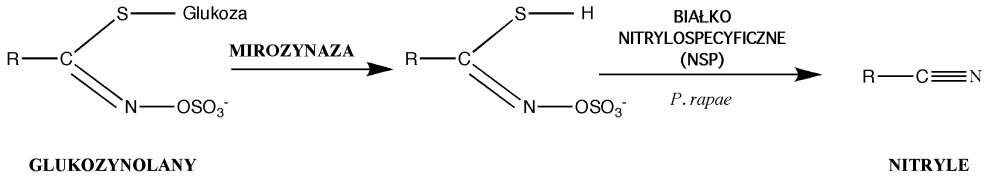
Wyniki licznych badań pokazują, że owady mogą wytworzyć system neutralizowania szkodliwego wpływu izotiocyjanianów. Oprócz opisanych wcześniej mszyc *Brevicoryne brasicae i Lipaphis erysimmi*, należy wspomnieć o motylu tantnisiu krzyżowiaczku (*Plutella xylostella*), żywiącym się roślinami z rodziny kapustowatych, który usuwa ugrupowanie siarkowe z glukozynolanów za pomocą sulfatazy, przez co przestają być one substratem dla mirozynazy (rys. 5) [72].



Rys. 5. Schematyczne przedstawienie sposobu działania roślinnej mirozynazy (A) oraz sulfatazy pochodzenia mikrobiologicznego (B) na glukozynolany

Fig. 5. Scheme of enzymatic activity of plant mirosinase (A) and microbial sulfatase (B) towards glucosinolates

Z kolei larwy bielinka (*Pieris sp*.), które żywią się wyłącznie roślinami z rodziny kapustowatych, wytworzyły inny mechanizm obronny. Produkują one nitrylospecyficzne białka NSP, przez co glukozynolany są hydrolizowane przez mirozynazę do nitryli, a nie do toksycznych izotiocyjanianów (rys. 6) [73-75].



Rys. 6. Schemat reakcji rozkładu glukozynolanów do nitryli przez larwy bielinka *Pieris rapae*

Fig. 6. Reaction of glucosinolate degradation to nitriles by *Pieris rapae* caterpillars

Larwy gnatarza rzepakowca (*Athalia rosae*) w celach obronnych w tylnej części tułowia kumulują wybrane glukozynolany ze spożywanej rośliny, głównie sinalbinę i synigrynę. Szkodniki te nie wykazują aktywności związanej z mirozynazą, desulfatazą ani nie mają białek nitrylospecyficznych. Glukozynolany są wydalane, a ewentualne produkty ich przemian nie są obecne w organizmie [76, 77].

Niektóre glukozynolany i produkty ich rozkładu mogą działać jak atraktanty podczas wyboru miejsca składania jaj przez owady. W przypadku samic *Piersi rapae* indolowe i aromatyczne glukozynolany stymulują wybór tego miejsca bardziej niż związki alifatyczne. Natomiast genetycznie modyfikowane odmiany rzodkiewnika (*A. thaliana)* cyp79B2 i cyp79B3, które nie są zdolne do syntezy indolowych glukozynolanów, są o wiele mniej atrakcyjne pod tym względem dla *P. rapae* niż tradycyjne odmiany tej rośliny [78].

Izotiocyjaniany uwalniane z glukozynolanów zawartych w roślinach z rodziny kapustowatych są toksyczne dla szkodników, ale mogą też hamować rozwój pożytecznej mikroflory glebowej. W badaniach przydatności nawozów z kapusty i gorczycy w eliminowaniu stonki *Leptinotarsa decemlineata*, pasożyta ziemniaków, okazało się, że zmniejszyła się również populacja nicieni *Steinernema feltiae* i *Steinernema riobrave*, naturalnych wrogów stonki. Niezbędne jest, jak widać, dokładne zbadanie działania biofumigantów na organizmy zarówno te szkodliwe, jak i pożyteczne oraz możliwości łączenia różnych metod ochrony roślin [79-81].

Problemy związane z wykorzystaniem biofumigacji w rolnictwie

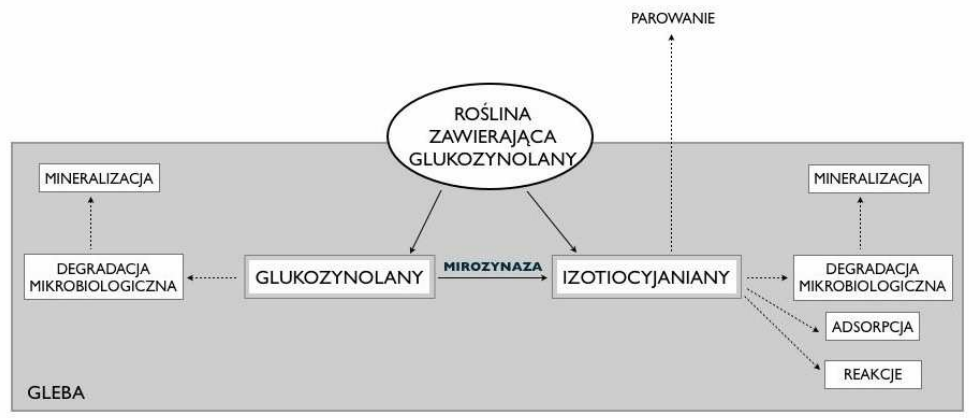
Naturalne właściwości biobójcze roślin z rodziny kapustowatych mogą być potencjalnie wykorzystane w ochronie upraw na większą skalę, dlatego w wielu ośrodkach naukowych prowadzone są szeroko zakrojone badania nad biofumigacją. Proponowane jest stosowanie preparatów otrzymanych z wybranych roślin przez ich wysuszenie i sproszkowanie. W jednym z doświadczeń wykorzystano odtłuszczoną mączkę z gorczycy etiopskiej (*B. carinata*), zawierającą glukozynolany, głównie synigrynę (98%), oraz odpowiednią ilość mirozynazy. Mączkę ręcznie rozprowadzono po polu, a następnie zwilżono na 6 dni przed zasadzeniem cukini. Efektem działania tego preparatu była poprawa jakości badanej gleby i zwiększenie plonu cukini o 14% w porównaniu z plonem uzyskanym z uprawy, w której wykorzystano syntetyczny preparat ochronny. Dodatkowo uzyskano wyraźne zahamowanie rozwoju szkodników *Meloidogyne incognita* [6]. Innym sposobem wprowadzenia glukozynolanów do gleby jest zasadzenie rośliny z rodziny *Brassicaceae* na chronionym polu przed uprawą właściwych warzyw.

Ze względu na duże spożycie ziemniaków na świecie ochrona upraw tej rośliny jest szczególnie ważna. Obserwacje doświadczalnej uprawy ziemniaków w stanie Maine (USA)

mogą być podstawą do stwierdzenia, że przed chorobami bulwy najlepiej chroniła gorczyca, a przed atakiem grzybów *Rhizpous* - rzepak. Rośliny te zasadzono w lipcu i po miesiącu zaorano jako nawóz naturalny, a wiosną następnego roku na tym samym polu posadzono ziemniaki i zbadano ochronny wpływ poprzedniej uprawy roślin z rodziny kapustowatych na jakość plonu [67]. Natomiast wyniki innych badań nad ochroną ziemniaków przed mątwikami *Globodera rostochiensis* za pomocą ekstraktów z roślin *Brassicaeae* pokazują, że najskuteczniejsze w hamowaniu rozwoju tych nicieni są rzeżucha, kalafior i kapusta (*B. rapa*) [34].

Wyniki licznych badań dowiodły zależności pomiędzy ilością glukozynolanów w roślinach od warunków prowadzenia uprawy. Jest to wiedza, którą można wykorzystać w kontrolowanym zwiększaniu zawartości tych związków w roślinach stosowanych w biofumigacji. Ważnym czynnikiem decydującym o zawartości glukozynolanów w roślinie jest żyzność gleby. Przykładowo, zawartość siarki i azotu ma wpływ na ilość glukozynolanów w nasionach rzepaku [72]. Mała zawartość wody w glebie zwiększa ilość glukozynolanów w rzepaku i jego nasionach oraz prawdopodobnie w większości roślin krzyżowych. Ograniczenie ilości wody, zwłaszcza we wczesnym stadium rozwoju rośliny (36-62 dni po wysianiu), może doprowadzić do wzrostu zawartości glukozynolanów nawet o ponad 40% w porównaniu z roślinami z uprawy kontrolnej bez ograniczenia wody [34]. Wyniki badań opisanych w literaturze mogą być również podstawą do wniosku, że temperatura uprawy roślin ma wpływ na zawartość glukozynolanów - rośliny rosnące w podwyższonej temperaturze (30ºC w dzień i 15ºC w nocy) zawierają więcej glukozynolanów niż te, uprawiane przy niższych temperaturach (22ºC/15ºC oraz 18ºC/12ºC) [82].

Można by przypuszczać, że glukozynolany i produkty ich hydrolizy, jako naturalne związki, będą szybciej ulegały procesowi degradacji niż syntetyczne środki ochrony roślin. Przeczą temu wyniki badań, które pokazują na przykładzie tropaeoliny i izotiocyjanianu benzylu, że połowa wprowadzonej ilości tych związków jest nadal obecna w glebie po 60 dniach [83]. Jednak inne badania przeprowadzone w podobnych warunkach wskazują na całkowitą degradację tych samych związków w ciągu 15 dni [84].



Rys. 7. Schemat przedstawiający los glukozynolanów i izotiocyjanianów w glebie

Fig. 7. The fate of glucosinolates and isothiocyanates in soil

Wpływ rodzaju gleby na efektywność biofumigacji można rozpatrywać w kilku aspektach. Los badanych związków w ziemi przedstawiono na rysunku 7. Rozkład glukozynolanów następuje szybciej w gliniastych niż w piaszczystych glebach, zależy także od ilości i rodzaju mikroflory występującej na danym terenie, temperatury i wilgotności. Glukozynolany są słabo adsorbowane w ziemi, co pozwala na ich migrację w glebie i skuteczniejsze działanie, ale powoduje także ryzyko przedostania się do wód gruntowych. Natomiast duża zawartość materii organicznej powoduje adsorpcję izotiocyjanianów, przez co obniża skuteczność ich działania. Wilgotność ziemi także jest ważnym czynnikiem w degradacji glukozynolanów. Mniejsza zawartość wody w glebie powoduje wydłużenie czasu hydrolizy tych związków, co znacznie opóźnia antybiologiczne działanie biofumigantów [33, 39, 85, 86].

Kluczową rolę w procesie biofumigacji odgrywają izotiocyjaniany, jako produkty hydrolizy glukozynolanów, dlatego ważna jest ilość mirozynazy, która umożliwia otrzymywanie tych bioaktywnych związków. Enzym ten może pochodzić bezpośrednio z użytych roślin lub z dodatkowego źródła w postaci gotowego preparatu. Należy także uwzględnić aktywność mirozynazy pochodzącej z glebowej mikroflory, która przyczynia się do hydrolizy glukozynolanów.

Skuteczność biofumigacji zależy także od interakcji produktów hydrolizy glukozynolanów w środowisku. Gdy uprawa na danym polu była wcześniej chroniona za pomocą soli sodowej kwasu ditiokarb-*N*-metyloaminowego, mikroorganizmy glebowe brały udział w procesie degradacji glukozynolanów i izotiocyjanianów pochodzących z roślin krzyżowych zbyt szybko, aby mogły one skutecznie chronić rośliny. Przykładowo, izotiocyjanian allilu uległ degradacji w ciągu 11 dni w glebie, w której nie było pestycydu, natomiast w glebie traktowanej pestycydem - w ciągu 21 godzin, co spowodowało mniejszą śmiertelność szkodników. Ważna jest więc wiedza na temat rodzaju i właściwości wcześniej wykorzystywanych pestycydów oraz opracowanie alternatywnych sposobów ochrony roślin, np. płodozmianu i biofumigacji [52].

Podsumowanie

Glukozynolany stanowią liczną grupę bioaktywnych związków o różnorodnych właściwościach wynikających z ich zdolności do wiązania się z molekułami i makromolekułami o znaczeniu biologicznym. Stanowią one naturalną ochronę roślin przed szkodliwymi drobnoustrojami i roślinożercami. Możliwość biodegradacji i brak toksyczności dla ludzi pozwala na wykorzystanie glukozynolanów jako biofumigantów w zwalczaniu szkodników w rolnictwie, szczególnie upraw roślin jadalnych. Aby zapewnić w pełni bezpieczne i efektywne stosowanie glukozynolanów i produktów ich hydrolizy jako biofumigantów, należy dokładnie poznać mechanizmy ich działania oraz interakcje, które wywołują w środowisku. Wyniki dotychczas prowadzonych badań ukazują złożoność tych procesów, ale jednocześnie wyniki doświadczeń zachęcają do kontynuowania prac w kierunku upowszechnienia stosowania biofumigacji w ochronie upraw roślinnych.

Badania nad wykorzystaniem kapusty w ochronie upraw są również prowadzone w ramach projektu AGROBIOKAP, w którym udział biorą: Politechnika Gdańska, Instytut Chemii Przemysłowej w Warszawie oraz Uniwersytet Rolniczy w Krakowie. Celem projektu jest opracowanie technologii wykorzystania kapusty białej w procesach biofumigacji oraz fitoremediacji prowadzonych na terenach zdegradowanych. Badania są

skoncentrowane na określeniu przydatności kapusty jako surowca do produkcji biopreparatu, który mógłby służyć do biofumigacji gleby oraz na opracowaniu wydajnej i przyjaznej środowisku technologii jego uzyskiwania na skalę przemysłową z uwzględnieniem maksymalnego zagospodarowania biomasy ze zbiorów kapusty. Opracowanie i wdrożenie prostej, niskoenergetycznej i niemalże bezodpadowej, a także taniej technologii oczyszczania gleb dodatkowo przyczyni się do stymulacji rozwoju rolnictwa ekologicznego. Zastosowanie w praktyce wyników badań realizowanych w ramach projektu przyczyni się do rozwiązywania najbardziej aktualnych problemów społecznych, do których należy spadek zdrowotności społeczeństwa, związany z postępującym zanieczyszczeniem środowiska i pogorszeniem jakości żywności.

Podziękowania

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BIOFUMIGATION AS AN ALTERNATIVE METHOD OF CROP PROTECTION

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Abstract: Health risks related to common use of pesticides and artificial fertilizers raised the interest in alteranative methods of crop protection, among them biofumigation is becoming the most important. In this process natural compounds, mainly glucosinolates degradation products from *Brassica* species are used to combat pests and microorganisms attacking crops. Moreover, in the case of glucosinolate degradation products also beneficial influence on soil quality and yield efficiency can be expected. This article reviews the information on compounds used in biofumigation, their biocidal activity and describes a few trials of practical application of this method.

Keywords: biofumigation, alteranative methods of crop protection, glucosinolates, isothiocyanates, myrosinase

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EUROPEJSKI FUNDUSZ
ROZWOJU REGIONALNEGO

WYKORZYSTANIE KAPUSTY BIAŁEJ NA POTRZEBY FITOREMEDIACJI I BIOFUMIGACJI GLEBY (AGROBIOKAP)

Projekt nr UDA-POIG.01.03.01-00-138/09
współfinansowany przez Unię Europejską ze środków Europejskiego Funduszu Rozwoju Regionalnego w ramach Programu Operacyjnego Innowacyjna Gospodarka, 2007-2013

Oś priorytetowa 1.:	Badania i rozwój nowoczesnych technologii
Działanie 1.3.:	Wsparcie projektów B+R na rzecz przedsiębiorstw realizowanych przez jednostki naukowe
Poddziałanie 1.3.1.:	Projekty rozwojowe
Rekomendowana kwota dofinansowania:	3 391 950,00 PLN
Termin realizacji projektu:	01.07.2007-01.07.2013

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- produkcja naturalnego środka do biofumigacji gleb użytkowanych rolniczo i ogrodniczo

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Usilnie prosimy o stosowanie układu jednostek SI. Zwracamy uwagę, że osie wykresów oraz główki tabel powinny bezwzględnie zawierać jednostki stosownej wielkości. W przypadku artykułów pisanych po polsku podpisy tabel i rysunków powinny być podane w językach polskim i angielskim.

Polecamy symbolikę zalecaną przez PTChem (Symbole i terminologia wielkości i jednostek stosowanych w chemii fizycznej, Ossolineum, Wrocław 1989; Pure Appl. Chem., 1979, **51**, 1-41).

Materiał graficzny (rysunki, wykresy), obok wersji na papierze, powinien również być dostarczony w postaci cyfrowych plików wektorowych, np. za pomocą programów: CorelDraw wersja 9.0, Grafer dla Windows lub przynajmniej bitowe (TIF, JPG, PCX, BMP).

Przypisy i tabele, podobnie jak rysunki, zapisujemy jako osobne pliki.

Literaturę prosimy zamieszczać wg poniższych przykładów:

[1] Kowalski J. i Malinowski A.: Polish J. Chem., 1990, **40**(3), 2080-2085.
[2] Nowak S.: Chemia nieorganiczna. WNT, Warszawa 1990.

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