SOCIETY OF ECOLOGICAL CHEMISTRY AND ENGINEERING

ECOLOGICAL CHEMISTRY AND ENGINEERING A

CHEMIA I INŻYNIERIA EKOLOGICZNA A

Vol. 17 No. 10

OPOLE 2010

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

ISSN 1898–6188

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ECOLOGICAL CHEMISTRY AND ENGINEERING A

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Joanna DŁUŻNIEWSKA[1] and Maria NADOLNIK

OCCURRENCE AND HARMFULNESS OF FUNGAL DISEASES ON ROSE BUSHES CULTIVATED IN THE AREA OF KRAKOW PART 1. POWDERY MILDEW (*Sphaerotheca pannosa*)

WYSTĘPOWANIE I SZKODLIWOŚĆ CHORÓB GRZYBOWYCH NA KRZEWACH RÓŻ UPRAWIANYCH NA TERENIE KRAKOWA CZ. 1. PORAŻENIE PRZEZ MĄCZNIAKA PRAWDZIWEGO (*Sphaerotheca pannosa*)

Abstract: The aim of the present paper objective was determination of the occurrence of powdery mildew on rose beds situated in convent gardens and a park in Krakow. The studies were conducted in 2002–2004. The obtained results demonstrated that powdery mildew is a serious disease on multiflower rose beds. The pathogen attacks the plants every year and in subsequent years the highest infection indices may reach between 37.9 and 100 %. The disease proved the gravest hazard in Carmelite Sisters' convent garden and in St. Bernard's monastery garden, except the year 2002. Rose from the Cistercians' monastery garden were the least affected.

Keywords: roses, *Sphaerotheca pannosa*, atmospheric conditions, urban green

Due to their decorative qualities and resistance to some harmful compounds, roses are bushes most useful for plantings, particularly in urban areas. Rose beds are an element of city landscape and an essential element of the urban green. The bushes grow and blossom even in places with high traffic density. However, only healthy and properly tended plants have decorative values [1].

Powdery mildew caused by *Sphaerotheca pannosa* (Wallr. ex Fr) Lev. var. rosae Wor. is one of the most dangerous and commonly occurring diseases in roses [2, 3]. Disease symptoms are visible mainly on the youngest shoots and leaves. Usually at the beginning of June white powdery coating appears on diseased organs, graying on shoots and pedicles. Leaves stop their development, crease, yellow or acquire pinkish colour and their edges curl down. The shoot ends become thickened and twisted. The fungus

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may also infect the flowers and disease symptoms are visible on sepal calyx and petals. Considerable intensification of disease symptoms may lead to inhibition of flowering. Diseased plants grow poorly, their photosynthesis and transpiration are disturbed. Very strong infection may lead to premature defoliation and inhibition of plant development. Blossoms and whole bushes lose their decorative value. Intensification of the disease symptoms depends among others on the atmospheric conditions [3–6].

The objective of the work was determination of the occurrence of powdery mildew on rose beds situated in convent gardens in Krakow.

Material and methods

The research was carried out in Krakow in 2002–2004 on rose beds situated in four convent gardens: Carmelite Sisters (40, Lobzowska str.), Felician Sisters (6, Smolensk str.), St. Bernard's order (2, Bernardynska str.), Cistercians' order (11, Klasztorna str.) and in Polish Aviators' Park (John Paul II Av.). Only the first three gardens are located in the very centre of Krakow, whereas Cistercians' garden and Polish Aviators' Park are located in the eastern part of the city in a sparsely build-up area. The plant husbandry in individual analysed places comprised agrotechnical measures, ie digging over the rose beds and NPK fertilization of flowers. No chemicals were used for pest or disease control during the vegetation season. Shoots were cut in autumn and the bushes were covered with earth to protect them against frost during the wintertime.

In the subsequent years observations were conducted from May until October. On each date 20 multiflower rose bushes were analyzed in three replication. Assessed was powdery mildew infection in shoots, leaves, flower buds and blossoms on a four degree scale [1]. The infection index was computed from the obtained data [7].

The results were verified statistically using analysis of variance for two-factor experiments (factor A – observation date, factor B – research point). The significance was verified using the Duncan test at significance level $p = 0.05$.

Meteorological data was obtained from State Meteorological Service Bulletins [8–10]. The hydrothermic coefficient was also computed and on its basis humidity was determined [11].

The data on air pollution were provided by the Department of Environment Monitoring and Automatic Air Analyses Laboratory in Krakow and presented in the paper by Dluzniewska and Nadolnik [12].

Results

The weather conditions during the research period were presented in Fig. 1 and Table 1. The lowest average temperature for the May to August period was registered in 2004. Considerable differences were noted also in the precipitation amount in the subsequent years of the research. In 2002 the greatest amount of precipitation was observed in July, when also excessive humidity was noted (Table 1). Too high a precipitation amount was also registered in October of the same year. However, average relative air humidity was the lowest in this season. The year 2003 was

Table 1

Meteorological data for the period of research in 2002–2004 [8–10]

Month	Hydrothermic coefficient			Content of humidity			Number of days with precipitation			Average relative air humidity [%]			Insolation [hours total]		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
April	1.5	1.5	0.9	optimal	optimal	deficient	11	16	12	53	—	67	153	171	188
May	1.3	2.5	1.1	optimal	excessive	optimal	11	11	17	49	70	70	224	242	194
June	1.8	0.6	1.1	optimal	deficient	optimal	12	11	16	59	66	69	206	281	180
July	2.2	2.1	1.8	excessive	excessive	optimal	10	16	22	57	76	74	226	186	201
August	1.5	0.4	1.4	optimal	deficient	optimal	8	8	16	58	66	76	212	281	229
Sept.	1.9	0.7	0.9	optimal	deficient	deficient	9	8	8	62	72	76	128	184	188
Oct.	3.5	2.6	1.3	excessive	excessive	optimal	21	23	10	71	84	82	70	85	117
Total							82	93	101	—	—	—	1219	1430	1297

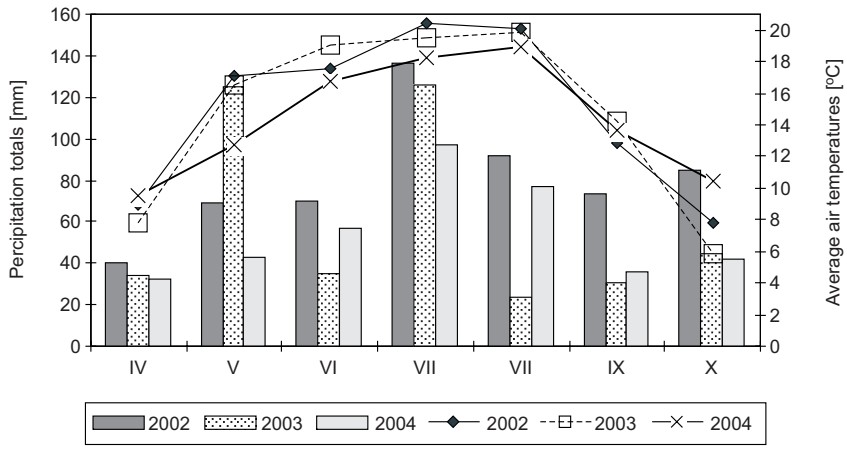

Fig. 1. Precipitation totals [mm] and average air temperatures [°C] in Krakow during the period of reserch [8–10]

characterized by the most unfavourable distribution of precipitation. Excessive humidity occurred in May and July. On the other hand, in April, June, August and September there was too little humidity and the number of sunshine hours was the highest in this season. In the third year of the research the relationships between the precipitation amount and the temperature were positive and the humidity amount was optimal during the whole vegetation season, except April and September. The greatest number of rainy days were noted in the same year.

In 2002 powdery mildew appeared by the end of May on rose bushes in the Carmelite Sisters' garden and in Polish Aviators' Park (Table 2).

Table 2

Dynamics of rose infection by *Sphaerotheca pannosa* in 2002

Date of observation 2002	Infection index [%] in respective observation points				
	Carmelite Sisters	Felicjan Sisters	St. Bernard's monastery	Cistercian monastery	Polish Aviators' Park
31.05	20.0 b-j	0.0 a	0.0 a	0.0 a	8.7 ad
14.06	25.3 d-m	0.0 a	0.0 a	3.3 ab	13.7 a-f
30.06	32.0 g-o	3.3 ab	0.0 a	4.3 ab	16.7 a-h
15.07	35.7 i-p	11.7 a-e	10.7 a-d	4.3 ab	23.0 c-l
30.07	41.3 m-p	17.3 a-h	10.7 a-d	6.3 a-c	29.0 e-n
14.08	48.0 o-q	24.7 d-m	15.0 a-g	12.0 a-e	32.7 g-p
28.08	61.0 q-r	30.3 f-o	18.7 b-i	19.3 b-j	41.0 l-p
12.09	71.0 r	40.0 k-p	22.7 c-k	24.0 c-m	45.7 n-k
27.09	76.7 r	45.3 n-q	25.0 d-m	34.7 f-o	46.7 n-q
11.10	100.0 s	50.3 p-q	33.3 n-p	37.3 j-p	46.7 n-q
26.10	100.0 s	67.7 r	50.0 p-q	43.3 n-p	50.0 p-q

Latest of all the pathogen attacked the plants in St. Bernard's monastery garden. The disease symptoms were spotted there in mid-July. On all invetsigated sites the disease was exacerbating significantly on the subsequent dates of observation and reached the highest level by the end of October. Roses in the Carmelite Sisters' garden were significantly the most diseased and there the infection index reached 100 %. On the other hand the notably lowest infection was observed in the gardens of St. Bernard's and Cistercian monastery.

In 2003 the disease appeared on rose bushes very late (Table 3). Only in mid-September were the diseased plants spotted in the gardens of the Carmelite and Felician Sisters' convets and in St. Bernard's monastery garden. The rose infection index was significantly lower than in 2002 and did not exceed 38 %. The disease most significantly affected the plants in St. Bernard's monastery garden, whereas the smallest disease symptoms were observed on roses growing in Cistercians' monastery garden.

Table 3

Dynamics of rose infection by *Sphaerotheca pannosa* in 2003

Date of observation 2003	Infection index [%] in respective observation points				
	Carmelite Sisters	Felicjan Sisters	St. Bernard's monastery	Cistercian monastery	Polish Aviators' Park
15.09	17.5 d	25.8 f	35.4 h	0.0 a	0.0 a
30.09	21.6 e	30.4 g	37.9 i	7.2 b	10.0 c

In 2004 vegetation period small numbers of bushes invaded by powdery mildew were noticed in mid-June (Table 4). In the next months the infection index was increasing slightly. Only in the middle of September did the pathogen invad more strongly the plants in the convent gardens of Carmelite and Felician Sisters' convents. In all convent gardens the most serious infection was registered on bushes in the middle of October. Roses from the Cistercian monastery garden and Polish Aviators Park revealed significantly the worst healthiness.

Table 4

Dynamics of rose infection by *Sphaerotheca pannosa* in 2004

Date of observation 2004	Infection index [%] in respective observation points				
	Carmelite Sisters	Felicjan Sisters	St. Bernard's monastery	Cistercian monastery	Polish Aviators' Park
11.06	1.6 a-c	0.0 a	3.3 a-c	0.0 a	0.0 a
27.06	0.9 ab	0.0 a	9.2 a-d	1.6 a-c	1.6 a-c
14.07	0.0 a	1.5 a-c	10.1 a-d	0.0 a	0.9 ab
4.08	0.0 a	5.9 a-c	13.3 b-d	0.0 a	0.9 ab
28.08	1.5 a-c	4.6 a-c	14.5 b-d	0.0 a	1.2 ab
18.09	20.9 de	5.9 a-c	19.2 de	0.0 a	5.9 a-c
1.10	28.8 ef	10.2 a-d	36.8 f	1.2 ab	1.2 ab
15.10	37.5 f	14.2 cd	58.3 g	1.6 a-c	0.9 ab
28.10	29.2 ef	10.9 a-d	50.0 g	0.0 a	0.0 a

Discussion

The reserch conducted demonstrated that powdery mildew is a serious disease of multiflower rose bed field plantations. The pathogen attacks plants every year and the highest infection indices in subsequent years may reach even between 37.9 and 100 %. The disease posed the gravest hazard in convent gardens of the Carmelite Sisters and except the year 2002 also in St. Bernard's monastery garden. Roses which were the least infected by this fungus were growing in the Cistercians monastery garden.

Depending on the weather course the first symptoms of powdery mildew in field conditions appear usually in mid-May and intensify during the vegetation season [3, 4]. In field cultivation the greatest intensification of the disease symptoms is observed in two periods: by the end of May and at the beginning of June at intensive bush growth, and in the second decade of August. In the second period roses, from which the blooms were not cut, start new shoots [13]. Particularly high level of bush infection by the end of summer and at the beginning of autumn is due to the susceptibility of young shoots and leaves to infection. Developing leaves up till the fifth day very easily give in to infection. 5-day-old and older leaves are less susceptible. It is considered that this fact is connected with the thickness of the cuticules of cell walls which is increasing with leaf age [14]. Moreover, young leaves of sensitive species contain β-alanine, necessary for fungi spore germination [3].

Also the weather conditions, dew at night and sunny weather during the day, favour the infection. A factor favouring *S. pannosa* development are great fluctuations of temperature. In field cultivation the conditions particularly favourable for the fungus development are at night, when the temperature is 15 ^{o}C and air humidity reaches 90–99 %. Fungus growth is favoured on strongly insolated walls of buildings with the southern aspect, where differences between day and night temperatures are very big [4]. The urban climate is characterized by a raised temperature and lowered humidity. A decrease in humidity results from a lack of a retention surface, drainage of the ground by excavations and channels. Excessive transpiration and evaporation are caused by the high temperature of heating buildings, and surfaces of pavements, streets or squares [15].

Powdery mildew mycelium develops best in quite dry air at high temperatures and in strong light [4]. Rose powdery mildew spreads during the vegetation season by means of conidial spores. The spore formation is enhanced by a decrease in air humidity and increase in temperature. At very high air humidity the temperature of 21 ^{o}C is optimal for spore germination and between 18 and 25^{o}C for mycelium growth. Spores germinate best at air humidity 97–99 %, whereas water on the leaf surface inhibits this process [3, 16, 17].

A great diversification among rose varieties considering their resistance to powdery mildew has also been pointed out [2, 5]. About 30 % of varieties with red and pink flowers were resistant to the disease whereas the others were medium resistant or highly susceptible. On the other hand among varieties with white or yellow blossoms between 60 and 75 % were prone or very prone to the disease. The variety susceptibility was connected with the size of the stomatal apparatus and the thickness of skin [18].

Pollutants from the atmosphere may also affect plant infection by pathogens [19]. Krakow belongs to the group of European cities with the worst air quality [20]. SO_2 concentrations were similar in all measuring points over the entire period of observations, whereas the suspended particulate matter, NO and NO_2 concentrations were higher in the Cistercians' monastery garden and in Polish Aviators' Park. The admissible level of airborne matter was exceeded only for the suspended particulate matter [12].

In the paper by Nadolnik [1] it was observed that the occurrence of rose powdery mildew was the most intensified on a lawn situated in a place with less heavy traffic. It shows that fungi causing powdery mildew may completely vanish at high concentrations of individual components of pollution [21].

The occurrence and development of fungi and symptoms of plant infection by parasitic fungi may increase or be inhibited under the influence of SO_2 fumigation. The species which benefit from SO_2 fumigation comprise *Sphaerotheca fuliginea* causing powdery mildew in cucumbers. This fungus revealed a greater capability of infecting the host plant and conidia spore germination at cucumber exposure to the activity of 143 μg SO_2 m^{-3}. Higher concentrations of SO_2 inhibited the fungus growth [19, 22].

Air pollution may also modify the plant-pathogen-endophyte relationship. *Hyalodendron album* endophyte present on lilac leaves act antagonistically on *Microspharea alni*. It was observed that the endophyte is most probably more sensitive to O_3 and SO_2 than the pathogen [19, 23].

The analysed dynamics of powdery mildew development makes possible the application of protection measures in rose gardens, which will reveal long-term activity and low toxicity. Cultivation measures which have just been completed make it possible to maintain the plants in good condition, which improves the flowers' decorative qualities.

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WYSTĘPOWANIE I SZKODLIWOŚĆ CHORÓB GRZYBOWYCH NA KRZEWACH RÓŻ UPRAWIANYCH NA TERENIE KRAKOWA CZ. 1. PORAŻENIE PRZEZ MĄCZNIAKA PRAWDZIWEGO (*Sphaerotheca pannosa*)

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Abstrakt: Celem pracy było określenie występowania mączniaka prawdziwego na skwerach różanych położonych w ogrodach przyklasztornych i parku Krakowa. Badania prowadzono w latach 2002–2004. Uzyskane wyniki potwierdziły, że mączniak prawdziwy jest groźną chorobą w uprawie polowej wielokwiatowych róż rabatowych. Patogen atakuje rośliny corocznie, a najwyższe indeksy porażenia w kolejnych latach mogą sięgać wartości 37,9–100 %. Choroba największe zagrożenie wywoływała w ogrodach karmelitanek i z wyjątkiem roku 2002 u bernardynów. Natomiast najmniej porażane przez grzyba były róże z ogrodu cystersów.

Słowa kluczowe: róże, *Sphaerotheca pannosa,* warunki atmosferyczne, zieleń miejska

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Michał GĄSIOREK[1] and Bernadetta ŁABUZ[2]

CONTENT OF HEAVY METALS IN SOIL TOP LAYERS FROM DISTRICT PLAYGROUNDS OF SOUTHERN AREAS OF KRAKOW

METALE CIĘŻKIE W WIERZCHNIEJ WARSTWIE GLEB OSIEDLOWYCH PLACÓW ZABAW POŁUDNIOWYCH REJONÓW KRAKOWA

Abstract: Excessive amounts of heavy metals can occur in the natural environment especially in urban areas. In big cities district playgrounds are very often the only places for rest and recreation. Children playing on playgrounds can be exposed to the negative impact of the chemical soil contamination. The object of the work was the evaluation of the level of pollution with heavy metals: Cd, Pb, Cu, Zn, Cr and Ni in top soil layers of playgrounds situated in southern Krakow districts and the estimation of the potential risk resulting from the excessive amounts of these elements. The analyzed playground soils were characterized in the straight majority of cases by natural contents of cadmium, nickel, chromium, lead and copper. The only heavy metal whose content was in general higher than natural was zinc.

Keywords: urban soils, playgrounds, heavy metals, Krakow

In big cities district playgrounds are very often the only places for rest and recreation. They play an important role as they influence the physical and emotional development of children [1]. Excessive accumulation of harmful substances in the environment results in the progress of diseases associated with civilization, thus in bad conditions for physical and mental development [2]. The studies carried out in urban [3–7] and industrial areas or located in the vicinity of roads [8] show that soils of these terrains are often characterized by an elevated level of heavy metals. The metals belong to the group of mineral pollutants of the environment the most dangerous for people, animals and plants. Therefore children, regular customers of playgrounds, can be particularly exposed to the negative impact of the chemical soil transformation. Up to now studies

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concerning heavy metal contents in playground soils have been carried out, among others, in Uppsala [9] and Madrid [10].

The present study aimed at the evaluation of the contamination with Cd, Pb, Cu, Zn, Cr and Ni of the top layers of soils in district playgrounds situated in southern parts of Krakow and the estimation of the potential risk, resulting from the excessive amounts of these elements, for children staying there.

Material and methods

Soil material for laboratory analyses was sampled from 9 playgrounds located in southern parts of Krakow (Fig. 1). From the selected playgrounds, depending on their area one or two soil samples were taken, in each case from the layer 0–1 cm (surface layer) and 0–20 cm (deeper layer). Such proceedings aimed at the determination of the impact of the depth from which the sample was taken on the content of heavy metals. It was guided also by the fact that children staying on playgrounds have a direct contact with the surface soil layer. The deeper soil layer was treated as a kind of a background for the surface layer.

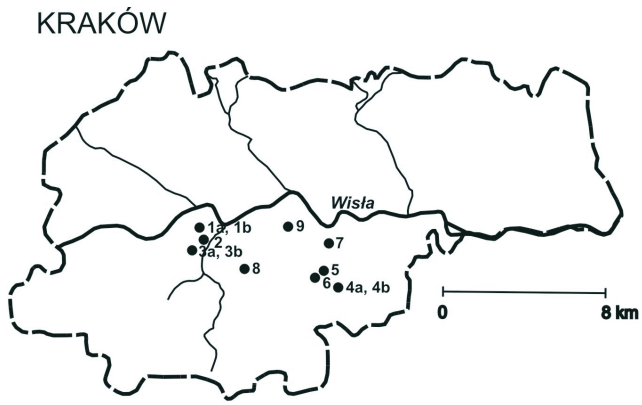

Fig. 1. Location of playgrounds in southern parts of Krakow: **1a, 1b – Debniki**; between Praska St. and Pietrasinskiego St., **2 – Debniki**; between Slomiana St. and Szwedzka St., **3a, 3b – Debniki**; between Slomiana and Szwedzka St., **4a, 4b – Prokocim**; J. Kurczaba St., **5 – Prokocim**; P. Sciegiennego St., **6 – Prokocim**; P. Sciegiennego St., **7 – Prokocim**; Klodzka St., **8 – Osiedle na Kozlowce**; Spoldzielcow St., **9 – Plaszow**; Krzywda St.

In the sampled soil material, after its drying and sieving through a plastic sieve with 1 mm mesh, the following analyses were performed: pH in 1 mol · dm^{-3} KCl [11], soil texture [12], organic carbon [13] and a level of total forms of: cadmium, lead, copper, zinc and chromium by the AAS method and nickel by the atomic emission spectrometry with inductively coupled plasma (ICP-AES), after previous digestion in the mixture of nitric(V) and chloric(VII) concentrated acids [14].

The results were subjected to statistical analysis. Simple correlation coefficients (r) between contents of heavy metals and selected soil properties were calculated.

A significance of correlation coefficients was estimated with the use of the t-Student's test. A significance of differences between mean contents of studied heavy metals in the layer 0–1 cm and 0–20 cm was evaluated by Tukey's test for the reasonable significant difference (RIR) at the significance level 0.05. The calculations were made using the STATISTICA program version 8.

Results and discussion

Heavy metal bounding in soil results mainly from its clay content, humus and soil reaction [15, 16]. The studied soils had sand texture in 3/4 cases, both in samples taken from the layer 0–1 cm and from the layer 0–20 cm. The remaining soils were light loams. Usually in soils from the deeper layer there were more fractions with ∅ < 0.002 mm (Table 1).

Table 1

Soil reaction and contents of fraction ∅ < 0.002 mm, organic carbon and heavy metals

Point No.	Layer [cm]	pH_{KCl}	Fraction ∅< 0.002 mm [%]	$C_{org.}$ [g · kg^{-1}]	Cd	Pb	Cu	Zn	Cr	Ni
					mg · kg^{-1}					
1a	0–1	6.6	0	35.4	0.06	45.7	23.2	171.3	17.9	9.1
	0–20	7.0	1	33.8	0.40	60.8	39.0	245.2	20.3	12.4
1b	0–1	6.5	1	18.1	0.00	10.9	6.0	71.5	10.1	2.9
	0–20	6.9	2	10.3	0.00	25.9	12.0	113.9	15.5	5.8
2	0–1	7.2	3	14.3	0.25	24.5	12.3	124.8	19.7	8.6
	0–20	7.3	5	10.9	0.14	26.8	11.5	111.4	14.2	9.8
3a	0–1	7.2	8	17.3	0.10	36.8	18.3	129.2	32.3	16.3
	0–20	7.0	8	19.4	0.19	38.5	20.5	135.6	27.3	14.6
3b	0–1	6.8	5	33.3	0.13	41.6	19.3	156.4	31.9	15.3
	0–20	7.1	6	22.3	0.11	60.4	26.8	181.2	28.4	17.1
4a	0–1	7.2	4	8.7	0.00	9.7	5.2	56.8	16.7	6.0
	0–20	6.9	4	5.4	0.00	10.2	6.5	55.9	14.2	7.1
4b	0–1	5.7	4	6.9	0.00	12.4	5.2	70.8	14.3	5.0
	0–20	6.7	2	5.9	0.07	17.4	5.2	69.6	13.3	5.8
5	0–1	6.4	1	10.2	0.00	9.6	4.0	71.3	7.7	3.0
	0–20	6.5	3	4.8	0.01	12.0	5.8	75.6	8.8	5.6
6	0–1	7.1	3	5.9	0.00	16.6	4.2	101.3	9.7	3.9
	0–20	7.1	3	8.0	0.00	10.6	5.9	80.0	11.5	5.5
7	0–1	7.4	4	29.3	0.01	36.2	22.8	126.8	31.1	9.4
	0–20	6.8	5	19.6	0.05	43.6	25.7	132.7	28.8	10.0
8	0–1	6.6	10	7.3	0.00	13.2	7.2	98.5	21.1	9.2
	0–20	7.3	9	5.4	0.00	15.2	7.8	94.7	20.1	9.7
9	0–1	6.8	1	9.7	0.00	5.8	2.4	33.5	10.1	2.6
	0–20	7.5	3	4.4	0.00	6.3	3.3	32.8	12.7	4.8

The majority of the studied soils, regardless of the depth from which they were taken, was characterized by a neutral reaction, even though there occurred also alkaline or slightly acid soils, and even acid in one case (a playground in Prokocim, point 4b, layer 0–1 cm). In the studied soils the organic carbon content ranged in large limits from 4.4 to 35.4 $g \cdot kg^{-1}$, and the depth from which the soil was sampled influenced its content and in consequence the content of humus. The highest levels of organic carbon were determined usually in the surface layer (0–1 cm). The exceptions were constituted by soils from research points 3a and 6, where in the layer 0–20 cm a slightly higher level of organic carbon was ascertained. Playground soils located in the western part of the studied area (Fig. 1) were characterized by a distinctly higher organic carbon content than those in the eastern part, except for the soil taken from the playground in Prokocim in Klodzka Street (point 7).

Anthropopressure and natural processes going on in the nature did not contribute, apart from a few exceptions, to the pollution of the analyzed soils with cadmium, lead, copper, chromium and nickel. Their content is presented in Table 1. On the basis of the division serving for the evaluation of the level of the soil pollution with heavy metals, proposed by IUNG [17], it can be ascertained that in almost all playground soils regardless of the sampling depth a natural content of Cd, Pb, Cu and Ni occurred. The chromium content in the studied soils is comparable with that determined in unpolluted soils with this element [16]. Also, according to the Directive of the Environment Ministry regarding soil quality standards and ground quality standards, issued on 9 September 2002 [18], amounts of heavy metals determined and named in this paper did not exceed the acceptable values of the concentration of these elements. It was only the soil taken from point 1a (playground between Praska Street and Pietrasinskiego Street) which was characterized by small enrichment in heavy metals. In a deeper layer of this soil elevated Cd and Ni contents were determined when in both layers elevated Pb contents with elevated Cu content in the surface layer and slight pollution with this element in the layer 0–20 cm were noticed. Among other soils, only in point 7 (in Prokocim) in the layer 0–20 cm, elevated content of copper occurred [17].

In the case of Zn whose content ranged from 32.8 to 245.2 (105.9 on average) $mg \cdot kg^{-1}$ (Table 1), the largest contribution among the studied soils had those with its elevated content. Soils sampled from both layers in points 4a, 8 and 9 as well as from the layer 0–20 cm in point 4b revealed a natural content of zinc [17]. The highest content of Zn (as it was in the case of Pb and Cu) occurred in both soil layers from the playground in Debniki in point 1a. According to IUNG criteria [17] it was, comparably to the soil in point 1b from the layer 0–20 cm and in point 6 from the layer 0–1 cm, slightly polluted with this element (II degree of pollution). However due to the Directive of the Environment Ministry from 9 September 2002 [18] in none of the studied soils was the acceptable level of zinc concentration exceeded.

Among analyzed soil properties, organic carbon content had the strongest impact on heavy metal bounding in studied soils, which confirms the calculated correlation coefficients (Table 2). In playground soils in Uppsala [9] the most important soil property influencing the amount of heavy metals was clay content, which in the case of Cr and Ni was also noticed in the present study (Table 2).

Table 2

Simple correlation coefficients (r) determining relations between total contents of Cd, Pb, Cu, Zn, Cr, Ni and selected physicochemical properties of studied soils

Soil properties	Heavy metals					
	Cd	Pb	Cu	Cr	Zn	Ni
pH_{KCl}	0.219	0.181	0.194	0.293	0.131	0.300
$C_{org.}$	0.553**	0.830***	0.851***	0.622**	0.813***	0.590**
Fraction $\varnothing < 0.002$	–0.011	0.109	0.080	0.575**	0.085	0.583**

** $p \leq 0.01$, *** $p \leq 0.001$.

Soils sampled from the deeper layer were usually characterized by a higher content of heavy metals than those taken from the surface layer, even though statistical analysis performed using the reasonable significant difference (RIR) of Tukey did not reveal any significant differences among the mean contents of studied heavy metals in layers 0–1 cm and 0–20 cm (Table 3).

Table 3

Differences between mean contents of heavy metals in layers 0–1 i 0–20 cm in studied playground soils from southern parts of Krakow

Heavy metals	Layer 0–1 cm	Layer 0–20 cm
Pb	21.92[a]	27.31[a]
Cd	0.045[a]	0.081[a]
Cu	10.84[a]	14.17[a]
Zn	101.02[a]	110.72[a]
Ni	7.60[a]	9.02[a]
Cr	18.55[a]	17.93[a]

Differences between means marked in superscripts by the same letter are statistically insignificant.

Alloway and Ayres [15] as well as Kabata-Pendias and Pendias [16] attributed the increase of the heavy metal content in soils mainly to the industrial activity and motorization. However, it seems that in the case of playground soils of southern parts of Krakow they have a minimal significance. Heavy metal contents determined in the studied soils are generally much lower than in soils in Krakow with a different way of use: allotments [3], convent gardens [6], or a city park [7]. The only heavy metal that occurred in higher amounts was zinc. Metal devices, especially covered by an anticorrosive layer could be one of the reasons for its considerable accumulation in the analyzed soils. As a result of corrosion and stripping zinc together with other heavy metals can find its way to soils. Nevertheless its content is lower than maximal found in city soils in Krakow which, as Pasieczna reports [19], can amount to 612.0 $mg \cdot kg^{-1}$ in the layer 0–20 cm. In the studied soils the determined contents of heavy metals are, except for zinc, at the similar level to those determined in the surface layer of playground soils in Madrid [10].

Small enrichment in heavy metals which occurred in some studied soils could have different sources that are difficult to establish. Regarding heavy metal contents, playground soils in southern parts of Krakow should not pose any serious danger to children playing on them but other risks must not be forgotten, among others those related with the technical condition of outdoor game devices.

Conclusions

1. The straight majority of playground soils in southern parts of Krakow was not polluted with cadmium, nickel, lead, copper and chromium.

2. Zinc was the only heavy metal whose content was generally higher than natural. The majority of soils revealed its elevated content.

3. The studied soils should not pose any danger regarding their contamination with heavy metals for children playing on playgrounds.

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METALE CIĘŻKIE W WIERZCHNIEJ WARSTWIE GLEB OSIEDLOWYCH PLACÓW ZABAW POŁUDNIOWYCH REJONÓW KRAKOWA

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Abstrakt: Nadmierne ilości metali ciężkich mogą występować w środowisku przyrodniczym zwłaszcza w obszarach miejskich. W wielkich miastach, bardzo często jedynym miejscem wypoczynku i rekreacji są osiedlowe place zabaw. Dzieci bawiące się na tych placach mogą być narażone na negatywne oddziaływania chemicznego skażenia gleb. Celem pracy była ocena stopnia zanieczyszczenia metalami ciężkimi: Cd, Pb, Cu, Zn, Cr i Ni wierzchnich warstw gleb placów zabaw położonych w południowych dzielnicach Krakowa oraz oszacowanie ewentualnego zagrożenia wynikającego z nadmiernej zawartości tych pierwiastków. Analizowane gleby placów zabaw charakteryzowały się, w zdecydowanej większości przypadków, naturalną zawartością kadmu, niklu, chromu, ołowiu i miedzi. Jedynym metalem ciężkim, którego zawartość była na ogół większa od naturalnej, był cynk.

Słowa kluczowe: gleby miejskie, place zabaw, metale ciężkie, Kraków

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 10 2010

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INFLUENCE OF CEMENT DUST ON SELECTED PROPERTIES OF SOILS AND THE MORPHOLOGY OF PINE (*Pinus sylvestris* L.) NEEDLES FROM THE FOREST STANDS IN THE SURROUNDINGS OF "LAFARGE" – CEMENT PLANT IN BIELAWY

WPŁYW PYŁÓW CEMENTOWYCH NA WYBRANE WŁAŚCIWOŚCI GLEB ORAZ BUDOWĘ MORFOLOGICZNĄ IGIEŁ SOSNY POSPOLITEJ (*Pinus sylvestris* L.) Z FITOCENOZ OTACZAJĄCYCH ZAKŁADY CEMENTOWO-WAPIENNICZE „LAFARGE" W BIELAWACH

Abstract: In the present research the impact of cement dust emitted by cement plant Lafarge S.A. – in Bielawy on soil and forest stands were investigated. Selected physicochemical properties of soils and the morphology of Scots pine needles were studied. The soils in the vicinity of the cement plant have elevated pH and contained $CaCO_3$ in the surface horizons due to alkaline dust accumulation. Shoots and needles of Scots pine were covered with thin layer of cemented dust. Pine needles from trees near the dust emitter were shorter and more narrow than needles from the trees beond the dust impact. Thus, pine trees in the vicinity of cement dust emitter have lower assimilation surfaces that might cause the lowering of photosynthesis process.

Keywords: cement dust, soil, Scots pine (*Pinus sylvestris* L.)

Numerous studies describe the damage done by acidic air pollution to forest areas in industrial regions [1–4]. The impact of alkaline air pollution on forests stands is much less recognized.

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The cement production process is accompanied with the emissions of considerably amount of dust. Thus, cement industry is a source of environmental pollution with alkaline dusts. Cement dusts influence the ecosystems including soil and plants, cause the imbalances in soil nutrients and reduce biodiversity. In some varieties of Portland cement (and in cement dust) traces of toxic metals such as chromium and lead are common. Elevated metal contents might also effect the plant cover and soil in the area being under the cement dust impact [5].

In the last decades, cement production has been rising in Poland and the problem is increasing. The objective of the study was to assess the impact of emitted cement dusts on selected soil properties and the condition of pine forest stands, particularly the morphology of pine needles, in the vicinity of cement plant Lafarge S.A. in Bielawy.

Materials and methods

The study area is situated at the central part of Poland, the relief is flat and the age of Scots pine stand is 20 years.

For the study soil samples and pine needles from pine forest stands nearly Lafarge Cement Plant in Bielawy have been sampled. Scots pine needles are used for monitoring environmental pollution [6, 7]. Soil from two soil profiles: typical podzolic soil (profile P1) located on the edge of pine forest and arable lessive soil (Profile P2) located near the forest in the direct vicinity of cement plant, were investigated. Soil samples from each soil horizon was analysed. The texture of the soils was determined using Boyoucose–Cassagrandč method with Proszynski modification and soil pH was measured in H_2O and 1 M KCl on pH-meter. Content of organic carbon was determined according to Tiurin method, basic exchangeable cations and cation exchange capacity – acc. to Kappen's method and calcium carbonate concentration – using Scheibler's method.

Plant material: pine needles of different age was sampled from *Pinus sylvestris* L.

Morphological features of plant material (after the segregation of needles in the laboratory on 3 group: one year old, two years and three years old) were characterized on the base of biometric data such as length and width of the needles. The surface area of needles was calculated assuming that each individual needle is cylinder shaped. Biometric data were collected and interpreted using computer programme DIGISHAPE (Cortex Nova 2005).

All analysis were made in triplicate, arithmetic mean values are presented in the paper.

Results and discussion

The results of the study on soil and plant material are presented in Table 1, 2 and 3, respectively. The most prominent effect of dust emissions in the investigated area is the elevated soil pH, which was in the range 5.99–7.26, with the highest values at the surface horizons: 7.22 and 7.26. Alkaline effect is not only restricted to the surface soil. Deeper samples showed elevated pH values, too – Table 2.

Table 1

Soil texture

Horizon	Depth [cm]	The percentage of the particle fractions ∅ [mm]					
		2–0.1	0.1–0.05	0.05–0.02	0.02–0.005	0.005–0.002	< 0.002
Site P1							
Ap	0–35	78	11	3	2	1	5
Ees	35–50	80	8	3	2	1	6
Bhfe	50–120	90	6	3	1	—	—
C	< 120	78	6	2	2	1	11
Site P2							
Ap	0–38	73	11	6	3	1	6
Eet	38–93	89	4	1	1	—	5
Bt	93–120	72	9	2	3	2	12
C	< 120	53	15	7	7	2	16

Normal pH in soils of the region classified as podzolic soils or luvisols is 4.5–5.0. High soil pH values indicate evident anthropogenic influence of cement dust emission rich in $CaCO_3$. Previous study [8] showed that parent material as well as soil from the upper horizons does not contain calcium carbonate. Cement dust contains high level of calcium carbonate and calcium oxide; other oxides like potassium silica and aluminium are also present.

Calcium oxide is very reactive and with water forms calcium hydroxide – alkaline compound responsible for the damage of the plant tissue. Calcium is described as the element tracer of the pollution due to the cement industry [9]. The pH of the cement dust is about 12.0. The average monthly emission of dust from the Lafarge plant is 13.2 g/m^2.

Naturally acid soils sorrounding the cement plant in Bielawy are rich in base exchangeable cations. The ratio of alkaline cations to the total cation exchange capacity ranged from 83 % to 99 % with the maximum at the surface horizons – Table 2.

Excess of base cations in naturally acid soils are considered to originate from the cement dust. The impact of cement dust accumulation was higher in site P2 located closer to the emmiter – Table 2. Similar observation have been made in area polluted by cement emissions in Niepolomnice [10]. In studied soils near Bielawy the consequence of alkalization might be significant due to sandy texture of the soils – Table 1. In soils enriched in alkaline cement dust the effect similar to overliming was reported [11, 12]. It is well documented that soil pH raising leads to decrease of microelements phyto-availability. Kreutzer [13] reported boron deficiency and damage of the mycorrhizae in pine forest.

Elevated pH observed in soils in the vicinity of Lafarge plant might lower the amount of other needed nutrients even considered to be tolerant to low soil nutrients like Scots pine [14].

Table 2

Physico-chemical properties of soils

Horizon	Depth [cm]	pH		Hh [mmol(+) · kg^{-1}]	$CaCO_3$ [%]	C-org. [g · kg^{-1}]	S [mmol(+) · kg^{-1}]	T [mmol(+) · kg^{-1}]	V [%]
		H_2O	KCl						
Site P1									
Ap	0–35	7.26	6.44	3.40	< 1	6.6	49.4	49.7	99
Ees	35–50	6.21	6.39	2.20	< 1	—	11.5	13.7	83
Bhfe	50–120	6.23	6.49	3.00	< 1	—	74.5	77.9	95
C	< 120	6.21	6.29	4.10	< 1	—	67.5	71.6	94
Site P2									
Ap	0–38	7.22	6.40	1.90	5.9	4.5	113.6	117.0	97
Eet	38–93	6.02	6.51	1.50	5.0	—	25.5	27.0	94
Bt	93–120	5.99	5.89	2.60	< 1	—	78.0	80.6	96
C	< 120	6.12	5.90	6.70	< 1	—	108.0	114.7	94

Like soil, pine trees are affected by cement dust. Hard incrustation of cement was observed on entired exposed surface of pine trees including bark, branches and needles. The main visible pollution generated by the cement industry on the vegetation corresponds to the dusts. Visual estimation showed that shoots and leaves (needles) of trees from the vicinity of cement plant are covered by a white thin layer of cement. Adhesive properties of alkaline dust favour formation a sort of incrustation on the leaf surface. Detailed study showed that. Young needles were longer and have larger surface area (Table 3, Fig 1). Thus, the negative effects of emission were more pronounced in older 2–3 years old needles. Site P2 was located closer to the source of dust emission compared with the site P2, and the reduction of length and width of pine needles from site P2 was larger than from P1, less affected site – Table 3. Other authors [15] reported very low amounts of available manganese and available phosphorus in soils affected by cement dust.The plant reactions differ in their rates of development and weaking the plant communities. Pine forest vegetation in the studied area is adapted to very acid soil, soil alkalization must negatively impact the whole ecosystem.

Table 3

Morphology of the pine needles

Age of the needles	Surface area [mm^2]	Width [mm]	Length [mm]
Site P1			
I*	256.13	1.43	82.18
II	250.32	1.45	80.10
III	208.96	1.48	74.04
Site P2			
I	169.39	1.20	63.35
II	171.59	1.58	51.98
III	158.17	1.34	59.13

* I, II, III, one year, two years and three years old, respectively.

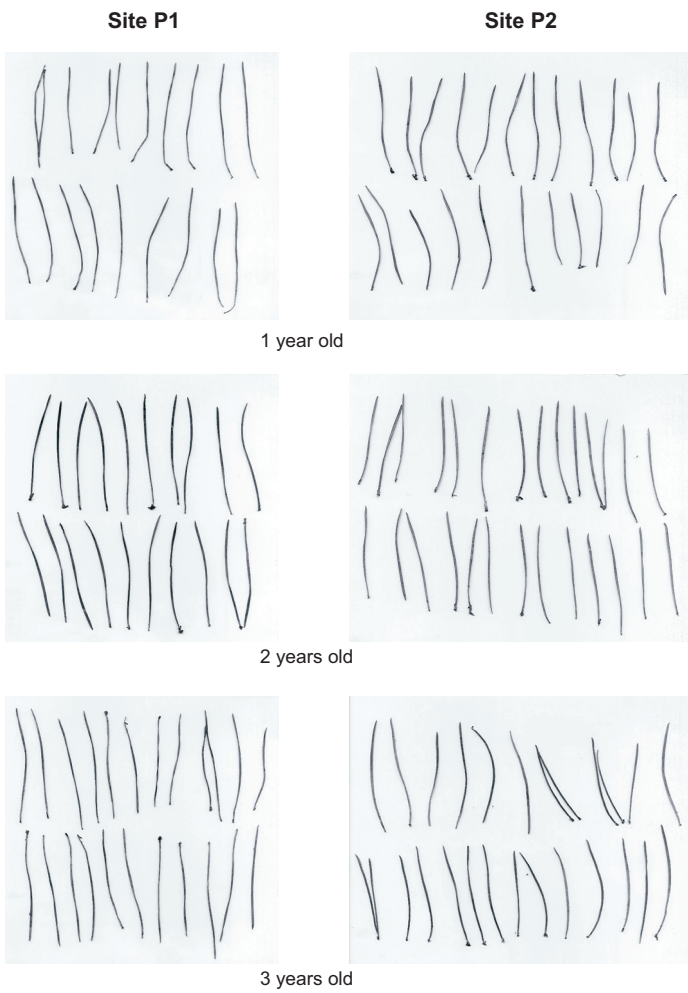

Fig. 1. Pine needles from investigated area

Brandt and Rhoades [16] report significant reduction in lateral growth of deciduous species: *Acer rubrum* and *Quercus rubra* in the cement dust-affected area as compared with control site. Foliar chlorosis, leaf scorch and general decline in growth and vigor was also observed.

The coniferous ecosystems are particularly sensitive to air contamination. Other authors observed a decrease in moss species and changes in the life cycle of mosses near the cement plant [17].

The cement crust on the needle surface limited also light conditions. Under the dust cover light shortage was detected and as a consequence disorders in physiological processes connected with assimilation was found. Increasing chlorosis and necrosis of needles and decreasing needle sizes was also reported. Changes were also observed in

the reproductive phase of Scots pine. The average number of cones on a single tree decreased [9].

Negative effects of cement dust were also reported on crop plants. Significant reduction of shoot length and total leaf area was observed in *Celosia argentea* (spinach) due to cement pollution [18].

Forest in the vicinity of cement plant Lafarge S.A. in Bielawy acts as pollutant sink, suffering from the effects of pollution but also eliminating these effects.

The long-term effect of soil alkalization on forests and the impact of cement layer on physiological processes of vegetation is still not well recognized. Because pine forest is adapted to very acid soil, soil alkalization must negatively impact these areas. In order to forecast and estimate the dynamics of changes it is necessary to continue the study in a longer period time regarding eventually modification in cement technology production, and the installation of dust collecting facilities.

Conclusions

1. Soils under the impact of cement dust contain calcium carbonate of anthropogenic origin and elevated pH compared with the soils beyond the cement plant emission.

2. Exchangeable cations dominate over the acid cations in studied soils and the degree of base saturation of colloidal complex is above 94 %, in spite of other features like eluviation horizons typical for podzolic soil (Profile 1) and lessive soil (Profile 2).

3. Pine needles from the forest stands in the vicinity of cement plant are covered with cement dust.

4. The analysis of morphology of needles show that their length and width are lower than needles sampled from the tree beyond the cement dust impact. The negative effect of cement dust was more pronounced in older needles (3 years old).

5. It is important to plant tolerant tree and shrub species in the forest ecosystem around the cement plant.

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WPŁYW PYŁÓW CEMENTOWYCH NA WYBRANE WŁAŚCIWOŚCI GLEB ORAZ BUDOWĘ MORFOLOGICZNĄ IGIEŁ SOSNY POSPOLITEJ (*Pinus sylvestris* L.) Z FITOCENOZ OTACZAJĄCYCH ZAKŁADY CEMENTOWO-WAPIENNICZE „LAFARGE" W BIELAWACH

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Abstrakt: Celem podjętych badań była ocena wpływu pyłów cementowo-wapienniczych na wybrane właściwości gleb oraz na stan ulistnienia drzewostanów sosnowych w otoczeniu Zakładów Cementowo-Wapienniczych „Lafarge" w Bielawach. Badania przeprowadzono na próbkach pochodzących z dwóch profili gleb uprawnych: gleba bielicowa właściwa (P1) zlokalizowana na skraju lasu sosnowego oraz gleba płowa typowa (P2) – oddalona o 200 m od lasu mieszanego, leżąca w pobliżu emitora. Materiał roślinny stanowiły igły sosny *Pinus sylvestris* L., zebrane z drzew rosnących w sąsiedztwie pól uprawnych, z których pochodziły próbki glebowe. W próbkach glebowych oznaczono uziarnienie, odczyn, C-organiczny, kationową pojemność sorpcyjną oraz zawartość $CaCO_3$. Ocenę materiału roślinnego (po segregacji wg wieku igieł) przeprowadzono na podstawie danych biometrycznych uzyskanych za pomocą programu komputerowy Digishape (Cortex Nova 2005). Analizowane gleby charakteryzują się uziarnieniem odpowiadającym piaskom słabogliniastym (P1) i piaskom gliniastym (P2). Badane gleby mają odczyn lekko kwaśny (pH_{KCl} 5,6–6,5). Poziomy powierzchniowe charakteryzują się wyraźnie większymi wartościami pH, co wynika ze wzbogacenia tych poziomów w $CaCO_3$. Zawartość próchnicy w poziomach Ap była typowa dla gleb badanego regionu i wynosiła 1,14 $g \cdot kg^{-1}$ (P1) i 0,78 $g \cdot kg^{-1}$ (P2). Z analizy biometrycznej igieł sosnowych wynika, że ich powierzchnia oraz długość maleją z biegiem lat, natomiast szerokość rośnie. Materiał roślinny pochodzący z drzew rosnących bliżej emitora charakteryzuje się znacznie mniejszą powierzchnią asymilacyjną. Igły sosnowe były w tym przypadku krótsze i węższe.

Słowa kluczowe: pył cementowy, gleba, sosna pospolita (*Pinus sylvestris* L.)

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Helena KUBICKA[1] and Wioletta KOPRAS[2]

ASSESSMENT OF OAT VARIETIES SENSITIVITY TO THE IMPACT OF ALUMINIUM

OCENA WRAŻLIWOŚCI ODMIAN OWSA (*Avena sativa* L.) NA DZIAŁANIE GLINU

Abstract: In this work, the influence of aluminium on the germination and growth of 9 oat varieties was researched The experiment was carried out in hydroponic cultures and four concentrations of aluminium were applied. The selected varieties of oat reacted to aluminium in various ways. Aluminium at concentrations of 100 and 200 μM reduced the growth of roots to the highest degree, however, its influence on the growth of shoots was negligible. A 75 μM concentration of aluminium had no effect on the growth of seedlings of chosen oat varieties in comparison with control. Amongst the assessed varieties of oat the following varieties were most tolerant to the toxic influence of aluminium: Flemingsprofi and Bohun at the lowest concentration (75 μM) as well as Borowiak and Akt at higher concentrations (100 and 200 μM). The obtained results will be used for cultivation selection in given areas.

Keywords: aluminium, oat, varieties, tolerant

In soil solutions aluminium ions may appear in various forms which depend on the pH balance and content of the soil. The most toxic element for plants is mobile aluminium Al^{3+}, whose harmful pH increases proportionally to the acidity of soil. 52 % of acidic soil in Poland is below pH = 5.5 [1, 2]. According to Furukawa et al [3] 30–40 % of the world's arable soil is acidic. On this soil a reduction of crop production has been observed.

The roots of plants are at highest risk of exposure to aluminium toxic stress. This harmful element interferes with the growth of roots which is caused by the reduced number of cell division in root meristems. If aluminium stress is maintained over a longer period of time, this may lead to a complete stunt of root growth, and next to the death of the plants.

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Aluminium also interferes with the mineral distribution in plants which is characterized by magnesium, potassium and phosphor deficiency [4]. Moreover, the toxic concentration of aluminium influences the process of respiration, DNA synthesis and proteolithic enzyme activity of cell membranes [5].

The aim of this work was to assess the influence of aluminium on the germination and growth of seedlings of oat varieties as well as the selection of tolerant varieties to the negative activity of this element.

Materials and methods

9 varieties of oat (7 Polish – Akt, Polar, Kasztan, Borowiak, Bohun, Deresz, Jawor and 2 German – Flaemingstern, Flemingsprofi) were used. At the first stage of the experiment, germination energy of the oat varieties was defined. 100 seeds of each variety were placed on Petri dishes (two repetitions) and left for 72 h in 4 aluminium combinations: control (0), 75, 100 and 200 μM. In the second part Aniola's [6] hydroponic nutrition growth test modified by Gallego i Benito [7] was applied. The germinated young seedlings (20 for each variety in three repetitions) were placed on the medium using the same concentrations for aluminium.

Germination energy of oat varieties after aluminium treatment was defined. The degree of the growth stunt for roots and shoots as well their number after 14 days of observation was also defined. Standard deviations were taken into account.

Results and discussion

In this paper, it was shown that aluminium lessens germination of seeds proportionally to its concentration levels. The highest germination energy was observed in Polish varieties Borowiak and Akt, ranging from 100 to 93 % (from the lowest to the highest aluminium concentration, accordingly) in comparison with control. However, the German Flaemingstern variety was most sensitive to the activity of aluminium at the germination phase in which its germination energy ranged from 88 % to 36 % in relation with control (Fig. 1).

A different response of the chosen oat varieties to the activity of aluminium was observed at the seedling development stage which was characterized by a differentiated growth of roots and shoots. The toxic influence of aluminium was first observed in the growth stunt of roots and for this reason it was basic criterion for tolerance assessment. Aluminium caused the shortening, browning and brittleness of roots. The greatest growth of root length in control was observed in Kasztan, Bohun and Flemingsprofi varieties. In the case of the last variety aluminium stimulated the growth of roots at concentration of 75 μM. Morever, the Jawor variety had a lower growth of roots in all combinations (Fig. 2).

The roots were directly exposed to the harmful influence of aluminium and for this reason their growth was significantly reduced in comparison with the growth of shoots. Aluminium concentration of 200 μM in the experiment lasting 14 days proved to be the threshold at which the first symptoms of plant death was already observed.

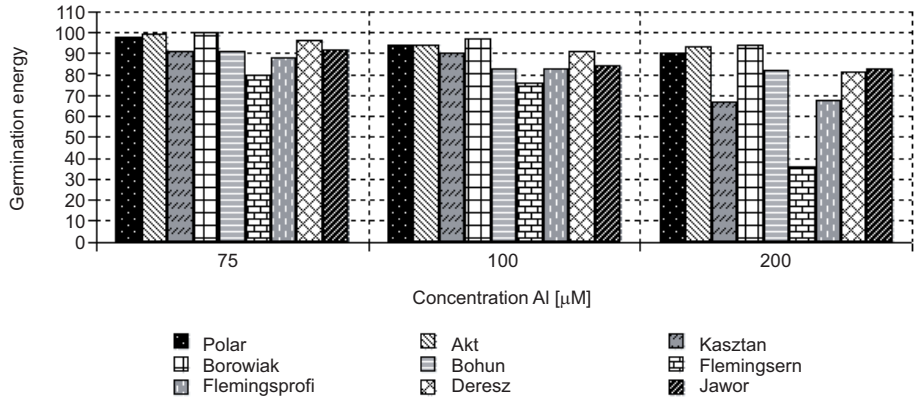

Fig. 1. Germination energy of seeds in oat varieties [in relation to control – %]

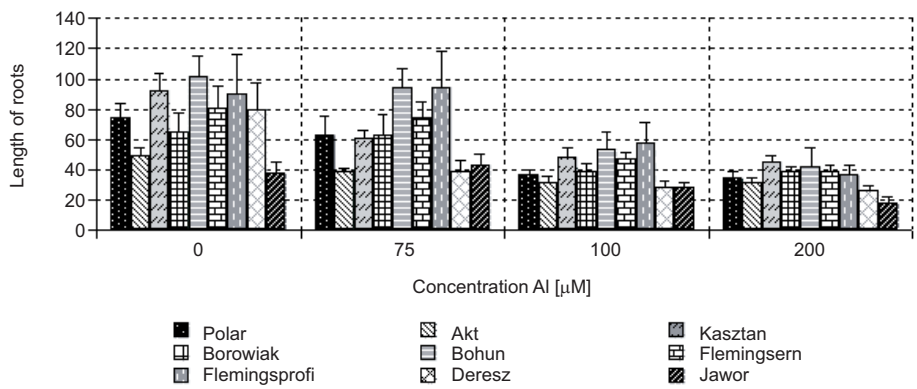

Fig. 2. The influence of aluminium on length [mm] of oat roots after 14 days

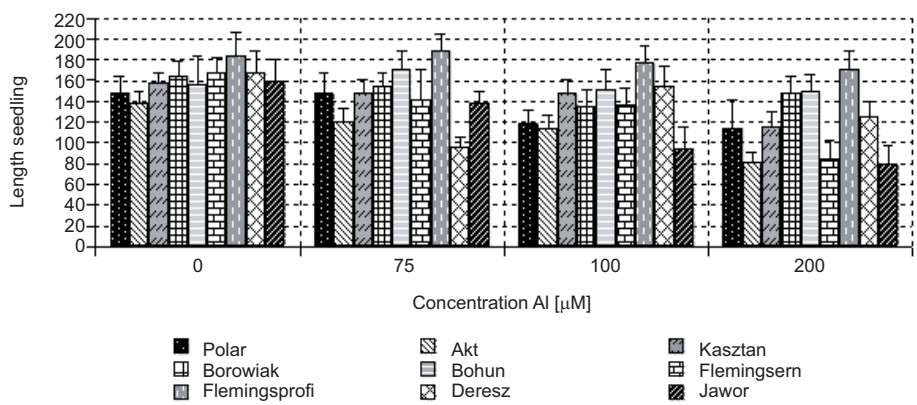

Fig. 3. The influence of aluminium on length [mm] of oat shoots after 14 days

Similarly as in the case roots, aluminium at concentration of 75 μm stimulated the growth of shoots in some varieties: Flemingsprofi, Bohun and Jawor. The greatest growth of shoots in all combinations was observed in the Flemingsprofi variety. This variety also created the highest number of roots – on average – 6, but in others around 4. The length of shoots in the remaining varieties lessened proportionally to the growth of aluminium concentration (Fig. 3).

On the basis of the obtained results, oat varieties tolerant to the activity of aluminium ions were identified: Flemingsprofi and Bohun – at lowest concentration (75 μM) as Borowiak and Akt – at higher concentrations (100 and 200 μM).

The researched oat varieties variously reacted to aluminum stress depending on the phase of plant development. The Flaemingstern and Flemingsprofi varieties proved the most sensitive to the toxic activity of aluminium ions at the germination stage, yet its harmfulness at the seedling phase of those varieties was significantly lower. The existence of similar differences among species and inbred lines of rye with regard to salinity stress sensitivity depending on the plant development stage has been reported by Noble [8] and Kubicka and Dec [9], eg the *Secale montanum* rye species was characterized by high sensitivity at the germination phase.

According to Aniol [10] and Zawada et al [11], some species and varieties of crops react differently to aluminium stress. It was demonstrated in the presented paper – seedlings of oat varieties differed in their root and shoot length after aluminium treatment.

A negative aluminium effect on the development phase was observed in the experiment, however it was less extensive for shoots.

The selected genotypes of oat tolerant to the harmful action of aluminium may be used in the breeding of varieties which are better adapted to acidic soils in Poland, However sensitive varieties can be used in genetic experiments to define the inheritance of this trait.

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OCENA WRAŻLIWOŚCI ODMIAN OWSA (*Avena sativa* L.) NA DZIAŁANIE GLINU

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Abstrakt: W pracy badano wpływ glinu na kiełkowanie i wzrost siewek 9 odmian owsa, w kulturach hydroponicznych, stosując cztery stężenia tego metalu. Wybrane odmiany owsa zróżnicowanie reagowały na jego działanie. W najwyższym stopniu glin wpływał na zahamowanie przyrostu długości korzeni w stężeniach 100 i 200 μM, zaś nieznacznie na wzrost części nadziemnych. Glin w stężeniu 75 μM prawie nie wpływał na wzrost siewek badanych odmian owsa, a nawet stymulował wzrost siewek u odmiany Flemingsprofi. Spośród ocenianych odmian owsa najbardziej tolerancyjne na toksyczne działanie glinu były odmiany: Flemingsprofi – w najniższym stężeniu (75 μM) oraz Borowiak i Bohun – w wyższych (100 i 200 μM). Uzyskane wyniki zostaną wykorzystane przy doborze odpowiednich odmian do uprawy na danym terenie.

Słowa kluczowe: glin, owies, odmiany, tolerancja

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 10 2010

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CONTENT OF SELECTED ELEMENTS IN THE LEAVES GROWING IN AN URBAN AGGLOMERATION

ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW W LIŚCIACH DRZEW ROSNĄCYCH W AGLOMERACJI MIEJSKIEJ

Abstract: In the study the content of K, Ca, Mg and Na in the leaves of *Acer platanoides* L., growing in the agglomeration of Gdynia and Gdansk, was shown and the mean ion equivalent ratios between these elements were determined. The obtained results showed large differentiation in the amount of the elements in the leaves of maple growing on particular sites in the urban environment. Correlation relationships between the concentration of heavy metals in the leaves of maple show a significant influence, particularly in the case of of Cd and Pb, on the content of determined elements in the assimilation apparatus. The results of the studies on the trees also showed changes in mutual ion ratios of the elements in the leaves.

Keywords: elements, *Acer platanoides* L.

Functioning of an urban agglomeration makes the conditions of trees vegetation in this area get worse and worse. An excessive concentration of heavy metals and impurities in urban environment leads, among other things, to disturbances in the element uptake by plants [1–3] and also in the ion balance [4–7]. As a result, there are considerable changes in the content of macro- and microelements in the assimilation apparatus [8, 9] causing mainly deficiency in elements indispensable for appropriate course of physiological processes. [10–15]. Maple (*Acer platanoides* L.), a characteristic feature of which is a wide ecological niche, belongs to the group of plants commonly used in urban agglomerations.

The studies aimed to determine the bioaccumulation of selected macroelements (K, Na, Ca and Mg) in the assimilation apparatus of maple (*Acer platanoides* L.) growing in the urban agglomeration of Gdansk and Gdynia and the average ion equivalent ratios between these elements. The obtained results will make it possible to determine if the studied physiological parameters can be used as the indicator reflecting the influence of the urban environment on the growth of maple.

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Material and methods

The studies were carried out in the area of urban agglomeration of Gdańsk and Gdynia in 2004. In each town seven research sites were set up in the centre of the city along the streets of high traffic volume, two in the housing estate, two in the park and the control point at a distance of about 25 km from the city. On each research site four representative trees of an approximate diameter at breast height (d.b.h.) of about 20 cm were selected. From each tree two one-year shoots were selected.

Material for the analysis consisted of fully formed leaves of the first and the second pair of a year's gain of *Acer platanoides* L. Leaves for chemical determination were collected in September at the height of about 2 metres from the southern side of the tree. After the leaves had been washed, dried and then subjected to wet mineralization, the content of K, Na, Ca and Mg in the leaves was determined using the atomic absorption spectrophotometry (AAS). Their mutual ion ratios were also calculated. The obtained results were worked out statistically, by calculating the correlation coefficient between the amount of macro- and microelements in the tree leaves. The analysis of variance was carried out and the significance of factors, was assessed by Tukey test at a significance level of $\alpha = 0.05$.

Results and discussion

The obtained results showed large diversity in the content of the determined elements in the leaves of maple growing both in the agglomeration of Gdansk and in that of Gdynia.

The statistical analysis showed a significant effect of the site location on the content of macroelements in the tree leaves. Lowered amounts of potassium were obtained in the leaves of maple trees growing along the streets, both in Gdansk and Gdynia. The leaves of trees from these research sites contained within 70 % less of potassium than the leaves from the control trees and about 60 % less of this element than the leaves of park trees (Tables 1, 2). The uptake of potassium from the soil by the leaves of trees growing in Szczecin is also insufficient [15]. The negative significant correlation relationships for this macroelement were observed between the concentration of Pb, Cd, Ni and Zn in the tree leaves from Gdynia and the amount of Pb, Cd, Ni, Zn, Cu and Fe in the tree leaves from Gdansk (Table 3). A slight increase in the content of magnesium was observed in the leaves of trees growing in Gdansk as compared with the control maple trees (Tables 1, 2). There were also correlation relationships between the amount of this macroelement in the leaves and the concentration of Pb, Cd and Zn in the leaves of trees growing in Gdynia and Cd and Mn in the leaves of maple trees from Gdansk (Table 3).

The amount of Cd was 46 times, Ni – 12 times, Pb and Zn – 4 times, Fe and Cu it was 3 times higher in the leaves of maple trees growing in the centre of Gdansk than in those from the control site [16]. Whereas the amounts of Cd and Pb recorded in the leaves of trees growing in Gdynia on the sites along the streets with heavy traffic exceed the upper range presented for these metals by Kabata-Pendias and Pendias [7] and

Markert [17]. The concentration of heavy metals in the tree leaves was increased on the sites located along the streets but their values did not exceed the upper limits for these metals [18].

The course of variability of Ca and Na in the leaves of trees from Gdansk was differentiated. It was shown that the content of calcium in the leaves of investigated trees from the centre of Gdansk was lower by 7.7 g · kg^{-1} of dry matter than that in the control leaves (Table 1).

Table 1

The average content of elements [g · kg^{-1} d.m.] in the leaves of maple in the area of Gdansk

Stand		Elements [g · kg^{-1} d.m.]			
		K	Na	Ca	Mg
ul. Kartuska	City centre	7.3	0.18	21.8	6.4
ul. Jabłoniowa		9.4	0.32	19.4	5.5
ul. Bieganskiego	Housing estates	14.5	0.29	27.3	3.8
ul. Dragana		13.0	0.35	20.3	4.2
Park MOSiR	Park	22.3	0.24	25.7	4.1
Park Orunski		24.1	0.22	26.9	3.7
Control stand		29.8	0.15	28.3	5.2
$LSD_{0.05}$		2.24	0.17	2.61	2.04

In Gdynia an approximate amount of calcium was observed in the leaves of the control, park and housing estate trees (average about 23 g · kg^{-1} d.m.). The content of Ca in the leaves of maple trees was lower than the content determined (35 g · kg^{-1} d.m.) by Insley et al in the leaves of lime tree [19]. However, a slight increase in this macroelement content was recorded in the tree leaves from the centre of the city (Table 2).

Table 2

The average content of elements [g · kg^{-1} d.m.] in the leaves of maple in the area of Gdynia

Stand		Elements [g · kg^{-1} d.m.]			
		K	Na	Ca	Mg
ul. Morska	City centre	9.7	0.49	25.9	5.8
ul. Kwiatkowskiego		11.3	0.23	24.4	6.1
ul. Swarzewska	Housing estates	12.9	0.53	26.3	5.3
ul. Bosmanska		14.3	0.41	19.7	4.9
Park Rady Europy	Park	25.5	0.34	22.3	4.3
Park na Kamiennej Gorze		23.1	0.40	24.0	3.9
Control stand		33.8	0.51	23.9	4.7
$LSD_{0.05}$		1.00	0.10	1.51	0.29

On the basis of the correlation coefficient a negative significant correlation relationship was observed between the concentration of Pb, Ni, Zn, Cu and Fe and the content of this element in the leaves of trees from Gdansk. The content of Ca in the leaves of trees from Gdansk is in significantly correlated with the concentration of heavy metals (Table 3).

Table 3

Relationships (r) between the content of macro- and microelements in the leaves of maple from Gdansk and Gdynia

	Gdansk				Gdynia			
	Potassium	Sodium	Calcium	Magnesium	Potassium	Sodium	Calcium	Magnesium
Lead	–0.84*	–0.07	–0.79*	0.56	–0.86*	–0.30	0.17	0.78*
Cadmium	–0.82*	–0.17	–0.68	0.79*	–0.89*	–0.27	0.25	0.82*
Nickel	–0.93*	0.09	–0.77*	0.61	–0.84*	–0.04	0.16	0.30
Manganese	–0.74	–0.22	–0.61	0.87*	–0.40	–0.44	0.17	0.37
Zinc	–0.78*	–0.21	–0.94*	0.44	–0.87*	–0.12	0.04	0.77*
Copper	–0.81*	–0.11	–0.89*	0.38	–0.85	0.24	0.38	0.71
Iron	–0.83*	–0.20	–0.96*	0.54	–0.38	–0.15	0.06	0.45
Magnesium	–0.44	–0.38	–0.46		–0.73	–0.11	0.38	
Calcium	0.81*	0.36			–0.21	0.32		
Sodium	–0.16				0.21			

* Significant for: $\alpha = 0.05$.

The leaves of the trees growing in the housing estate were marked by a higher amount of sodium in comparison with the leaves of the remaining research sites. An average content of this element in these leaves amounted to 0.32 g · kg^{-1} d.m. in Gdansk and on average 0.47 g · kg^{-1} d.m. in Gdynia (Tables 1, 2). The level of sodium content (ranging from 0.34 to 0.63 g · kg^{-1} d.m.) was similar in the leaves of small-leaved lime growing in the centre of Szczecin [15]. The value of correlation coefficient showed a negative insignificant relationship between the concentration of heavy metals and the content of this element in the leaves of studied trees from the agglomeration of Gdansk and Gdynia (Table 3).

Correlation relationships between the content of macro- and microelements in the leaves of maple show a significant effect of trace metals, particularly those unused in the plant metabolism (Cd, Pb), on the chemical composition of the assimilation apparatus. These relationships are probably connected with phenomena of synergism and antagonism, the processes of which are slightly different in polluted soils than in typical arable soils. The phenomenon of interaction plays a significant role because it causes disturbances of chemical balance in plants. In the studied leaves of trees, changes in mutual ion ratios of the defined elements were observed. In the trees growing in the city centre these relations were considerably narrowed as compared with the control trees (Tables 4, 5). The changes in mutual relations may cause the acceleration of some processes and simultaneously the delay the other [7].

Table 4

Mean ion equivalent proportions in the leaves of maple growing in Gdansk

Stand	Ion proportions				
	K : (Ca + Mg)	K : Ca	K : Mg	K : Na	Ca : Mg
ul. Kartuska	0.12	0.17	0.36	23.75	2.06
ul. Jabloniowa	0.17	0.25	0.52	17.14	2.11
ul. Bieganskiego	0.17	0.27	0.49	21.76	1.80
ul. Dragana	0.25	0.32	0.94	22.01	2.91
Park MOSiR	0.35	0.44	1.68	57.00	3.79
Park Orunski	0.37	0.46	2.00	68.89	4.35
Control stand	0.41	0.54	1.77	108.57	3.30

Table 5

Mean ion equivalent proportions in the leaves of maple growing in Gdynia

Stand	Ion proportions				
	K : (Ca + Mg)	K : Ca	K : Mg	K : Na	Ca : Mg
ul. Morska	0.14	0.19	0.52	9.26	2.69
ul. Kwiatkowskiego	0.17	0.24	0.57	29.01	2.39
ul. Swarzewska	0.19	0.25	0.75	14.35	3.00
ul. Bosmanska	0.27	0.38	0.30	20.55	2.39
Park Rady Europy	0.45	0.58	1.91	43.33	3.29
Park na Kamiennej Gorze	0.39	0.49	1.84	34.71	3.75
Control stand	0.55	0.73	2.23	39.54	3.05

Conclusions

1. A significant impact of the site location on the amount of K, Na, Ca and Mg was observed in the leaves of maple.

2. Ion ratios were remarkably narrowed in the trees from the city centre as compared with the control trees.

3. A significant correlation relationship was shown between the concentration of cadmium and lead and the content of potassium and magnesium in the leaves of the trees investigated.

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ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW MINERALNYCH W LIŚCIACH DRZEW ROSNĄCYCH W AGLOMERACJI MIEJSKIEJ

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Abstrakt: W pracy przedstawiono zawartość K, Ca, Mg i Na w liściach *Acer platanoides* L., rosnącego w aglomeracji miejskiej Gdyni i Gdańska oraz określono średnie równoważnikowe proporcje jonowe między tymi pierwiastkami. Uzyskane wyniki wykazały duże zróżnicowanie w zawartości oznaczanych pierwiastków w liściach klonu rosnącego na poszczególnych stanowiskach w środowisku miejskim. Zależności korelacyjne między koncentracją metali ciężkich w liściach klonu wskazują na znaczący wpływ szczególnie Cd i Pb na zawartość oznaczanych pierwiastków w aparacie asymilacyjnym. U badanych drzew uzyskane wyniki wykazały również zmiany we wzajemnych proporcjach jonowych pierwiastków w liściach.

Słowa kluczowe: pierwiastki, *Acer platanoides* L.

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INFLUENCE OF BLACK LOCUST (*Robinia pseudoacacia* L.) MIDFIELD SHELTERBELTS ON THE CONTENT OF BIOAVAILABLE FORMS OF PHOSPHORUS AND POTASSIUM IN ARABLE SOIL DEVELOPED FROM LOESS

WPŁYW ZADRZEWIEŃ ŚRÓDPOLNYCH ROBINII AKACJOWEJ (*Robinia pseudoacacia* L.) NA ZAWARTOŚĆ PRZYSWAJALNYCH FORM FOSFORU I POTASU W GLEBIE UPRAWNEJ WYTWORZONEJ Z LESSU

Abstract: The aim of the paper was evaluation of influence of the black locust trees afforestation on loess soil available forms content of phosphorus and potassium. Samples were collected from arable land located in the area of the Proszowice Plateau. Investigated field was divided to 12 (2 m wide) zones in growing distance from black locust. It was found significant influence of afforestation of trees on available potassium content. Its content was the highest in zones located in neighborhood of afforestation. Phosphorus content showed not so strong correlation with the distance from trees, but it was found visible higher concentration of this element in zones distanced to 12 m from robinia.

Keywords: available elements, black locust, loess soil

Midfield shelterbelts are an element of agricultural landscape created by man. They can be remains of forest vegetation, a result of spontaneous colonization by arborescent vegetation and bushes or an effect of plantings [1].

Shelterbelts are treated as a forest substitute, which ought to be commonly used in the areas where, due to good quality of soils, afforestation should be discouraged. Planting shelterbelts is treated as an equivalent to afforestation factor of protection and use of the environmental space.

In our climatic zone shelterbelts are an element of landscape weakening the effect of destabilizing factors [2]. Midfield shelterbelts, in agricultural landscape composed of false acacia or other species, under specific conditions of relief may function as barrier

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ecosystems, which owing to their high sorption capacity resulting from the presence of litter and nutrient uptake by growing trees, are a buffer for biogenic elements [3].

There is no detailed data on the influence of midfield shelterbelts on soil environment, particularly the ploughlands adjoining the shelterbelts. An interesting object of investigations are midfield shelterbelts composed of black locust (*Robinia pseudoacacia* L.), the plant from the papilionaceous family, whose presence causes an increase in nitrogen content in soil [4, 5]. The influence of robinia on the contents of other elements in arable soils has not been investigated so far. Therefore, the presented work aimed at determining the effect of black locust on the content of bioavailable forms of potassium and phosphorus in arable soil formed from loess adjoining the robinia shelterbelts and establishing the range of its influence.

Material and methods

A microplot situated in Krolewice village on the Proszowice Plateau was selected for the research. The microplot was delineated in an arable field in the vicinity of midfield shelterbelts composed of false acacia plantings. The trees forming the shelterbelt are between 20 and 30 year old, the stripe of the shelterbelt is narrow, about 2–3 m in width, the trees are about 8–10 m high with crown width about 6–8 m. The selected arable field is flat, which excludes a potential effect of surface wash and runoffs on soil properties.

An area of 20 m × 24 m was marked out on the microplot and further divided into rectangular surfaces of 2 m × 4m. In this way 12 zones were identified (in five replications) at increasing distance from the black locust. Collective soil samples were taken from the depth of 0–20 cm using Egner's stick. The samples were collected in the early-spring season, before fertilization was applied to the fields.

In 60 collective samples collected in this way the content of bioavailable potassium and phosphorus forms were assessed using the Egner-Riehm method.

Spatial maps of bioavailable component content in the soil were created using Surfer 8.0 programme. Statistical analysis of results was conducted using Tukey test (at the significance level 0.05) in Statistica 6.0 programme.

The soil cover of the microplot consists of typical brown soil formed from loess, which is a dominant type of soil on the Proszowice Plateau. Brown soils, like soils lessivés, are considered an optimal site for black locust [4].

Results

The highest concentrations of bioavailable phosphorus were assessed in the samples collected in zones located between 22 m and 24 m from the robinia shelterbelts (Fig. 1). Elevated content of the element in some samples collected from the zones situated at between 2 and 12 m from the shelterbelts is visible on the presented spatial map of bioavailable phosphorus content (Fig. 2). Mean content of bioavailable phosphorus in these zones ranges from 52.6 to 57.3 mg · kg^{-1}. The lowest phosphorus concentration was assessed in the zone immediately adjoining the shelterbelts (42.3 mg · kg^{-1}). The highest phosphorus content in the zones at 20–22 and 22–24 m is most probably connected not with the shelterbelt effect but with some soil variability.

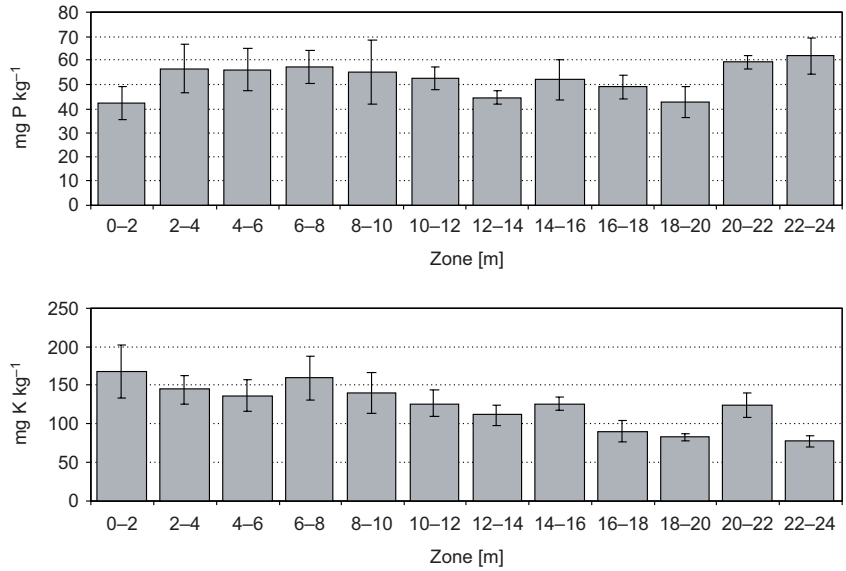

Fig. 1. Available phosphorus and potassium content in particular zones

Statistical analysis revealed significant differences in phosphorus content between the zones in which the content of the analyzed element was the lowest (zones 0–2, 12–14 and 18–20 m) and the highest (zones 20–22 and 22–24 m) (Table 1). The significance level of differences between the other zones was higher than 0.05, which denotes a lack of statistical dependence.

Table 1

Significance level of differences between average phosphorus content in soils of particular zones distanced from black locust trees (differences are statistically significant if significant level < 0.05)

Zone [m]	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24
0–2		0.92	0.69	1.00	0.81	0.26	0.03	0.26	.01	.01	0.21	0.12
2–4	0.92		1.00	1.00	1.00	0.99	0.62	0.99	0.05	0.01	0.98	0.92
4–6	0.69	1.00		0.94	1.00	1.00	0.88	1.00	0.15	0.05	1.00	0.99
6–8	1.00	1.00	0.94		0.98	0.58	0.11	0.59	.01	.01	0.51	0.33
8–10	0.81	1.00	1.00	0.98		1.00	0.79	1.00	0.09	0.03	1.00	0.98
10–12	0.26	0.99	1.00	0.58	1.00		1.00	1.00	0.50	0.22	1.00	1.00
12–14	0.03	0.62	0.88	0.11	0.79	1.00		1.00	0.97	0.80	1.00	1.00
14–16	0.26	0.99	1.00	0.59	1.00	1.00	1.00		0.49	0.22	1.00	1.00
16–18	.01	0.05	0.15	.01	0.09	0.50	0.97	0.49		1.00	0.57	0.75
18–20	.01	0.01	0.05	.01	0.03	0.22	0.80	0.22	1.00		0.27	0.44
20–22	0.21	0.98	1.00	0.51	1.00	1.00	1.00	1.00	0.57	0.27		1.00
22–24	0.12	0.92	0.99	0.33	0.98	1.00	1.00	1.00	0.75	0.44	1.00	

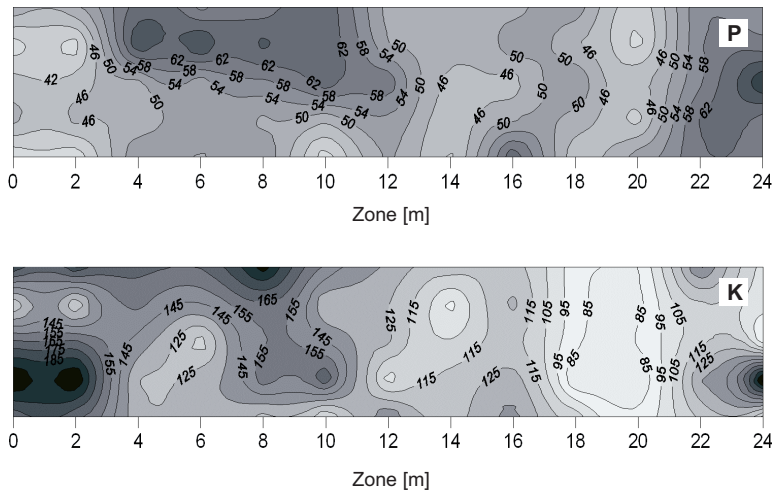

Fig. 2. Spatial distribution of available phosphorus (above) and potassium (below) content [mg · kg^{-1}] in dependence to black locust trees distance

The content of bioavailable potassium was adversely proportional to the distance from the robinia shelterbelts. The highest mean content of this element (168.1 mg · kg^{-1}) was assessed in the samples collected from the 0–2 m zone, ie the one situated in the immediate vicinity of the shelterbelts (Fig. 2). Elevated content of this element was noted in the samples from the zones distanced 12 m from the trees. Mean content of the element declines with the distance from the shelterbelts. The lowest concentration was registered in the 18–20 and 22–24 m zones.

Table 2

Significance level of differences between average potassium content in soils of particular zones distanced from black locust trees (differences are statistically significant if significant level < 0.05)

Zone [m]	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24
0–2		0.14	0.18	0.10	0.24	0.57	1.00	0.65	0.96	1.00	0.03	0.01
2–4	0.14		1.00	1.00	1.00	1.00	0.35	1.00	0.90	0.17	1.00	0.99
4–6	0.18	1.00		1.00	1.00	1.00	0.42	1.00	0.94	0.22	1.00	0.98
6–8	0.10	1.00	1.00		1.00	1.00	0.27	0.99	0.83	0.12	1.00	1.00
8–10	0.24	1.00	1.00	1.00		1.00	0.52	1.00	0.97	0.29	1.00	0.96
10–12	0.57	1.00	1.00	1.00	1.00		0.86	1.00	1.00	0.64	0.96	0.74
12–14	1.00	0.35	0.42	0.27	0.52	0.86		0.91	1.00	1.00	0.11	0.03
14–16	0.65	1.00	1.00	0.99	1.00	1.00	0.91		1.00	0.72	0.93	0.66
16–18	0.96	0.90	0.94	0.83	0.97	1.00	1.00	1.00		0.98	0.57	0.25
18–20	1.00	0.17	0.22	0.12	0.29	0.64	1.00	0.72	0.98		0.04	0.01
20–22	0.03	1.00	1.00	1.00	1.00	0.96	0.11	0.93	0.57	0.04		1.00
22–24	0.01	0.99	0.98	1.00	0.96	0.74	0.03	0.66	0.25	0.01	1.00	

The map of spatial distribution of abundance in bioavailable potassium reveals an increased content of the studied element in the zones close to the robinia trees.

On the basis of conducted Tukey test statistically significant differences were revealed between mean content of bioavailable potassium in the zones situated at the distance of 0–10 m from the shelterbelts and in the zones 16–20 m far from the trees, where this element concentrations were the lowest (Table 2).

Discussion

Research on the effect of black locust on soil focuses mainly on forest habitats. The investigations on robinia role in shaping the properties of forest soils demonstrated a positive effect of this plant on improvement of hydrophysical conditions in light soils, which is connected with increasing their concentrations of organic matter [6]. The Authors obtained similar results concerning robinia effect on increasing organic carbon and total nitrogen content in loess arable soil [7]. Especially elevated level of N content is visible in soil samples taken from the zones situated 12 m from the black locust. Increasing nitrogen content in soil is connected with symbiosis of these trees with nodule bacteria (*Rhizobium*, *Bacillus radicola*) fixing nitrogen from the atmosphere [4, 8].

According to von Holle et al [9] the presence of black locust trees on forest sites also leads to an increase in phosphorus and potassium content in the topsoil.

Presented investigations corroborate this thesis also for arable soils adjoining the robinia shelterbelts. Soil samples collected from the zones localized close to robinia were characterized by an elevated content of bioavailable phosphorus in comparison with more distanced zones. The content of bioavailable phosphorus and potassium was markedly increased in the zones located between 2 and 12 m from the robinia. The range of robinia trees influence on arable soil properties is connected with the tree height, which in turn affects the distance to which leaves, pods and branches, which are the source of raised element content in soil, are carried [10].

Higher concentrations of bioavailable components in the soils adjoining the shelterbelts should be taken into consideration when planning fertilization regime on such fields. Diversification of fertilization on the fields with considerable variability with respect to their chemical composition complies with the rules of so called precision agriculture [11].

Conclusions

1. Statistically significant effect of black locust shelterbelts on the content of bioavailable potassium was found; its highest content was assessed in the zones situated in the immediate vicinity of the robinia trees. Elevated content of this element was determined in the samples collected in the zones located 12 m from the trees.

2. No such obvious dependence was determined for bioavailable phosphorus, however this element content was markedly elevated in some soil samples collected from the zones situated between 2 m and 12 m from the shelterbelts.

3. Higher contents of bioavailable components in arable soils adjoining the plantings should be considered at the application of fertilization.

Acknowledgement

The work has been financed by Ministry of Sciences and Higher Education in the frame of research project No. N N3104352 33.

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WPŁYW ZADRZEWIEŃ ŚRÓDPOLNYCH ROBINII AKACJOWEJ (*Robinia pseudoacacia* L.) NA ZAWARTOŚĆ PRZYSWAJALNYCH FORM FOSFORU I POTASU W GLEBIE UPRAWNEJ WYTWORZONEJ Z LESSU

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Abstrakt: Celem pracy było określenie wpływu zadrzewień robinii akacjowej na zawartość przyswajalnych form potasu i fosforu w uprawnej glebie lessowej. Na podstawie oznaczeń laboratoryjnych i analizy statystycznej wyników stwierdzono statystycznie istotny wpływ zadrzewień robinii na zawartość przyswajalnego potasu; jego największą zawartość oznaczono w strefach położonych w bezpośrednim sąsiedztwie drzew robinii. Podwyższona zawartość tego składnika występuje w próbkach pobranych ze stref oddalonych do 12 m od drzew. W przypadku przyswajalnego fosforu nie stwierdzono tak wyraźnej zależności, jednak zawartość tego składnika była wyraźnie zwiększona w próbkach glebowych pobranych ze stref odległych od 2 do 12 m od zadrzewień.

Słowa kluczowe: składniki przyswajalne, robinia akacjowa, gleba lessowa

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EFFECT OF NUTRITION WITH MAGNESIUM IN VARIOUS MOISTURE CONDITIONS OF SOIL ON THE DYNAMICS OF ELONGATION GROWTH OF MEDICAL SAGE (*Salvia officinalis* L.)

WPŁYW DOGLEBOWEGO ŻYWIENIA MAGNEZEM W ZRÓŻNICOWANYCH WARUNKACH WILGOTNOŚCIOWYCH GLEBY NA DYNAMIKĘ WZROSTU ELONGACYJNEGO SZAŁWII LEKARSKIEJ (*Salvia officinalis* L.)

Abstract: The aim of the studies was to determine the effect of nutrition of the soil with magnesium in differentiated soil moisture conditions on the dynamics of the elongation growth of medical sage. The first factor of the two year pot experiment was the level of the nutrition with magnesium (0; 0.30; 0.90 g of Mg per pot), the other factor was the level of soil moisture (30 and 60 % of full water volume). Large usefulness of the logistic function for the description of the elongation growth of *Salvia officinalis* L. was statistically confirmed.

Keywords: medical sage, magnesium, soil moisture, elongation growth, logistic function

Medical sage belongs to herb plants of relatively large significance. The measure of intensity of plants physiological processes is, among other things, their elongation growth. The most natural measure of plants growth are the changes in their height. The elongation growth is most often presented graphically in a form of curves which are plots of some mathematical functions. To describe the growth of medical sage, a continuous sigmoidal logistic function, common in biometry, was used [1]. A basic factor affecting growth of plants is balanced mineral nutrition. It is estimated that about 60 % of arable land in Poland is characterised by a small amount of available magnesium and therefore mineral nutrition of plants with this element becomes more and more significant [2–5].

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The aim of the study was to determine the effect of in-soil nutrition with magnesium in various moisture conditions on the dynamics of elongation growth of medical sage. Usefulness of the logistic function for the description of this plant was also assessed.

Material and methods

A two year pot experiment (Mitcherlich pots) were carried out in the vegetation hall (greenhouse) of University of Agriculture in Szczecin. A method of complete randomization in the two factor system was used in ten replications, where: the 1st factor – the level of nutrition with magnesium (Mg0, Mg1, Mg2), the 2nd factor – the level of soil moisture content (30 % and 60 % of full water volume).

The medium for plants consisted of the soil material taken from the arable humus level of a post-cultivated soil of 6th quality class and its mechanical composition was light loamy silty sand.

The same doses of indispensable macro- and microelements, except magnesium, were used. The doses of magnesium in a form of $MgSO_4 \cdot 7H_2O$ were differentiated in the following way: Mg0 – 0 g Mg per pot, Mg1 – 0.30 g Mg per pot, Mg2 – 0.90 g Mg per pot. Doses of the remaining mineral elements per pot were: N – 1.0 g in a form of NH_4NO_3, K – 1.66 g in a form of K_2SO_4 and KC1 (1:1), P – 0.44 g in a form of NaH_2PO_4, Ca – 0.36 g in a form of $CaCO_3$, a solution of microelements according to Hoagland – 5 cm^3, a solution of 1 % $FeCl_3$ – 5 cm^3. In the course of filling the pots, current moisture and full water volume were annually determined. During the whole period of plants vegetation, the soil moisture was maintained at the level of 30 or 60 % of full water volume, depending on the combination of the experiment, using a gravimetric method [6].

In order to determine the dynamics of elongation growth in both vegetation seasons, the height of six (from each combination of the experiment) randomly selected plants (always the same) was measured every seven days since the moment the thinning of plants in the pots was made.

To describe the growth process, a logistic function was used:

$$h = \frac{h_{max}}{1 + b \cdot \exp(-kt)}$$

where: h – height of plants,
h_{max} – maximum height of plants,
t – vegetation time,
b, k – coefficients.

Coefficients of the logistic model were estimated using a method of the smallest squares after linearization of a function by means of logarithmic transformation [7, 8]:

$$\ln [(h_{max}/h) - 1] = \ln b - kt$$

Additionally, other parameters of the logistic function were calculated, ie initial value h_0, coordinates of the inflexion point (t_i, h_i) and characteristic of this point maximum growth rate $(dh/dt)_{max}$.

These calculations were made on the basis of the following formulae $h_0 = h_{max}/(1 + b)$: $t_i = (\ln b)/k$; $h_i = 0.5h_{max}$; $(dh/dt)_{max} = 0.25\ kh_{max}$.

Using an analytical form of the logistic function, curves showing the elongation growth of plants h = h(t) were plotted for each combination of the experiment during the 1st and the 2nd year of the studies (Fig. 1). In order to compare the degree of the fitting of theoretical curves to the experimental data, values of determination coefficient R^2 were calculated.

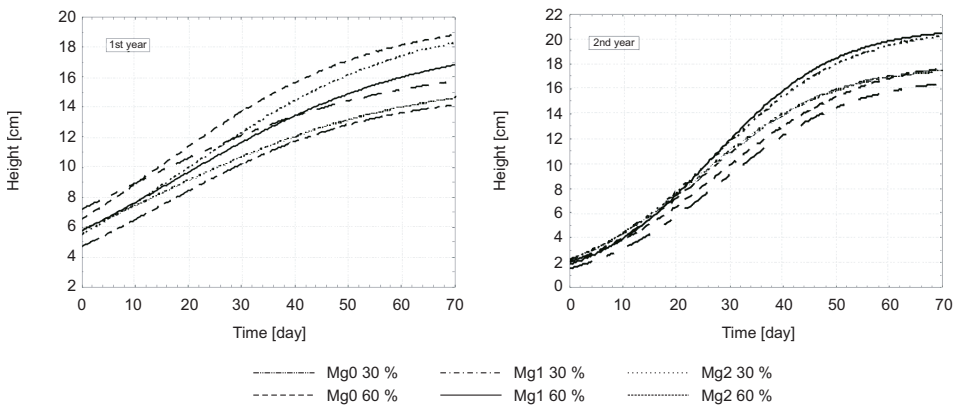

Fig. 1. Growth curves of medical sage plants from combinations of the experiment

Results and the discussion

Parameters of the logistic function for individual combinations of the experiment are shown in Table 3. The determination coefficient R^2 ranges from 0.931 to 0.994. The data in Table 3 show that the logistic function describes sufficiently enough the actual course of the elongation growth of medical sage. This is reflected in the values of determination coefficients R^2, very close to one. Similar results of the studies were also obtained by Gregorczyk [9]. He confirmed usefulness of the logistic function for mathematical modelling of the elongation growth of herb plants.

During the first year of the studies, intensive growth of plants lasted from the 8th to the 20th day of vegetation (counting from the thinning of the plants in the pots) depending on the combination of the experiment; at this time the curve of absolute growth is at the point of inflexion. The earliest, ie on the 8th day of vegetation, maximum growth rate was observed in medical sage in the combination of Mg1 30 % of full water volume, whereas the latest (ie on the 20th day of vegetation), in plants in the combination of Mg2 60 % of full water volume. During the second year of the studies the growth curve of sage plants reached the point of inflexion later than in the first year.

Table 1

Kinetics of the elongation growth of medical sage plants from individual combinations of the experiment (the averages of the measurements of 6 plants) 1st year of the experiment

Measurement	Day of vegetation*	Height of plants [cm]					
		Mg0 30 %	Mg1 30 %	Mg2 30 %	Mg0 60 %	Mg1 60 %	Mg2 60 %
1	1	4.0	6.0	5.0	6.0	6.0	6.0
2	8	7.0	9.0	7.0	8.0	7.0	7.0
3	15	8.0	10.0	9.0	10.5	9.0	9.0
4	22	9.0	12.0	10.0	13.0	10.0	10.5
5	29	10.0	12.5	11.0	14.0	12.0	12.0
6	36	11.0	13.0	12.0	15.0	12.5	13.5
7	43	11.5	13.5	12.5	16.0	13.0	14.5
8	50	13.0	14.0	13.0	16.5	15.0	16.0
9	57	13.5	15.0	13.5	18.0	16.0	17.5
10	64	15.0	17.0	16.0	20.0	18.5	20.0

* From the day of thinning the plants in pots.

Table 2

Kinetics of the elongation growth of medical sage plants from individual combinations of the experiment (the averages of the measurements of 6 plants) 2nd year of the experiment

Measurement	Day of vegetation*	Height of plants [cm]					
		Mg0 30 %	Mg1 30 %	Mg2 30 %	Mg0 60 %	Mg1 60 %	Mg2 60 %
1	1	2.0	2.0	2.5	2.0	2.0	2.0
2	8	4.0	3.0	4.0	3.5	4.0	4.0
3	15	6.0	4.0	6.0	5.0	5.5	6.0
4	22	8.5	6.5	9.0	8.0	8.5	10.0
5	29	10.0	8.0	10.0	10.0	11.0	11.5
6	36	12.0	10.0	12.0	11.5	14.0	14.0
7	43	14.0	13.0	14.0	14.0	16.0	16.0
8	50	16.0	14.0	16.0	15.0	18.0	18.0
9	57	17.0	16.0	17.0	16.5	20.0	19.0
10	64	18.0	17.0	18.0	18.5	21.0	21.0

* From the day of thinning the plants in pots.

The earliest, ie on the 24th day of vegetation, appearance of the maximum growth rate was observed in sage plants in the combination of Mg2 30 % of full water volume, whereas the latest appearance of that rate occurred on the 28th day in sage in the combination of Mg1 30 % of full water volume and in Mg0 60 % of full water volume. The maximum value of the growth rate of sage plants during the first year of the studies ranged from 0.17 cm · day^{-1} (combination of Mg1 30 % of full water volume and Mg2 30 % of full water volume) to 0.25 cm · day^{-1} (combination of Mg0 60 % of full water volume) – Table 3. During the second year almost two times larger maximum

Table 3

Parameters of the logistic function approximating the elongation growth of medical sage plants from individual combinations of the experiment $h = h_{max}/(1 + b \cdot \exp(-kt))$

Experimental combination	Year of experiment	h_{max} [cm]	k [day^{-1}]	b	t_i [day]	h_i [cm]	h_0 [cm]	$(dh/dt)_{max}$ [cm · day^{-1}]	Coeficient of determination R^2
Mg0 30 %	I	15.0	$5.13 \cdot 10^{-2}$	2.19	15.24	7.50	4.71	0.19	0.964
	II	18.0	$8.18 \cdot 10^{-2}$	7.72	25.00	9.00	2.06	0.37	0.991
Mg1 30 %	I	17.0	$4.06 \cdot 10^{-2}$	1.36	7.51	8.50	7.22	0.17	0.931
	II	17.0	$8.08 \cdot 10^{-2}$	9.84	28.29	8.50	1.57	0.34	0.988
Mg2 30 %	I	16.0	$4.21 \cdot 10^{-2}$	1.73	13.07	8.00	5.85	0.17	0.947
	II	18.0	$7.91 \cdot 10^{-2}$	6.68	24.35	9.00	2.29	0.36	0.989
Mg0 60 %	I	20.0	$5.02 \cdot 10^{-2}$	2.07	14.52	10.00	6.51	0.25	0.971
	II	18.5	$7.29 \cdot 10^{-2}$	7.82	28.19	9.25	2.10	0.34	0.990
Mg1 60 %	I	18.5	$4.43 \cdot 10^{-2}$	2.22	17.98	9.25	5.75	0.21	0.958
	II	21.0	$8.61 \cdot 10^{-2}$	10.16	26.91	10.50	1.88	0.45	0.994
Mg2 60 %	I	20.0	$4.80 \cdot 10^{-2}$	2.62	20.04	10.00	5.53	0.24	0.972
	II	21.0	$7.78 \cdot 10^{-2}$	8.19	27.03	10.50	2.29	0.41	0.990

daily increases were recorded in plants in all combinations of the experiment. The largest daily maximum growth ($(dh/dt)_{max}$= 0.45 cm · day^{-1}) was characteristic of the plants in the combination of Mg1 60 % of full water volume, whereas the smallest (0.34 cm · day^{-1}) was recorded in plants in the combination of Mg1 30 % of full water volume and in Mg0 60 % of full water volume) – Table 3. From the 8th to the 20th day of vegetation during the first year of the studies and from the 24th to the 28th day during the second year (the end of the intensive growth stage) the growth rate of sage decreased gently. In both years of studies *S. officinalis* L. reached a similar maximum height, ranging from 15 cm (combination of Mg0 30 % full water volume – 1st year of studies) to 21cm (combination of Mg1 60 % of full water volume – 2nd year of studies) (Table 3). The observed final height of sage corresponds to the data found in literature [10, 11]. It should be mentioned that sage plants growing on a soil with moisture of 60 % of full water volume reached a larger maximum height in both years of studies than those growing on a soil with moisture of 30 % of full water volume. The difference in a mean height of plants in successive years amounted to 3.5 and 2.5 cm, respectively. Analyzing the elongation growth of sage in both years of studies, no significant differences were observed in its dynamics in plants to which various doses of magnesium were applied. On the whole, it can be said that the course of the elongation growth of *S. officinalis* L. was typical, ie sigmoidal. From the curves of the growth three characteristic stages can be distinguished: initial slow growth, the stage of maximum growth and final slow growth.

Conclusions

1. The elongation growth of medical sage was typical ie sigmoidal, independently of the experimental combination studied.

2. No significant differences were observed in the dynamics of the elongation growth in sage fed with differentiated doses of magnesium.

3. Large usefulness of the logistic function for the description of the growth of the researched plants was confirmed.

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WPŁYW DOGLEBOWEGO ŻYWIENIA MAGNEZEM W ZRÓŻNICOWANYCH WARUNKACH WILGOTNOŚCIOWYCH GLEBY NA DYNAMIKĘ WZROSTU ELONGACYJNEGO SZAŁWII LEKARSKIEJ (*Salvia officinalis* L.)

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Abstrakt: Celem pracy było określenie wpływu doglebowego żywienia magnezem, w zróżnicowanych warunkach wilgotnościowych gleby na dynamikę wzrostu elongacyjnego szałwii lekarskiej. Pierwszym czynnikiem doświadczalnym był poziom żywienia magnezem (0; 0,30 i 0,90 g na wazon), drugim – poziom uwilgotnienia gleby (30 i 60 % pełnej pojemności wodnej). Statystycznie potwierdzono dużą przydatność funkcji logistycznej do opisu wzrostu elongacyjnego badanej rośliny. Wzrost *Salvia officinalis* L., w obu latach badań, niezależnie od kombinacji doświadczenia miał typowy, sigmoidalny przebieg. Nie stwierdzono różnic w dynamice wzrostu elongacyjnego szałwii żywionej zróżnicowanymi dawkami magnezu.

Słowa kluczowe: szałwia lekarska, magnez, wilgotność gleby, wzrost elongacyjny, funkcja logistyczna

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PHYSIOLOGICAL RESPONSE OF STRAWBERRY (*Fragaria ananassa* Duch.) TO FOLIAR APPLICATION OF POTASSIUM AND SILICON FERTILIZER

REAKCJA FIZJOLOGICZNA TRUSKAWKI (*Fragaria ananassa* Duch.) NA DOLISTNE NAWOŻENIE POTASOWO-KRZEMOWE

Abstract: The aim of the performed studies was to assess the physiological reaction of two cultivars of strawberry to foliar application of potassium-silicon fertilizer. In 2005–2006 at the Experimental station of Orchard Department, University of Agriculture in Szczecin in Rajkowo (a locality near Szczecin), a two-factor vegetation experiment in the system of random blocks was carried out in three replications. The experimental factor was foliar application of potassium Alkaline with silicon (variant I – with fertilization, variant II – control, spraying with distilled water). Strawberry Senga–Sengana c.v. was chosen as biological material for the studies. The following gas exchange parameters of the investigated variety were determined: assimilation rate of CO_2, transpiration rate, index of water use efficiency in the photosynthesis.

Keywords: *Fragaria ananassa* Duch., potassium alkaline with silicon, assimilation, transpiration

Poland belongs to the leading strawberry growers. Along with growing popularity of the cultivation of these plants, the demand for their quality also increases. The quantity and quality of the yield are, to a large extent, determined by an appropriate supply of nutritive components and water to the plants. Foliar nutrition, being supplementation of traditional fertilization, is more and more often applied to strawberry growing. This way of supplying these elements to a plant may then result in obtaining stable yields of high quality [1].

The applied potassium Alkalin with silicon is a source of potassium and silicon, it is characterized by an alkaline reaction that has a favourable influence on limiting mainly fungous diseases. Productivity of plants is mainly dependent on their photosynthetic activity. The intensity of physiological processes in plants is mainly determined by genetic properties, but it can be modified by such factors as temperature and light intensity,

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availability of mineral components and the supply of water [2–5]. Potassium is an indispensable element directly affecting water management of plants. It affects the size and firmness of strawberry fruit. Whereas silicon, despite the fact that it does not belong to the group of indispensable elements, according to some authors, has a favourable effect on plant health, stable ion balance, biomass production and transpiration rate limitation. In plants this element is found in the cells of epidermis, sclerenchyma, mesophyll and xyleme, mainly in the cell walls [6, 7]. It is believed that the potassium silicon Alkalin increases the synthesis of salicylic acid, a growth substance released as a result of the effect of stress factors.

The objective of the studies was the assessment of physiological activity of strawberry variety Sanga–Sengana under the influence of foliar application of potassium alkaline with silicon.

Material and methods

In 2005–2006, at the Experimental station of Orchard Department, University of Agriculture in Szczecin in Rajkowo (a locality near Szczecin), a two-factor vegetation experiment in the system of random blocks was carried out in three replications. 20 plants were included in one replication. The first experimental factor was foliar application of potassium Alkaline with silicon (variant I – with fertilization, variant II – control, spraying with distilled water); the second factor (II) was the date of measurement of the physiological features examined. Strawberry Senga–Sengana c.v. was chosen as biological material for the studies. The experiment was carried out on strawberries planted in 2005 on beds covered with a polyethylene white sheet at a spacing of 0.2 m × 1 m, on the grey-brown podsolic soil of talus origin. For the irrigation of the plantation a drip T-line mounted under the polyethylene sheet was used. Irrigation needs were determined by means of a soil contact tensiometer. Potassium Alkaline with silicon (43 g of $N\text{-}NH_2$, 360 g K_2O and 15 g of $SiO_2 \cdot dm^{-3}$), was used in the form of foliar spraying with a solution of 1 % concentration, at two dates: at the beginning of April (first decade of May) and directly after flowering of plants (first decade of June).

The studies included the measurements of assimilation rate of CO_2 in the leaves (A) and transpiration rate (E). The measurements were taken at three dates: in the third decade of May (1st date), 3rd decade of June (2nd date) and 3rd decade of July (3rd date). Parameters of gas exchange were measured using a portable IRGA analyzer, an LCA-4 model (ADC Bioscentific LTD. Hoddeson, Great Britain), equipped with a PLC-4 leaf chamber, at constant lighting by a halogene lamp (Xenophot HLX, OSRAM) – 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR on the leaf surface. On the basis of the obtained results of assimilation and transpiration rates, the photosynthetic efficiency of water use (ω_W) was calculated and it was assessed by the A:E ratio.

For the comparison of the experimental objects, a two-factor ANOVA was used. In order to determine the differences between means and for the interaction, semi-intervals of Tukey confidence at a level of $\alpha = 0.05$, were calculated. For the sake of homogeneity of variance of error, a synthesis of the results of two years' studies was carried out [8]. Coefficients of linear correlation between assimilation and transpiration rates were

also computed. When the coefficient of linear correlation between variables was significant at the level of $\alpha = 0.05$, the relationship was presented in the diagrams.

Results and the discussion

Leaves of strawberry fertilized with potassium Alkaline and the control leaves were characterised by approximate rate of CO_2 assimilation. Whereas a significant effect of the date of measurement on the rate of this physiological process was shown, for the highest rate of assimilation (1.91 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was observed at the first date of the measurement (2.08 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Table 1). Some authors report that plants which take larger amounts of silicon are characterised by a smaller coefficient of transpiration. According to Brogowski [9] rice fertilized with this component shows lower transpiration by 12 to 15 %, wheat by about 10 %. The applied fertilization with potassium Alkalin and silicon did not modify significantly the intensity of the transpiration process in the strawberry variety studied, whereas a significant decrease in the intensity of this process was observed at successive dates of the measurement, both in the plants fertilized and in the control variant. The rate of transpiration at the third date (3rd decade of July) was by 23 % lower than at the first date (3rd decade of May) (Table 1).

Table 1

Intensity of CO_2 assimilation ($\mu mol \cdot m^{-2} \cdot s^{-1}$), transpiration ($mmol \cdot m^{-2} \cdot s^{-1}$) and water use photosyntetic efficiency ($mmol \cdot m^{-2} \cdot s^{-1}$) *Fragaria ananassa* Duch.

	Time			Mean II
	I	II	III	
Assimilation (A)				
Control	1.745 ab*	0.758 ab	0.500 a	1.001 a
Fertilization	2.083 b	0.683 a	0.553 a	1.107 a
Mean I	1.914 b	0.7208 a	0.527 a	
Transpiration (E)				
Control	0.515 bc	0.403 abc	0.197 ab	0.372 a
Fertilization	0.678 c	0.338 abc	0.085 a	0.367 a
Mean I	0.597 c	0.370 b	0.141 a	
Water use photosyntetic efficiency (ω_w)				
Control	2.92 a	1.88 a	5.67 ab	3.49 a
Fertilization	2.36 a	3.45 a	9.52 b	5.11 a
Mean I	2.64 a	2.66 a	7.59 b	

* Averages denoted with the same letters do not differ significantly at the level of significance $\alpha = 0.05$.

An important indicator of plant productivity is the photosynthesis of water use efficiency (ω_W) [10–12]. The results presented in the table show that despite the fact that no statistically significant differences were observed in the strawberry variety studied, larger efficiency of gas exchange (5.11) was characteristic of the plants fertilized with

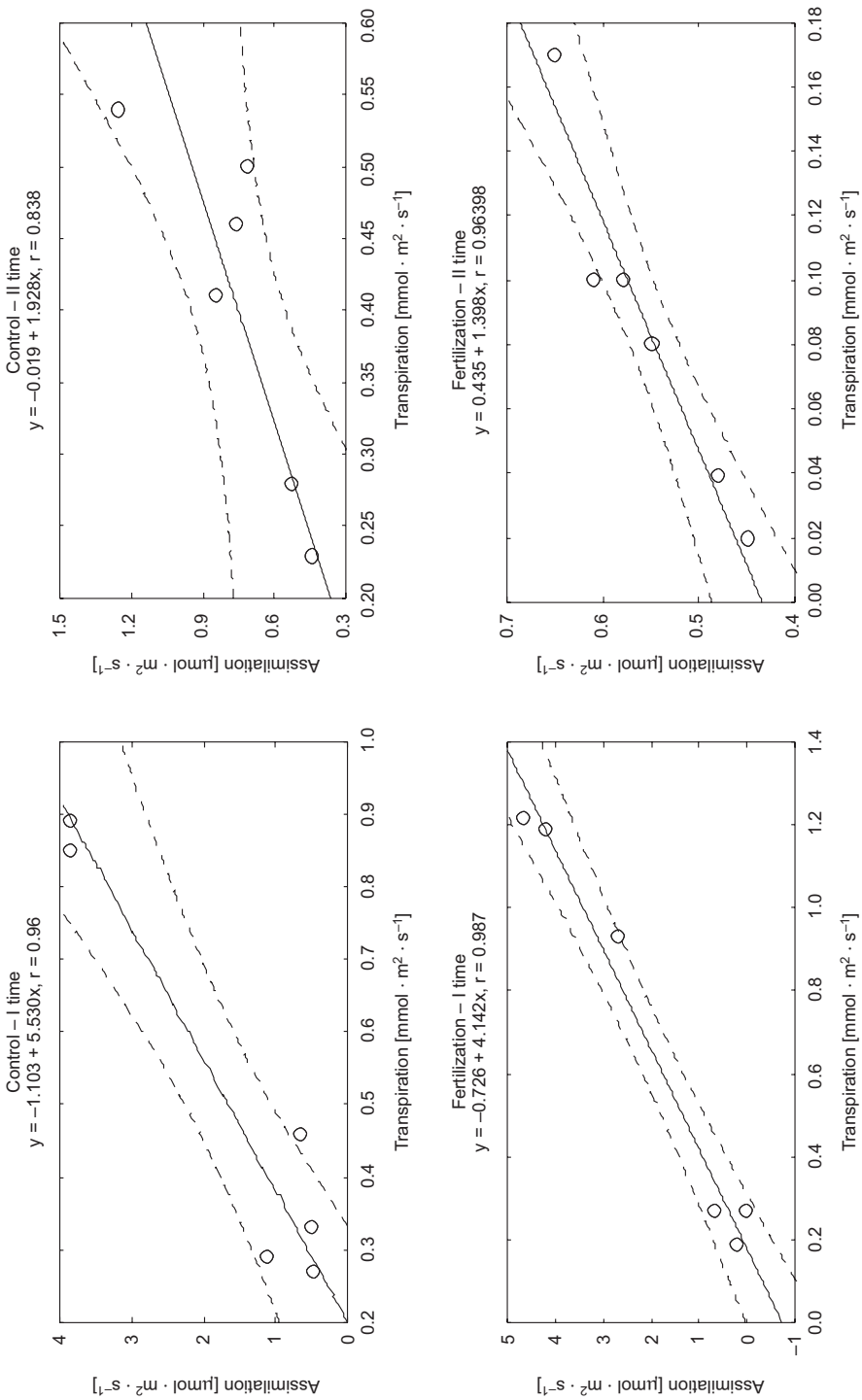

Fig. 1. Dependence of CO_2 assimilation on transpiration of *Fragaria ananassa* Duch.

potassium Alkalin. Photosynthetic efficiency of water use shown at the third date of measurement, was twice as large in comparison with the remaining two. A high value of this parameter at the third date resulted first of all from a low rate of transpiration.

On the basis of the results of the gas exchange parameters, an analysis of straight-line correlation between assimilation (A) and transpiration (E) was performed – Table 2, Fig. 1.

Table 2

Equations of linear regression and values of coefficients of correlation between the parameters of the parameters of the gaseous exchange of *Fragaria ananassa* Duch.

Character (y)	Character (x)	Time	Regression equation	Correlation coefficients (r)
Control				
A	E	I	$y = -1.103 + 5.530 \cdot x$	0.96**
		II	$y = -0.019 + 1.928 \cdot x$	0.84*
		III	—	0.73
Fertilization				
A	E	I	$y = -0.726 + 4.142 \cdot x$	0.99**
		II	—	0.34
		III	$y = 0.434 + 1.398 \cdot x$	0.96**

The analysis of correlation coefficients showed a significant positive relationship between assimilation and transpiration in strawberry at the first date of measurement, both in the plants fertilized with Alkalin and in the control variant. The correlation coefficient (r) was in these cases very high, close to the unity, and it amounted to 0.99 and 0.96, respectively. In the fertilized plants, a significant positive correlation between discussed parameters of gas exchange was also recorded at the third date of measurements (coefficient r – 0.96), while in the control variant such relationship was observed at the second date. In the other cases coefficients were insignificant.

Conclusions

1. The applied fertilization with potassium Alkalin and silicone did not modify in a significant way the processes of CO_2 assimilation and transpiration rates in the strawberry variety studied.

2. The highest rates of assimilation and transpiration in strawberry were recorded at the first date of measurement (3rd decade of May).

3. The highest photosynthetic efficiency of water use was characteristic of the investigated strawberry variety at the third date of measurement (3rd decade of July).

4. In the plants fertilized with potassium Alkalin and silicon, a significant positive correlation was observed between assimilation and transpiration at the third date of measurement.

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REAKCJA FIZJOLOGICZNA TRUSKAWKI (*Fragaria ananassa* Duch.) NA DOLISTNE NAWOŻENIE POTASOWO-KRZEMOWE

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Abstrakt: Celem przeprowadzonych badań była ocena reakcji fizjologicznej dwóch odmian truskawki na dolistne żywienie nawozem potasowo-krzemowym. W latach 2005–2006 w Stacji Doświadczalnej Katedry Sadownictwa Akademii Rolniczej w Szczecinie w miejscowości Rajkowo koło Szczecina, przeprowadzono dwuczynnikowe doświadczenie wegetacyjne, w układzie bloków losowanych, w trzech powtórzeniach. Czynnik doświadczalny stanowiło nawożenie dolistne alkalinem potasowym z krzemem (wariant I – z nawożeniem, wariant II – kontrola, oprysk wodą destylowaną). Biologiczny materiał badań stanowiła odmiana truskawki: Senga–Sengana. Oznaczono parametry wymiany gazowej badanej odmiany truskawki: intensywność asymilacji CO_2, natężenie transpiracji, wskaźnik efektywności wykorzystania wody w fotosyntezie.

Słowa kluczowe: *Fragaria ananassa* Duch**.,** alkalin potasowy z krzemem, asymilacja, transpiracja

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EFFECT OF THE DIVERSE CONCENTRATION OF SODIUM CHLORIDE IN THE MEDIUM ON THE CONTENT OF ASSIMILATION PIGMENTS AND THE BIOMETRIC FEATURES OF BASKET WILLOW (*Salix viminalis* L.) CULTIVATED IN HYDROPONICS

WPŁYW ZRÓŻNICOWANEGO STĘŻENIA CHLORKU SODU W POŻYWCE NA ZAWARTOŚĆ BARWNIKÓW ASYMILACYJNYCH I CECHY BIOMETRYCZNE WIERZBY WICIOWEJ (*Salix viminalis* L.) UPRAWIANEJ W HYDROPONICE

Abstract: In 2006 a hydroponic vegetation experiment was carried out under controlled conditions in the laboratory of the Department of Plant Physiology University of Agriculture in Szczecin. The experiment was conducted using a complete randomization method in a two-factor system in three replications Three series of experiments were performed during the period from April to June. The first experimental factor was a concentration of NaCl in the medium (0.068, 0.136 and 0.170 mol NaCl · dm^{-3}, control test – complete Hoagland's medium), whereas the other factor was a willow clone. The following biometric features were determined: the length of a shoot, the number of leaves on the shoot, fresh and dry matter of shoots. The content of assimilation dyes in the leaves of willow was also determined, 48 and 168 hours after the salinization of the media.

Keywords: *Salix viminalis*, clones 'Bjor', 'Jorr' and 'Tora', salinity stress, assimilation pigments, fresh matter, dry matter

Basket willow (*Salix viminalis*) is used in the protection of environment and in the management of wasteland. Its biomass becomes a very important and economically effective aspect of agricultural production [1]. Its characteristic feature is a low ash content (1–3 %), the ash formed during combustion can be used as a mineral fertilizer [2–4]. The phenomenon of a too strong concentration of salt in a medium resulting in

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a salt stress is caused, first of all, by anthropogenic factors, eg by application an excessive amount of salt to fertilizers or by using these substances to clear the roads of ice in winter [5, 6]. Particularly toxic to trees are chlorine and isoosmotic solutions of NaCl [7]. Considering the fact that there is not much information on the effect of salt stress on various genotypes of *Salix viminalis* in available literature, particularly in regards of a juvenile vegetative stage, studies were started in order to assess the physiological reaction of three clones of basket willow ('Bjor', 'Jorr' and 'Tora') to an increased concentration of NaCl in hydroponics.

Material and methods

Biological material for the studies consisted of three clones of basket willow (*Salix viminalis*): 'Bjor', 'Jorr' and 'Tora', the cuttings of which were from the plantation of the Department of Plant Physiology University of Agriculture in Szczecin.

The hydroponic vegetation experiment was carried out under controlled conditions in the laboratory of the Department of Plant Physiology, University of Agriculture in Szczecin in 2006. A method of complete randomization in the two factor system was used in three replications. The first experimental factor was the concentration of NaCl in a medium (0.068, 0.136 and 0.170 mol NaCl · dm^{-3}, the control test – complete Hoagland's medium), the second was a clone of willow. Three independent series of the experiment were carried out in April, May and June and three replications in each series. Willow cuttings were placed in 1 dm^3 glass containers (2 pieces in a container) filled with complete Hoagland's medium. After about 14 days when the cuttings had rooted and the shoots had reached the length of several centimeters, the concentration of NaCl in the media was differentiated according to the experimental combination.

During the juvenile period of willow, ie 168 hours (7 days) from the differentiation of the concentration of NaCl in the media, the following biometric features of the studied clones (the average of the three replications of each experimental combination) were determined: the length of each shoot, the number of leaves on a single shoot, the complete yield of fresh and dry matter of shoots in each plant.

The content of assimilation pigments in the leaves of willow were determined after 48 hours (2 days) and 168 hours (7 days) from the differentiation of the concentration of NaCl in the media, using Lichtenthaler and Wellburn's method [8]. Material for the studies was taken from three representative plants of each experimental combination.

The obtained results of the studies were verified by means of a two-factor analysis of variance in the system of complete randomization. In order to determine the differences between the averages and for the interaction, Tukey's half-intervals at confidence of $\alpha = 0.05$ were calculated. Due to the uniformity of variance of error, a synthesis of the results of three experimental series was performed.

The results and their analysis

The length of individual shoots of *Salix viminalis* growing in the media of differentiated concentration of NaCl did not differ significantly. The largest difference of the

length of a single shoot was observed between the combination of 0.170 mol NaCl · dm^{-3} (19.0 cm) and the control combination (26.4 cm). No significant differences were observed in the length of shoots of the compared clones of willow, either. A slightly larger length of the clone shoots than that of the remaining clones was observed in clone 'Jorr' (25.2 cm) – Table 1.

Table 1

The mean length of a single shoot of *Salix viminalis* [pieces]

Concentration of NaCl [mol · dm^{-3}]	Clone			Mean I
	'Bjor'	'Jorr'	'Tora'	
Control	25.2	30.0	24.2	26.4
0.068	17.0	28.0	26.7	23.9
0.136	22.5	23.8	20.0	22.1
0.170	23.2	18.8	15.0	19.0
Mean II	21.9	25.2	21.4	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns			

ns – non-significant.

A significant effect of NaCl concentration in the medium on the average number of leaves on individual shoots of basket willow was recorded, for the largest number of leaves on a shoot was characteristic of the plants growing under the controlled conditions (24 pieces) and in the medium of the smallest concentration of NaCl (21 pieces). Whereas the smallest number of leaves (18 and 16 pieces) was characteristic of the plants grown hydroponically in the medium of the following concentration: 0.136 and 0.170 mol NaCl · dm^{-3} (Table 2). Similar results of the studies were obtained by Stark [6]. She observed that the strongest inhibition of the growth of leaves occurred in the plants that were in saline stress.

Table 2

The mean number of leaves on a shoot *Salix viminalis* [pieces]

Concentration of NaCl [mol · dm^{-3}]	Clone			Mean I
	'Bjor'	'Jorr'	'Tora'	
Control	21	25	25	24
0.068	15	25	24	21
0.136	18	21	15	18
0.170	19	15	14	16
Mean II	18	21	19	
$LSD_{0.05}$ for:	concentration of NaCl (I) – 5.2 clone (II) – ns interaction I × II and II × I – ns			

ns – non-significant.

Significantly the largest yield of fresh and dry matter of shoots was obtained by willows growing under controlled conditions, whereas the smallest by those growing in the medium of 0.170 mol NaCl · dm^{-3} concentration (Figs. 1, 2). Significantly a smaller amount of fresh and dry matter of shoots than in the remaining clones, was observed in clone 'Bjor'. Comparing all the experimental variants, it can be concluded that the largest yield of fresh matter of shoots was obtained in all the clones of willow under the controlled conditions and in clones 'Jorr' and 'Tora' growing in the medium of 0.068 mol NaCl · dm^{-3} concentration, whereas the smallest yield was observed in all the clones at the highest concentration of NaCl in hydroponic media (Fig. 1). Significantly the largest content of dry matter of willow shoots was recorded under

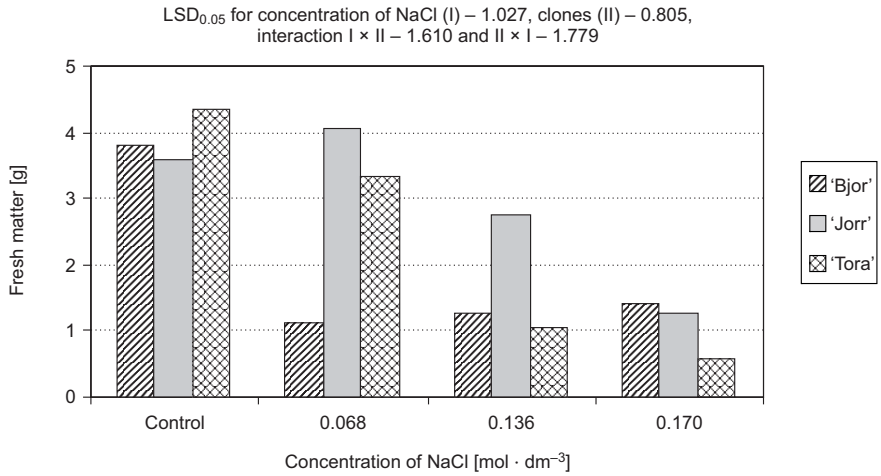

Fig. 1. The total yield of fresh matter of shoots *Salix viminalis* [g · $plant^{-1}$]

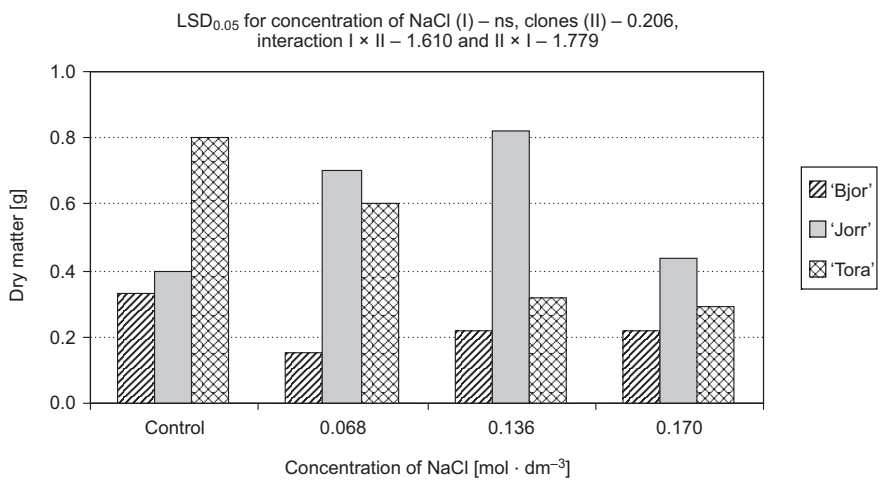

Fig. 2. The total yield of dry matter of shoots *Salix viminalis* [g · $plant^{-1}$]

controlled conditions (0.51 g), whereas the smallest, at the highest dose of NaCl (0.32 g). Significance of the influence of experimental factors on the yield of dry matter of willow shoots was also determined, for the largest dry matter was characteristic of clones 'Tora' and 'Jorr' under conrolled conditions and at the lowest concentration of NaCl and clone 'Jorr' at 0.136 mol NaCl · dm^{-3} concentration (Fig. 2).

It was observed that an increase in the concentration of NaCl in the medium resulted in a decrease in the yield of both fresh and dry matter of *Salix viminalis*. Similar results were obtained by Gregorczyk et al [1].

Under the conditions of excessive salinity of the medium, the photosynthetic apparatus of a plant is damaged. Then the efficiency of both phases of photosynthesis decreases [9]. According to many authors, the reaction to salt stress is also a decrease in the content of assimilation pigments – chlorophyll and carotenoids – in plant leaves [5, 10, 11]. At the first time of the determination (48 hours from the salinization of hydroponic media) no significant influence of experimental factors on the content of all the studied assimilation dyes in the leaves of *Salix viminalis* was observed. Whereas at the second time of the determination, the highest concentration of chlorophyll (*a*, *b* and complete), was characteristic of the leaves of willow growing in the highest concentration of NaCl. A stimulating effect of this concentration of salt on the production of chlorophyll in willow leaves was observed in clone 'Jorr' in particular. Also at this time, clone 'Jorr' was characterized by the largest amount of both chlorophyll and carotenoids, as compared with all the clones. 168 hours after the salinization of hydroponic media, a significant effect of NaCl concentration on the concentration of carotenoids in willow leaves, was also observed, for the largest amount of these dyes was recorded in plants growing in the medium of 0.170 mol NaCl · dm^{-3} concentration (Table 3).

Table 3

Content of chlorophyll *a*, *b*, *a* + *b* and carotenoids in leaves of *Salix viminalis* [mg · g^{-1} f.m.]

Clone	I time					II time				
	Concentration of NaCl [mol · dm^{-3}]				Mean II	Concentration of NaCl [mol · dm^{-3}]				Mean II
	Control	0.068	0.136	0.17		Control	0.068	0.136	0.17	
Chlorophyll *a*										
'Bjor'	1.156	0.757	1.323	0.975	1.053	1.655	1.406	1.563	0.966	1.398
'Jorr'	1.184	1.108	0.899	1.246	1.109	1.523	1.362	1.657	3.436	1.995
'Tora'	1.498	1.332	1.484	1.223	1.384	1.705	1.613	1.581	1.599	1.625
Mean I	1.280	1.066	1.235	1.148		1.628	1.460	1.600	2.000	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns					concentration of NaCl (I) – 0.408 clone (II) – 0.323 interaction I × II i II × I – 0.646 and 0.707				
Chlorophyll *b*										
'Bjor'	0.510	0.302	0.569	0.439	0.455	0.631	0.551	0.669	0.401	0.563
'Jorr'	0.524	0.498	0.382	0.616	0.505	0.607	0.546	0.727	1.745	0.906

Table 3 contd.

Clone	I time					II time				
	Concentration of NaCl [mol · dm^{-3}]				Mean II	Concentration of NaCl [mol · dm^{-3}]				Mean II
	Control	0.068	0.136	0.17		Control	0.068	0.136	0.17	
'Tora'	0.707	0.599	0.708	0.591	0.651	0.704	0.652	0.663	0.738	0.689
Mean I	0.508	0.466	0.553	0.549		0.648	0.583	0.686	0.961	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns					concentration of NaCl (I) – 0.198 clone (II) – 0.156 interaction I × II and II × I – 0.313 and 0.343				
Chlorophyll *a* + *b*										
'Bjor'	1.666	1.059	1.892	1.414	1.508	2.286	1.957	2.232	1.368	1.961
'Jorr'	1.707	1.610	1.280	1.863	1.615	2.130	1.908	2.385	5.181	2.901
'Tora'	2.206	1.930	2.192	2.008	2.084	2.410	2.265	2.243	2.336	2.314
Mean I	1.860	1.533	1.788	1.762		2.275	2.043	2.287	2.961	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns					concentration of NaCl (I) – 0.605 clone (II) – 0.478 interaction I × II and II × I – 0.956 and 1.047				
Carotenoids										
'Bjor'	0.478	0.497	0.509	0.440	0.481	0.642	0.570	0.674	0.461	0.587
'Jorr'	0.471	0.455	0.376	0.551	0.463	0.611	0.541	0.731	1.557	0.860
'Tora'	0.619	0.528	0.620	0.614	0.595	0.674	0.632	0.670	0.750	0.682
Mean I	0.523	0.493	0.502	0.535		0.642	0.581	0.691	0.923	
$LSD_{0.05}$ for:	concentration of NaCl (I) – ns clone (II) – ns interaction I × II and II × I – ns					concentration of NaCl (I) – 0.170 clone (II) – 0.134 interaction I × II and II × I – 0.269 and 0.295				

ns – non-significant.

Similar results of the studies concerning the impact of salt concentration on the content of assimilation pigments in leaves of various genotypes of basket willow were obtained by Wrobel and Gregorczyk [5]. According to these authors, clone 'Jorr' in differentiated concentrations of NaCl of the medium was distinguished by the largest amount of photosynthetic dyes.

Conclusions

1. Increasing concentration of NaCl in hydroponic media resulted in the reduction of the mean number of leaves on a shoot of basket willow.

2. Willows growing under the conditions of the highest salinity gave the lowest yield of fresh matter of shoots.

3. The applied concentration of NaCl did not cause any increases in either chlorophyll or carotenoids in willow leaves. Particularly high concentration of assimilation pigments was characteristic of clone 'Jorr'.

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WPŁYW ZRÓŻNICOWANEGO STĘŻENIA CHLORKU SODU W POŻYWCE NA ZAWARTOŚĆ BARWNIKÓW ASYMILACYJNYCH I CECHY BIOMETRYCZNE WIERZBY WICIOWEJ (*Salix viminalis* L.) UPRAWIANEJ W HYDROPONICE

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Abstrakt: Hydroponiczne doświadczenie wegetacyjne przeprowadzone zostało w 2006 roku, w kontrolowanych warunkach, w laboratorium Katedry Fizjologii Roślin Akademii Rolniczej w Szczecinie. Doświadczenie założono metodą kompletnej randomizacji w układzie dwuczynnikowym, w trzech powtórzeniach. Wykonano trzy serie doświadczeń w miesiącach kwiecień – czerwiec. Pierwszy czynnik doświadczalny stanowiło stężenie NaCl w pożywce (0,068; 0,136 i 0,170 mol NaCl · dm^{-3}, kontrola – pożywka pełna Hoaglanda), drugi natomiast klon wierzby. Określono następujące cechy biometryczne roślin: długość pędu, liczba liści na pędzie, świeża i sucha masa pędów. Oznaczono również zawartość barwników asymilacyjnych w liściach wierzby, 48 oraz 168 godzin po zasoleniu pożywek.

Słowa kluczowe: *Salix viminalis*, klony ‘Bjor’, ‘Jorr’, ‘Tora’, stres solny, barwniki asymilacyjne, świeża masa, sucha masa

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Adam RADKOWSKI[1]

EFFECT OF FERTILIZATION WITH MICROELEMENTS ON THE MACROELEMENT CONTENT IN TIMOTHY GRASS (*Phleum pratense* L.)

WPŁYW NAWOŻENIA MIKROELEMENTAMI NA ZAWARTOŚĆ MAKROELEMENTÓW W TYMOTCE ŁĄKOWEJ (*Phleum pratense* L.)

Abstract: A one-factor field experiment established by means of random block sampling, in four replicants, was located on the degraded chernozem with loess subsoil. The kind of fertilization with microelements was an assessed factor. Foliar fertilizers were applied in the form of single microelements (copper, zinc, manganese) and the multicomponent formulation Plonvit P containing elements in the form of chelates. It was found that applied fertilization had the most spectacular effect on the magnesium content in timothy grass. As a result of foliar fertilization with the multicomponent formulation, copper and manganese, significant increase of the average content of this element was observed (by 52 %, 79 %, 85 %, respectively). Moreover, it was determined that foliar fertilization with the examined microelements caused elevation of the calcium and potassium level by 31 % and 22 % (respectively) when compared with the control object. Fertilization with copper had a negative effect on the phosphorus content, whereas application of manganese negatively influenced the level of sodium. Fertilization with the multicomponent fertilizer, copper and manganese narrowed proportions between the sum of univalent and divalent cations in timothy grass.

Keywords: timothy grass, fertilization with microelements, chemical composition

Fertilization is considered as one of the most important factors influencing the content of mineral components in timothy grass [1]. Not only fertilization with macroelements is of great importance but also application of microelements [2–4]. These components are responsible for regulation of enzymatic processes proceeding in plants as well as affect the content of macroelements [5]. The quality of plant yield is influenced not only by the mineral component content but also by the proportions between them [6].

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The aim of the three-year study was an estimation of foliar fertilization with microelements in the form of a chelated multicomponent preparation or single microelement on the macroelement content and their proportions in timothy grass.

Material and methods

The 3-year field experiment was run in the Plant Cultivation Station in Skrzeszowice near Krakow, in the years 2004–2006. The experiment was set up by the method of random blocks as one-factor, in four replicants, on the degraded chernozem. The content of assimilable forms of phosphorus, potassium, zinc, manganese and copper was characterised with average level.

In the research the following forms of microelements were applied as foliar fertilizers:

– Zinc chelate 14 % Zn (chelator EDTA + DTPA) in a dose of 100 g Zn · ha^{-1} for each swath.

– Manganese chelate 14 % Mn (chelator EDTA + DTPA) in a dose of 100 g Mn · ha^{-1} for each swath.

– Copper chelate 12 % Cu (chelator EDTA + DTPA) in a dose of 60 g Cu · ha^{-1} for each swath.

– Plonvit P in a dose of 2 dm^3 · ha^{-1}.

Plonvit P is a multicomponent, concentrated microelement fertilizer containing elements in the chelated form. Doses of single microelements and the dose of Plonvit P were adjusted in such proportions as to achieve the same content of selected microcomponents in the single fertilizers or in the multicomponent preparation. The fertilizer dose of 2 dm^3 recommended by the producer, contains: 100 g Zn, 100 g Mn and 60 g Cu. The solutions for spraying were prepared by dissolution of respective doses of microelements chelates in such an amount of water as to achieve the working fluid volume of 300 dm^3 · ha^{-1}. Tap water of medium hardness was used for this purpose. Spraying of plants was done annually as follows: I spraying – in spring, after the beginning of the vegetation period, then after the harvesting during the first stage of the sward regrowth, but not later than 3 weeks before the following mowing. In each year of the experiment the following basic mineral fertilization was performed: under the I swath 80 kg N · ha^{-1}, for II and III 60 kg N · ha^{-1} (for each swath) as ammonium saltpetre, phosphorus once in the spring in the amount of 120 kg P_2O_5 · ha^{-1} as triple superphosphate and potassium for the first and third regrowth 60 kg K_2O · ha^{-1} (each swath) as 57 % potassium salt.

In the experiment timothy grass of the Skald cultivar was cultivated on the fields of the 10 m^2 area. The harvested plant material was each time subjected to the analysis of the forage chemical composition. The dry matter content was determined by the drying method at 105 ^oC. The phosphorus and magnesium content was analysed by the colorimetric vanadium-molybdenic method, whereas the content of potassium, sodium and calcium using the flame photometry method [7].

On the basis of the results obtained proportions between the sum of univalent and divalent cations (K + Na):(Mg + Ca) in timothy grass were determined.

Presentation of the results was limited to the average values from all investigated years. All results were subjected to the analysis of variance considering the Tukey test at a significance level of $\alpha = 0.05$.

Results and discussion

This work revealed the important influence of applied fertilization on the content of microelements in timothy grass (Table 1). As a result of foliar spraying with mangenese and multicomponent fertilizer significant increase of the phosphorus content in timothy grass was found. Fertilization with the multicomponent preparation, copper, zinc and manganese contributed to a significant increase of the potassium content. The differences of its level in comparison with the control object amounted to 16, 21, 38 and 14 %, respectively.

Table 1

Weighted mean of macroelements and ionic proportions in timothy grass as affected by the fertilization with microelements (means for three years of the study)

Examined parameter	Fertilized objects					Mean	$LSD_{0.05}$
	Control	Multicomponent fertilizer	Cu	Zn	Mn		
P content [g · kg^{-1} d.m.]	2.40	2.66	2.13	2.39	2.56	2.43	0.252
K content [g · kg^{-1} d.m.]	21.71	25.27	26.37	30.02	24.65	25.61	3.771
Ca content [g · kg^{-1} d.m.]	1.92	3.34	1.92	2.79	2.03	2.40	0.869
Mg content [g · kg^{-1} d.m.]	1.70	2.58	3.04	1.83	3.15	2.46	0.780
Na content [g · kg^{-1} d.m.]	0.175	0.204	0.181	0.328	0.167	0.21	0.085
(K+Na) : (Ca+Mg)	2.38	1.73	1.97	2.69	1.77	2.06	0.520

In our study we found that foliar application of the multicomponent fertilizer as well as zinc and manganese resulted in significant growth of the calcium content in timothy grass (74, 45 and 6 %, respectively) when compared with the control object.

As a result of fertilization with the multicomponent preparation, copper, zinc and manganese a significant elevation of the magnesium level was achieved, whose concentration increased respectively by 52, 79, 8 and 85 % in comparison with its content in timothy grass harvested from the control, non-fertilized object. Magnesium is an important grass component, its presence in a plant affects the metabolism and energy transformation. Magnesium takes place in about 300 enzymatic reactions but also constitutes the active center of a chlorophyll molecule [6]. Copper plays an important role in chlorophyll synthesis and stabilization. Manganese acts in the process of photosynthesis, in absorption and assimilation of several nutrients as well as in regulation of plant hormones [8, 9].

In the present work a visible impact of foliar application of the multicomponent fertilizer and zinc preparation on the higher sodium level in timothy grass was observed. The difference in relation to the control object amounted to 17 and 87 %, respectively. The physiological role of sodium in plants is not well known, however according to

physiologists this element, like zinc, acts in transformational processes of nitrogen compounds in plants.

The quality of harvested plant crops is defined not only on the basis of optimal levels of particular elements but, when estimating the feeding value, relations between them are also of great importance [10, 11].

On the basis of the results obtained it was concluded that fertilization with the multicomponent fertilizer, copper and manganese narrowed proportions between the sum of univalent and divalent cations (K + Na):(Mg + Ca) (Table 1). It is worth emphasizing that the lower value of the mentioned above ratio occurred in the timothy grass sprayed with the solution of multicomponent preparation and with manganese solution.

Conclusions

1. Foliar fertilization with microelements positively influenced the potassium, calcium, magnesium and sodium content in timothy grass.

2. Significant increase of potassium and sodium content in timothy grass was achieved after foliar application of zinc, whereas the calcium content was positively affected by the treatment with Plonvit P and zinc and magnesium after sprying plants with copper and manganese preparations.

3. Timothy grass spraying with microelements in the form of the multicomponent preparation – Plonvit P contributed to the significant increase of the calcium and magnesium content.

4. Copper and zinc fertilization led to the reduction of the phosphorus content in the examined plant material.

5. Fertilization with the multicomponent fertilizer, copper and manganese narrowed proportions between the sum of univalent and divalent cations in timothy grass.

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WPŁYW NAWOŻENIA MIKROELEMENTAMI NA ZAWARTOŚĆ MAKROELEMENTÓW W TYMOTCE ŁĄKOWEJ (*Phleum pratense* L.)

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Abstrakt: Jednoczynnikowe doświadczenie polowe założono metodą losowanych bloków, w czterech powtórzeniach, na czarnoziemie zdegradowanym o podłożu lessowym. Czynnikiem doświadczenia był rodzaj nawożenia mikroelementowego. Stosowano dolistnie pojedyncze mikroelementy miedzi, cynku i manganu oraz wieloskładnikowy nawóz Plonvit P, zawierający pierwiastki w formie schelatowanej. Wykazano, że zastosowane nawożenie największy wpływ wywierało na zawartość magnezu w tymotce łąkowej. W wyniku dolistnego stosowania wieloskładnikowego nawozu, miedzi i manganu stwierdzono znaczny wzrost średniej zawartości tego pierwiastka – w porównaniu z obiektem nienawożonym – odpowiednio o 52 %, 79 % i 85 %. Stwierdzono, że nalistne stosowanie badanych mikroelementów spowodowało średnio wzrost zawartości wapnia i potasu odpowiednio o 31 % i 22 % w porównaniu z obiektem kontrolnym. Wykazano, że nawożenie miedzią spowodowało spadek zawartości fosforu, a nawożenie manganem zmniejszenie zawartości sodu w stosunku do kontroli. Nawożenie wieloskładnikowym nawozem, miedzią i manganem zawęziło stosunek sumy kationów jednowartościowych do sumy kationów dwuwartościowych w tymotce łąkowej.

Słowa kluczowe: tymotka łąkowa, nawożenie mikroelementami, skład chemiczny

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Adam RADKOWSKI[1] and Iwona RADKOWSKA[2]

QUALITY AND NUTRITIONAL VALUE OF SILAGES MADE FROM GRASSES DERIVED FROM THE FARMS LOCATED IN THE REGION OF KRAKOW-CZESTOCHOWA JURA PART II. CONTENT OF MACROELEMENTS

JAKOŚĆ I WARTOŚĆ POKARMOWA KISZONEK SPORZĄDZONYCH Z TRAW POCHODZĄCYCH Z GOSPODARSTW POŁOŻONYCH NA TERENIE JURY KRAKOWSKO-CZĘSTOCHOWSKIEJ CZ. II. ZAWARTOŚĆ MAKROELEMENTÓW

Abstract: This paper presents an estimation of the mineral composition of silages derived from farms specialized in milk production from the region of Krakow-Czestochowa Jura. The samples of silages, four from each farm, were collected for the chemical analysis before grazing. The phosphorus and magnesium content was estimated by the colorimetric, vanadium-molybdenic method, whereas the potassium, sodium and calcium content using flame photometry.

Silages from all investigated farms did not reach the optimal P, Ca and Na content, which indicates too low a level of phosphorous fertilization as well as limited liming of grasslands located in the investigated farms. On the other hand, an excessive amount of potassium results from fertilization with liquid manure, leading to the accumulation of this element, which is calcium and magnesium antagonist.

Proportions between mineral component content in the examined silages were diversified. Only 42 % of samples had Ca:Mg weight ratio and K: (Ca + Mg) ionic ratio at the optimal level and 33 % of silage trials were characterised with the optimal K:Mg ratio. The other proportions, especially K:Ca and K:Na, were unfavourably too high.

Keywords: silages, macroelement content, antagonism of elements

Among all chemical components of silages derived from grasslands not only organic compounds but also macroelements play an important role in animal feeding [1, 2]. 80–85 % of the total phosphorus content of animal organisms occurres in blood and

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bones. Deficiency of this element has a negative effect on the appetite, animal growth, process of ossification, intensity of metabolism and animal reproduction. Potassium can lower the level of magnesium and calcium, what can lead to grass tetany occurrence in animals. Calcium in animal organisms can be found in bones, which contain about 98 % of its whole content. Magnesium deficiency induces response from the nervous system, convulsions and tetany. On the other hand, sodium deficiency affects perturbations of water balance, blood pressure drop, excessive licking behavior, hematuria, muscle tremors and fertility disturbances [3, 4].

The aim of the present paper was an estimation of the mineral composition of silages from farms specialized in milk production in the region of Krakow-Czestochowa Jura, situated in the southern part of Poland.

Material and methods

The research was conducted under production conditions in 12 farms specialized in milk production in the region of Krakow-Czestochowa Jura.

The examined silages were derived mostly from the first swath of grass flora, less frequently from the second and third swath. The material was harvested in two stages, first the green fodder was mown at the turn of stages of heading and flowering of grasses (silages from the first swath) with a rotational mower and then the fodder was shortly dried by single teding. The material was raked 30 minutes before collection. Harvesting was done with a constant chamber baler and the bales formed were transported to the storage place, where they were wrapped using the wrapping machine. Average time from the bale forming to the protection with plastic wrapping never exceeded 4 hours.

Before feeding samples of silages were collected (4 from each farm) for chemical analysis, which comprise: the phosphorus and magnesium content estimated by the colorimetric, vanadium-molybdenic method as well as the potassium, sodium and calcium content using the flame photometry method.

Presentation of the results was limited to the average values from all investigated years. The results obtained were subjected to the analysis of variance and significance of differences was estimated by the Duncan test at the significance level of $\alpha = 0.05$.

Results and discussion

The content of mineral components in the examined silages was diversified. The weighted mean macroelement content fluctuated in the range of: 1.39–2.68 g P; 24.84–68.38 g K; 3.03–6.26 g Ca, 1.42–3.16 g Mg; 0.35–0.88 g Na $\cdot$ kg^{-1} d.m. (Table 1). However, forage of high quality should contain at least 3.0 g P; 17–20 g K; 7.0 g Ca; 2.0 g Mg i 1.5–2.5 g Na $\cdot$ kg^{-1} d.m. [1, 5]. In the conducted study the potassium content in silages from all farms reached desired values, whereas the magnesium content was at the satisfactory level in 58 % of all samples. On the other hand, phosphorus, calcium and sodium amounts were below the optimal level.

Table 1

Weighted mean of macroelement content and ionic proportions in silages

Item	Investigated farm											
	1	2	3	4	5	6	7	8	9	10	11	12
	[g · kg^{-1} d.m.]											
P content [g · kg^{-1} d.m.]	1.39 a*	2.24 b	2.68 c	2.53 c	1.87 ab	2.16 b	2.57 c	2.21 b	2.37 bc	1.79 ab	1.47 a	2.14 b
K content [g · kg^{-1} d.m.]	39.99 ab	25.93 a	68.38 c	28.28 a	46.68 b	24.84 a	51.73 bc	29.14 a	34.34 ab	30.92 a	44.82 b	30.83 a
Ca content [g · kg^{-1} d.m.]	4.32 b	3.25 a	6.26 c	3.03 a	3.35 a	3.66 ab	3.57 ab	3.18 a	3.89 ab	3.70 ab	4.89 b	3.50 ab
Mg content [g · kg^{-1} d.m.]	1.64 a	1.70 a	2.96 b	1.42 a	1.64 a	2.36 ab	2.14 ab	1.86 a	2.29 ab	3.16 b	2.97 b	2.22 ab
Na content [g · kg^{-1} d.m.]	0.44 a	0.46 a	0.88 b	0.46 a	0.35 a	0.80 b	0.42 a	0.40 a	0.58 ab	0.79 b	0.58 ab	0.47 a
Ca:Mg	2.63 c	1.91 bc	2.11 c	2.13 c	2.04 c	1.55 b	1.67 b	1.71 b	1.70 b	1.17 a	1.65 b	1.58 b
K:Mg	7.63 b	4.77 a	7.23 b	6.23 ab	8.91 c	3.29 a	7.57 b	4.90 a	4.69 a	3.06 a	4.72 a	4.35 a
K:Ca	4.74 ab	4.09 a	5.60 b	4.78 ab	7.14 c	3.48 a	7.43 c	4.70 ab	4.52 a	4.28 a	4.70 ab	4.51 a
K:(Ca + Mg)	2.92 b	2.19 a	3.14 b	2.70 b	3.95 c	1.68 a	3.73 c	2.39 a	2.29 a	1.78 a	2.35 a	2.21 a
K:Na	53.44 bc	33.14 ab	45.69 b	36.15 ab	78.42 c	18.26 a	72.42 c	42.83 b	34.81 ab	23.01 a	45.44 b	38.57 ab

* Means marked with the same letter are not statistically different following verification with Duncan test ($p = 0.05$).

Quantitative weight or ionic ratios between elements are crucial to the estimation of forage quality [6]. The Ca:Mg weight ratio, which should amount to 2–3:1, was on an appriоprate level only in the case of 42 % of samples. About 33 % of silage samples had optimal K:Mg weight proportions (close to 6–8:1) as opposed to other trials, which were characterised by its unproper range. The 2:1 K:Ca weight ratio is assumed as optimal, in our research it was always very high. The K:(Ca + Mg) ionic proportions in good forage derived from grasslands should range between 1.6–2.2:1 [7]. It was stated that 42 % of samples fulfilled these requirements. The K:Na weight ratio in high quality fodder should amount to 5–7:1 [7]. In the examined forage its range was too wide, from 4 to 11 times higher than the optimal value. Serious sodium deficiency in silages was the major reason of poor forage quality.

The fact of a low content of mineral components in analysed silages can be due to unproper fertilization of the grassslands in examined farms. Low phosphorus and calcium fertilization was determined, which indicates application of liquid manure, which is an excessive product in such farms. Under high treatment with this fertilizer higher yield is achieved but on the other hand the effect of component dilution ocurres. This phenomenon is accompanied by the changes in nutrient availability due to strong acidification of soil and is manifested by the lower level of basic cations, especially Ca and Mg in plants [5]. As a result most of the investigated silages were characterised with unfavourable weight and ionic proportions.

Complementation of macroelement deficiency, especially P, Ca and Na, with mineral formulas in the diet of ruminents is highly recommended in examined farms [8].

Conclusions

1. Silages from all of the investigated farms did not reach the optimal P, Ca, Na content, which indicates too low a level of phosphorous fertilization as well as limited liming of the grasslands located in the investigated farms.

2. An excessive level of potassium indicates that grasslands were fertilized with liquid manure. As a result there occurred accumulation of this element which is calcium and magnesium antagonist.

3. The Ca:Mg weight ratio and the K:(Ca + Mg) ionic ratio in 42 % of samples as well as the K:Mg weight proportion in 33 % of samples reached the optimal level. Other proportions between elements were characterised by unfavourable values.

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JAKOŚĆ I WARTOŚĆ POKARMOWA KISZONEK SPORZĄDZONYCH Z TRAW POCHODZĄCYCH Z GOSPODARSTW POŁOŻONYCH NA TERENIE JURY KRAKOWSKO-CZĘSTOCHOWSKIEJ CZ. II. ZAWARTOŚĆ MAKROELEMENTÓW

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Abstrakt: Praca prezentuje ocenę składu mineralnego kiszonek z gospodarstw specjalizujących się w produkcji mleka z terenu Jury Krakowsko-Częstochowskiej. Przed skarmianiem z kiszonek pobrano próbki (po 4 z każdego gospodarstwa) do analizy chemicznej. Zawartość fosforu i magnezu oznaczono kolorymetrycznie metodą wanadowo-molibdenową, potasu, sodu i wapnia metodą fotometrii płomieniowej.

Kiszonki ze wszystkich gospodarstw nie miały optymalnej zawartości P, Ca, Na, wskazuje to na zbyt niskie nawożenie fosforem i ograniczenie wapnowania użytków zielonych w badanych gospodarstwach. Z kolei zbyt wysoka zawartość potasu wskazuje na nawożenie użytków zielonych gnojowicą. W wyniku tego nawożenia następuje kumulacja potasu, który jest antagonistą wapnia i magnezu.

Wartość stosunków ilościowych między pierwiastkami w kiszonkach była zróżnicowana. Jedynie stosunek masowy Ca:Mg i jonowy K:(Ca + Mg) w 42 % próbek oraz stosunek masowy K:Mg w 33 % próbek kiszonek miał wartość optymalną. Pozostałe stosunki, szczególnie K:Ca i K:Na odznaczały się niekorzystnymi wartościami – zbyt wysokimi.

Słowa kluczowe: kiszonki, zawartość makroelementów, antagonizm pierwiastków

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IMPACT OF THE WAY OF LAND USE ON DEHYDROGENASE ACTIVITY IN THE SOILS OF THE SILESIAN FOOTHILLS

WPŁYW SPOSOBU UŻYTKOWANIA NA AKTYWNOŚĆ DEHYDROGENAZ GLEB POGÓRZA ŚLĄSKIEGO

Abstract: The objective of the research was to check the relations between dehydrogenase activity and the way of land use. Soil material collected from 6 soil profiles located in the area of the Silesian Foothills was used in the research work. The results showed higher dehydrogenase activity in the surface horizons of grassland soils (12.40–17.34 $cm^3 \cdot kg^{-1} \cdot 24^{-1}$) than in the analogous horizons of arable soils (4.58–7.06 $cm^3 \cdot kg^{-1} \cdot 24^{-1}$). It was observed that in all profiles dehydrogenase activity was higher in surface horizons than in the deeper ones. Dehydrogenase activity depended mainly on the way of land use and the soil concentrations of organic carbon and total nitrogen.

Keywords: Silesian Foothills, soil biological activity, dehydrogenases, land use

Enzymatic activity is one of the indirect forms of soil biological activity assessment. The source of enzymes in soil are microorganisms, plant roots and soil fauna, but the main role in creating enzymatic activity is ascribed to microorganisms [1]. Dehydrogenases occurring in live cells are a group of oxyreductases responsible for the catalysis of organic compound oxidation reaction, which makes them a good indicator of the intensity of respiration metabolism in soil microorganisms [2]. Dehydrogenase activity depends on moisture, temperature and soil reaction but also on its concentrations of organic carbon, micro- and macroelements [3]. Moreover it is also affected by the heavy metal content in soil [4].

The research was conducted to determine the impact of the way of land use on soil enzymatic activity through comparing dehydrogenase activity in individual horizons of soil profiles used as arable lands and meadows, as well as testing the dependency between their activity and essential chemical soil properties.

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Materials and methods

The work used soil material collected from 6 soil profiles formed from weathering material of the Silesian unit of the Carpathian Flysch, localized in the Silesian Foothills. Soil pits were arranged in pairs (P1R-P1Z, P2R-P2Z, P3R-P3Z) on the neighbouring arable lands and grasslands so as to eliminate the influence of soil forming factors, other than land use. The soil samples were subjected to standard soil analyses to determine their texture (using the areometric-sieve method acc. to PN-R-04032), pH in H_2O and KCl (using electrometric method), total exchangeable bases (TEB) through determining individual cations after their extraction from the soil with CH_3COONH_4 (where Ca^{2+}, K^+ and Na^+ were assessed using flame photometry and Mg^{2+} using AAS method), hydrolytic acidity (by Kappen method), organic carbon content (by means of modified oxydimetric Tiurin's method), total nitrogen (using Kjeldahl method) and dehydrogenase activity (using Caside et al method) [5].

Spearman rank coefficients between dehydrogenase activity and analyzed soil properties were computed as part of the statistical elaboration of results.

Results and discussion

The analyzed soils belong to brown soils – *Haplic Cambisols* (*Eutric*) and to pseudogley soils – *Haplic Stagnosols* [6, 8]. The soils were characterized by a texture of silt loam and sandy silt (PN-R-04032) and their pH varied from very acid to slightly acid (Table 1). The soils differed in their cation exchange capacity (CEC) in the top horizons of P1R and P2R used as arable lands (and limed), which was visibly higher than in the analogous parts of P1Z and P2Z soil profiles managed as meadows (without liming) (Table 1). In P3R and P3Z profiles the base saturation ratio (V) was approximate and reached about 60 % in the surface horizons.

Dehydrogenase activity in the top A1h horizons of sodded soils (12.40–17.43 cm^3 $H_2 \cdot kg^{-1} \cdot 24^{-1}$) was between twice and thrice higher than in deeper A2h humus horizons of these soils (4.61–5.93 cm^3 $H_2 \cdot kg^{-1} \cdot 24^{-1}$) and in the arable Ap layer of the arable lands (4.58–7.06 cm^3 $H_2 \cdot kg^{-1} \cdot 24^{-1}$). The sod horizon of the limed soil (P3Z) revealed the highest value of enzymatic activity. These results confirm an important role of roots in developing enzymatic activity, among others mentioned by Pancholy and Rice [7]. The rhizosphere is characterized by a numerous population of microorganisms accompanied by heightened activity of fauna feeding on microorganisms and roots.

In the subsurface horizons of the analyzed soils dehydrogenase activity was much lower (0.05–1.51 cm^3 $H_2 \cdot kg^{-1} \cdot 24^{-1}$) and did not reveal diversification in individual soil pairs. The only exception was the 55–69 cm horizon of the P3Z profile which revealed higher enzymatic activity (Fig. 1) and content of C_{org} and N_{tot} (Table 1). It might have been caused by numerous fauna – turbulences occurring there. The obtained results were in accordance with those obtained by Doran [after 7], which demonstrated that activity of dehydrogenases, water content, organic substance, organic C and total N

were higher in the topsoil horizons of soils fallowed for long periods of time than in cultivated soils. In lower situated horizons these regularities were not observed.

Table 1

Selected chemical properties of studied soils

Profil	Depth [cm]	Horizon	pH		DHA [cm^3 $H_2 \cdot kg^{-1} \cdot 24^{-1}$]	H_h	S	CEC	BS	$C_{org.}$	$N_{tot.}$
			H_2O	KCl		[mmol(+)kg^{-1} soil]			[%]		
PR1	0–22	Ap	6.2	5.0	4.58	53.7	95.4	149.11	64.0	1.75	0.22
	22–35	Ah	6.3	5.1	5.21	50.8	98.9	149.64	66.1	1.77	0.19
	35–45	Bbr1C	6.3	4.9	1.30	41.8	71.7	113.51	63.2	0.67	0.08
	45–61	Bbr2C	6.1	4.7	0.49	35.8	71.1	106.93	66.5	0.50	0.09
	50–75	IIBbrC	5.8	4.5	0.30	34.3	50.1	84.4	59.3	0.25	0.04
P1Z	0–10	A1h	5.5	4.4	12.40	74.6	62.2	136.82	45.5	2.16	0.22
	10–22	A2h	6.0	4.7	5.93	50.8	72.3	123.08	58.8	1.43	0.17
	22–40	B1br	6.1	4.6	1.26	44.8	69.3	114.03	60.7	0.62	0.07
	40–57	B2br	5.9	4.4	0.72	44.8	56.1	100.89	55.6	0.56	0.09
	57–87	IIBbrC	5.9	4.7	0.09	35.8	47.1	82.9	56.8	0.31	0.08
P2R	0–25	Ap	5.7	4.3	7.06	56.7	78.6	135.30	58.1	1.68	0.17
	25–44	A/Gg	4.9	4.0	0.15	38.8	67.0	105.85	63.3	0.34	0.06
	44–62	G1g	4.7	3.8	0.11	58.2	61.4	119.58	51.3	0.19	0.05
	62–87	G2g	5.8	4.5	0.30	77.6	44.9	122.48	36.6	0.16	0.04
P2Z	0–8	A1h	5.5	4.3	14.05	88.1	43.4	131.43	33.0	2.40	0.21
	8–25	A2h	5.3	3.9	4.61	65.7	53.6	119.29	45.0	1.69	0.17
	25–58	G1g	5.1	3.7	0.18	56.7	66.5	123.23	54.0	0.16	0.02
	58–84	G2g	5.2	3.6	0.34	71.6	50.3	121.91	41.2	0.10	0.03
	84–125	G3g	5.1	3.7	0.05	65.7	60.7	126.39	48.0	0.12	0.02
P3R	0–20	Ap	5.6	4.2	5.95	58.2	89.0	147.24	60.5	1.69	0.2
	20–41	G1g	5.3	4.0	0.82	38.8	51.1	89.9	56.8	0.38	0.06
	41–62	G2g	5.0	3.7	0.05	53.7	68.3	122.00	56.0	0.21	0.03
	62–80	C1g	4.9	3.5	0.09	71.6	69.0	140.65	49.1	0.22	0.05
P3Z	0–8	A1h	5.6	4.2	17.34	65.7	98.6	164.27	60.0	2.44	0.29
	8–32	A2h	5.6	4.1	5.74	71.6	74.7	146.34	51.1	1.84	0.20
	32–55	A2h/Gg	5.7	4.2	0.52	62.7	74.3	136.99	54.2	0.68	0.10
	55–69	Gg	5.2	3.8	1.51	59.7	65.8	125.45	52.4	0.84	0.11
	69–95	C1g	5.2	3.8	0.13	59.7	63.7	123.39	51.6	0.22	0.04

DHA – dehydrogenase activity, H_h – hydrolitic acidity, S – total exchangeable bases, CEC – cations exchange capacity, BS – base saturation.

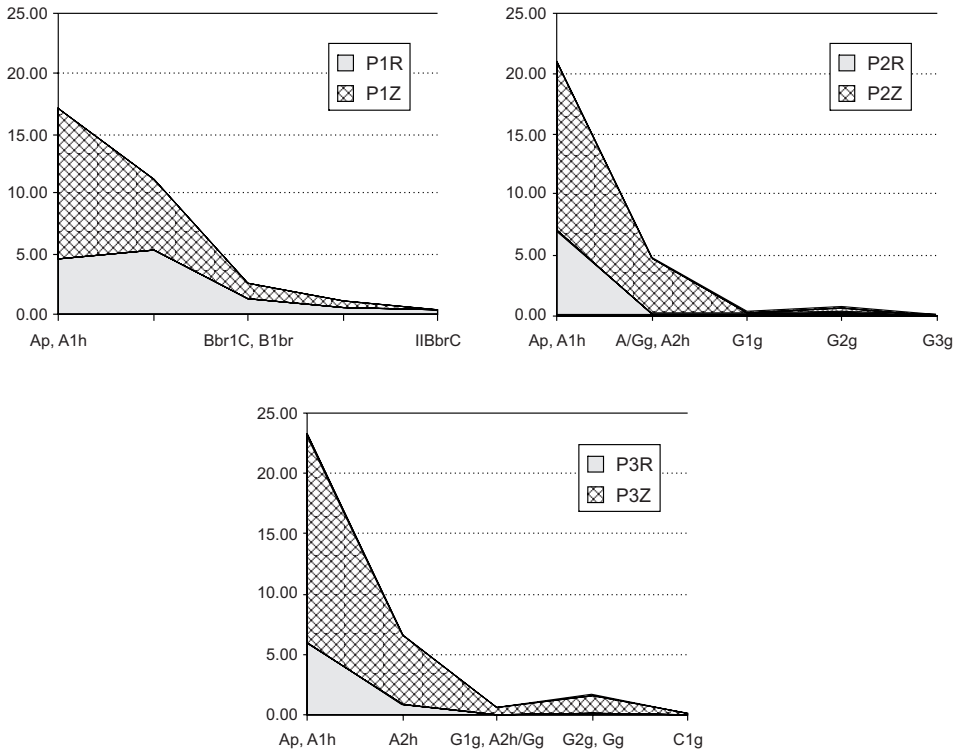

Fig. 1. Comparison of dehydrogenase activity in selected pairs

The computed Spearman rank coefficients confirmed a high and statistically significant dependency between dehydrogenase and organic carbon activity ($r = 0.8783$) and total nitrogen ($r = 0.8675$).

Conclusions

1. Dehydrogenase activity in soils depended on the way of land use – it was much higher in the surface horizons of sodded soils than in the analogous horizons of arable lands.

2. Surface horizons of the analyzed soils were characterized by much higher dehydrogenase activity than the subsurface horizons.

3. Dehydrogenase activity to a great degree depended on organic carbon and total nitrogen content in soil.

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WPŁYW SPOSOBU UŻYTKOWANIA NA AKTYWNOŚĆ DEHYDROGENAZ GLEB POGÓRZA ŚLĄSKIEGO

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Abstrakt: W przeprowadzonych badaniach sprawdzono zależności pomiędzy aktywnością dehydrogenaz a sposobem użytkowania gleb. W pracy wykorzystano materiał glebowy z 6 profili glebowych, zlokalizowanych na terenie Pogórza Śląskiego. Aktywność dehydrogenaz w powierzchniowych poziomach gleb zadarnionych była większa (12,40–17,34 $cm^3H_2 \cdot kg^{-1} \cdot 24^{-1}$) niż w analogicznych poziomach gleb gruntów ornych (4,58–7,06 $cm^3H_2 \cdot kg^{-1} \cdot 24^{-1}$). We wszystkich profilach stwierdzono większą aktywność dehydrogenaz w powierzchniowych poziomach w porównaniu do poziomów niżej leżących. W badanych glebach aktywność dehydrogenaz zależała głównie od sposobu użytkowania oraz od zawartości węgla organicznego i azotu ogólnego.

Słowa kluczowe: Pogórze Śląskie, aktywność biologiczna gleb, dehydrogenazy, sposób użytkowania

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 10 2010

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WATER AND IONIC BALANCE IN THE LEAVES OF BASKET WILLOW (*Salix viminalis* L.) CULTIVATED IN HYDROPONICS WITH DIFFERENT SALINITY LEVELS

BILANS WODNY ORAZ JONOWY W LIŚCIACH WIERZBY WICIOWEJ (*Salix viminalis* L.) UPRAWIANEJ W KULTURACH WODNYCH O RÓŻNYM STOPNIU ZASOLENIA

Abstract: In the study, a significant effect of different NaCl concentration in Hoagland's medium was showed on the water and ionic balance in the leaves of three clones of *Salix viminalis* L., ie 'Bjor', 'Jorr' and 'Tora'. The measurements of water indicators (RWC and WSD) as well as the content of monovalent (K^+ and Na^+) and bivalent (Ca^{2+} and Mg^{2+}) cations in the leaves of basket willow clones allow to conclude that under high salinity of hydroponics with sodium chloride the 'Bjor' clone was characterised by more favourable water and ionic balance when compared with the 'Jorr' and 'Tora' clones, as well as by effective mechanism of decreasing the uptake of sodium ions in leaves under the highest salt concentration in the medium. The results indicate that the 'Bjor' clone is best adapted to survival under salt stress conditions.

Keywords: hydroponics, salinity, *Salix viminalis* L., water balance, ionic balance

The basket willow (*Salix viminalis* L.), know also as the energy willow, is characterised not only by a quick and large increase in biomass but also by a broad tolerance to unfavourable environmental conditions. Considering its specific genetic and physiological properties, this plant can be used both for energy production purposes and reclamation of anthropogenically degraded land [1–3]. At present, one of the main stressors for plants is substratum salinity induced by excessive fertilisation or NaCl use for glazed ice control. The most frequently used compound, and at the same time the most toxic one, is sodium chloride. The excess of sodium and chlorine ions in nutritive environment induces a decrease in water chemical potential in the soil solution,

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reducing the same its availability for plants. Furthermore, it disturbs ion economy and normal plant feeding, bringing about in addition the oxidative stress [4–6].

Due to little information in the available literature on the effect of salt stress on new forms of energy willow, studies were taken with the aim to determine the volume of changes in the water and ionic balance in three clones of *Salix viminalis* under the influence of different NaCl doses added to hydroponics.

Material and methods

The biological study material was three clones of basket willow (*Salix viminalis* L.): 'Bjor', 'Jorr' and 'Tora', coming from a plantation of the Department of Plant Physiology of the Agricultural University in Szczecin. In 2006–2007, a hydroponics experiment was carried out in the Vegetation Room of the Agricultural University in Szczecin under controlled conditions. The experiment was set up as a two-factor one in randomised complete block design in three series (ie in May, June and July) and in three replications. The first experimental factor was different NaCl concentration in Hoagland's medium, ie 0.068, 0.136 and 0.170 mol NaCl · dm^{-3}, and the control, which was a complete Hoagland's medium, whereas three clones of *Salix viminalis* were the second one.

The willow cuttings were placed at first in appropriately prepared hydroponics, with a capacity of 2 dm^{-3} each (2 cuttings per each container), filled with complete Hoagland's medium. Next, after about 14 days, when the cuttings rooted themselves and developed shoots, about 20 cm long, the mediums were replaced, differentiating in them the NaCl concentration, in conformity with to the experiment variants adopted.

After 72 hours from exposing the hydroponics to salinity, the water balance was determined in the leaves of examined willow clones basing on the *relative water content* (RWC) and *water saturation deficit* (WSD) indicators according to Bandurska [7]. Then, after 168 hours from exposing the medium to salinity, the content of selected macroelements, ie sodium, potassium, calcium and magnesium, was assayed in the collected and subsequently wet-mineralised leaves. The determination was made with atomic absorption spectrophotometry method (AAS).

Results referring to the content of mineral elements were processed statistically using two-factor analysis of variance. In order to determine the significance of differences between means for interactions, Tukey's confidence semi-intervals were calculated at the significance level $\alpha = 0.05$ ($LSD_{0.05}$). In case of RWC, homogenous groups were determined, basing on the results given in grams. Considering the homogeneity of error variance, the synthesis of results from two years and three experiment series was performed.

Results and discussion

The salinity of medium with sodium chloride had a significant effect on the water balance of leaf tissues in two basket willow clones, out of three examined ones, which was measured by relative water content (RWC) and water saturation deficit (WSD) indicators (Table 1). The largest amount of water was a characteristic of the leaves of

'Tora' and 'Jorr' clones under control conditions (93.6 and 89.8 %, respectively) and in the lowest NaCl concentration (88.2 and 89.4 %, respectively. On the other hand, at the highest salinity of hydroponics, ie 0.170 mol NaCl · dm^{-3}, the same clones showed a significantly higher water loss, up to 20 %, when compared with the 'Bjor' clone. In case of that particular clone, irrespective of NaCl concentration in the medium, the water balance in leaves was at a similar level, ie about 82–85 % RWC and 15–18 % WSD. This may be evidence of larger resistance of the 'Bjor' clone to relatively high salinity of medium with sodium chloride when compared with the 'Jorr' and 'Tora' clones, which responded under the same conditions with a rapid decrease in leaf tissue hydration.

Table 1

Relative water content (RWC) [%] and water saturation deficit (WSD) indicators in the leaf tissues of *Salix viminalis* L.

Clone	RWC [%]				WSD [%]			
	Concentration of NaCl in culture [mol · dm^{-3}]				Concentration of NaCl in culture [mol · dm^{-3}]			
	0.0 (Control)	0.068	0.136	0.170	0.0 (Control)	0.068	0.136	0.170
'Bjor'	82.4b	85.1b	84.0b	82.0b	17.6b	14.9b	16.0b	18.0b
'Jorr'	89.8a	89.4a	80.3b	61.7c	10.2a	10.9a	19.7b	38.3c
'Tora'	93.6a	88.2ab	84.9b	62.6c	6.4a	11.8ab	15.1b	37.4c

a, b, c – The values denoted by the same letter do not differ significantly at the level $\alpha = 0.05$.

The plant hydration is affected by many different factors, including the supply of mineral components to plants and how these compounds are managed. This is because the disturbance of ionic balance in medium, brought about by overabundance of Cl^- and Na^+ ions, induces a disturbance in the ionic balance in plants. According to Marosz [5], Starck et al [8] and Greszta et al [9] the salinity of natural environment with sodium chloride brings about changes in the cell metabolism since the absorbed sodium ions increase the hydrophilic ability of plasma colloids, which induces the binding of larger quantity of water. The potassium ions have a similar effect. This is explained by high hydration of leaf tissues observed in the examined willow clones at lower NaCl concentrations in the medium (Table 1).

In the carried out study, a significant increase in the content of Na^+ ions in the leaves of basket willow was found together with the increase of NaCl concentration in the medium, but only to 0.136 mol NaCl · dm^{-3} (Fig. 1). At the highest salinity, the content of sodium in the 'Jorr' and 'Tora' clones was maintained at the same level. On the other hand, the content of that element in the 'Bjor' clone decreased significantly. This attests to setting off a defensive mechanism by this willow clone, protecting it from absorption of excessive and harmful for cells quantity of Na^+ ions. The constant and invariable level of hydration in the 'Bjor' clone can be explained by this. On the other hand, a decrease in the hydration of the 'Tora' and 'Jorr' clones can be explained by intoxication by excessive accumulation of sodium ions which probably induced destruction of cell

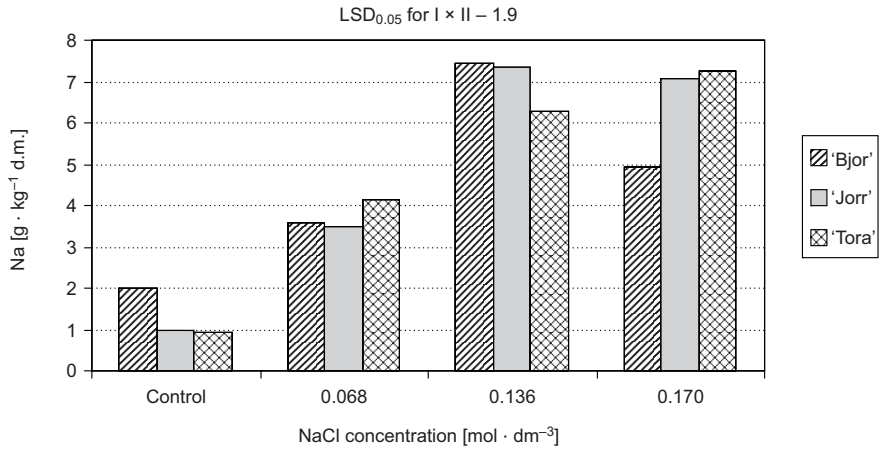

Fig. 1. Content of sodium [g · kg^{-1} d.m.] in leaves of *Salix viminalis* L. in relation to concentration NaCl in culture

structures and photosynthetic apparatus, which in turn could significantly affect a reduction in water intake.

The increased content of sodium and a small decrease in potassium content in the leaves of *Quercus robur* under NaCl salinity was found by Sehmer et al [10]; in addition, they observed a lower accumulation of some bivalent cations, in particular of Ca^{2+}.

The conducted experiment showed that, the salinity did not have any significant effect on the accumulation of potassium in the leaves of the 'Bjor' and 'Jorr' clones (Fig. 2). Only in the 'Tora' clone, a significant increase in the potassium content was found at the concentration of 0.136 mol NaCl · dm^{-3} in the medium (by about 40 %). On the other hand, no significant effect of medium salinity was found on the content of bi-

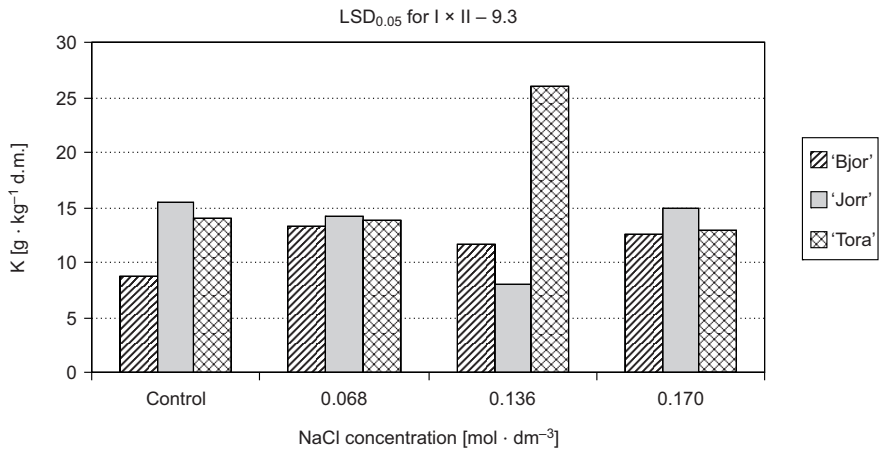

Fig. 2. Content of potassium [g · kg^{-1} d.m.] in leaves of *Salix viminalis* L. in relation to concentration NaCl in culture

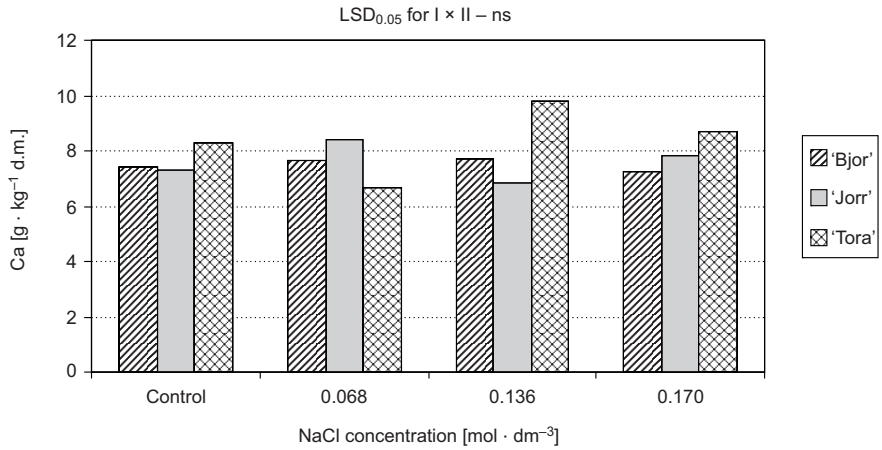

Fig. 3. Content of calcium [g · kg^{-1} d.m.] in leaves of *Salix viminalis* L. in relation to concentration NaCl in culture

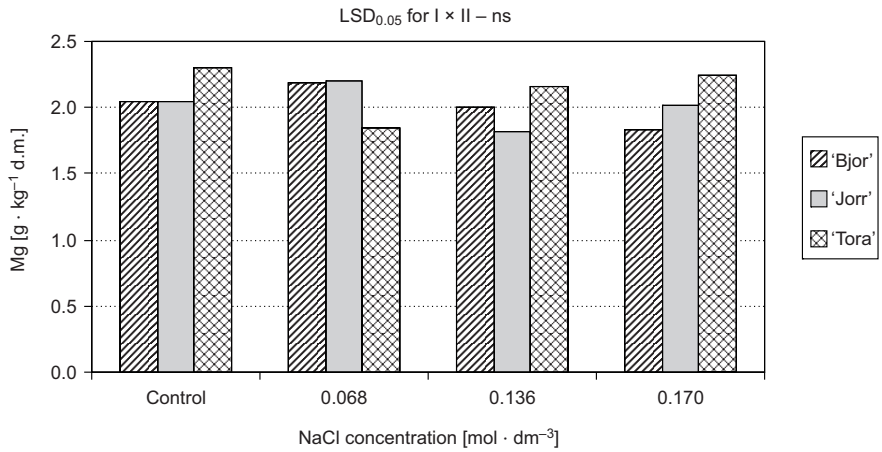

Fig. 4. Content of magnesium [g · kg^{-1} d.m.] in leaves of *Salix viminalis* L. in relation to concentration NaCl in culture

valent cations, ie calcium and magnesium (Figs. 3 and 4). Wrobel and Gregorczyk [11] showed in their study a significant correlation between calcium and magnesium accumulation in the leaves of the same basket willow clones and soil salinity. However, they applied two times higher doses of NaCl and carried out their experiment under different conditions.

Thus, it appears that the equal level of potassium in the leaf tissues of the 'Bjor' clone, with the limited uptake of excessive quantity of sodium, was decisive for the optimum hydration of leaf tissues at the high concentration of salt in the medium. Additionally, in the leaves of the 'Bjor' clone, out of three clones examined, were observed the most favourable equivalent ion proportions between K:Ca + Mg (0.4 to 0.6), K:Ca

(0.6 to 0.9) and K:Mg (1.3 to 2.1), which were characterised by the smallest fluctuations in the proportion of monovalent to bivalent cations in respective salt concentrations (Table 2). This is exactly them which decide about the optimum hydration of cell plasma and of whole plants.

Table 2

Mean equivalent ion proportions in the leaves of three clones of *Salix viminalis* L.

Clone	Ionic proportions	Concentration of NaCl in culture [mol · dm^{-3}]			
		0.0 (Control)	0.068	0.136	0.170
'Bjor'	K:(Ca + Mg)	0.4	0.6	0.5	0.6
	K:Ca	0.6	0.9	0.8	0.9
	K:Mg	1.3	1.9	1.8	2.1
	K:Na	2.4	2.1	0.9	1,5
	Ca:Mg	2.2	2.1	2.3	2.4
'Jorr'	K:(Ca + Mg)	0.7	0.6	0.4	0.7
	K:Ca	1.1	0.9	0.6	1.0
	K:Mg	2.4	0.5	1.3	2.2
	K:Na	10.0	0.5	0.6	1.2
	Ca:Mg	2.2	2.3	2.3	2.3
'Tora'	K:(Ca + Mg)	0.6	0.8	1.0	0.5
	K:Ca	0.9	1.1	1.3	0.8
	K:Mg	1.9	2.4	3.7	1.7
	K:Na	9.0	2.0	2.4	1.1
	Ca:Mg	2.2	2.2	2.7	2.3

Conclusions

1. Under high NaCl salinity conditions in the medium, the 'Bjor' clone was characterised by more favourable water and ionic balance when compared with the 'Jorr' and 'Tora' clones.

2. From among three basket willow clones examined, only the 'Bjor' clone showed an effective mechanism of decreasing the accumulation of sodium ions in the leaves under the highest salinity of hydroponics.

3. High values of RWC indicator and low values of WSD indicator at the highest concentration of sodium chloride in hydroponics as well as suitable ion proportions, irrespective of the degree of medium salinity, found in the 'Bjor' clone show that it is best adapted to survival under salt stress conditions when compared with the 'Jorr' and 'Tora' clones.

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BILANS WODNY ORAZ JONOWY W LIŚCIACH WIERZBY WICIOWEJ (*Salix viminalis* L.) UPRAWIANEJ W KULTURACH WODNYCH O RÓŻNYM STOPNIU ZASOLENIA

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Abstrakt: W badaniach wykazano ważny wpływ zróżnicowanego stężenia NaCl w pożywce Hoaglanda na bilans wodny i jonowy w liściach trzech klonów *Salix viminalis* L.: 'Bjor', 'Jorr' i 'Tora'. Pomiary wskaźników wodnych RWC i WSD oraz zawartości kationów jednowartościowych (K^+ i Na^+) oraz dwuwartościowych (Ca^{2+} i Mg^{2+}) w liściach klonów wierzby wiciowej pozwalają wnioskować, że w warunkach dużego zasolenia hydroponiki chlorkiem sodu klon 'Bjor' w porównaniu z klonami 'Jorr' i 'Tora' charakteryzował się korzystniejszym bilansem wodnym oraz jonowym, a także skutecznym mechanizmem zmniejszania pobierania jonów sodu w liściach w warunkach największego stężenia soli w podłożu. Wyniki badań wskazują, że klon 'Bjor' jest najlepiej przystosowany do przetrwania w warunkach stresu solnego.

Słowa kluczowe: hydroponika, zasolenie, *Salix viminalis* L., bilans wodny, bilans jonowy

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 10 2010

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RESPONSE OF MICROORGANISMS TO SOIL CONTAMINATION WITH CADMIUM, NICKEL AND LEAD

REAKCJA DROBNOUSTROJÓW NA ZANIECZYSZCZENIE GLEBY KADMEM, MIEDZIĄ, CYNKIEM I OŁOWIEM

Abstract: In pot experiment the effect of loamy sand contaminated with cadmium, copper, zinc and lead on number of oligotrophic bacteria, actinomycetes and fungi was studied. Heavy metals were applied to soil as a single pollution and in mix each other. Two level of heavy metals pollution were examined: 1st level (mg · kg^{-1} of soil): Cd – 4, Cu – 150, Pb – 100 and Zn – 300; 2nd level (mg · kg^{-1} of soil): Cd – 12, Cu – 450, Pb – 300 and Zn – 900. It was found that oligotrophic bacteria were the most sensitive on contamination of soil with cadmium, copper, zinc and lead, slightly less – actinomycetes and the least susceptible were fungi. The negative effect of heavy metals on microorganisms was not the sum of single heavy metals action.

Keywords: heavy metals, bacteria, fungi, actinomycetes

In most cases, heavy metals have negative influence on microorganisms. They reduce microbial biomass, counts, structure and activity [1–4]. They cause disorders in enzymatic activities by forming complexes with substrates or blocking reactive functional groups of enzymes [3]. They also impair the resistance of microorganisms to other stresses [1, 5]. Heavy metals disturb proper functioning of ecosystems by producing adverse effect on the course of many processes, such as those involved in transformations of carbon and nitrogen compounds [6, 7]. The actual effect of heavy metals on microbiological properties of soil depends on the degree of soil contamination, properties of chemical compounds which occur in the soil and on any other types of pollution present in the environment.

Unlike model studies, which often focus on the effect of individual heavy metals on the microbiological activity of soil, this experiment dealt with the influence of mixtures on microorganisms. Thus, our objective has been to analyze the effect of cadmium, copper, zinc and lead on oligotrophic bacteria, actinomycetes and fungi. The effect produced by individual heavy metals was compared with that exerted by their mixtures.

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Material and methods

The experiments (in four replications) were conducted in polyethylene pots placed in a greenhouse. The test soil was loamy sand soil with pH in 1 mol KCl · dm^{-3} – 5.60; hydrolytic acidity (Hh) – 1.31 cmol(H^+) · kg^{-1} soil; C_{org} – 5.00 g · kg^{-1}; total exchangeable bases (S) – 5.71 cmol(+) · kg^{-1}; exchangeable capacity of the sorptive complex (T) – 7.02 cmol(+) · kg^{-1}; base cation saturation ratio (V) – 81.34 %. Prior to the trials, soil samples (3 kg each) were mixed with mineral fertilizers and heavy metals. All the treatments received identical macro- and microelement fertilization consisting of N –100 [$CO(NH_2)_2$], P – 44 [KH_2PO_4]; K – 83 [KH_2PO_4 + KCl], Mg – 20 [$MgSO_4 \cdot 7H_2O$], Zn – 5 [$ZnCl_2$], Cu – 5 [$CuSO_4 \cdot 5H_2O$], Mn – 5 [$MnCl_2 \cdot 4H_2O$], Mo – 5 [$Na_2MoO_4 \cdot 2H_2O$], B – 0.33 [H_3BO_3] expressed as pure element in mg per kg soil. The soil samples prepared as described above were contaminated with cadmium ($CdCl_2 \cdot 2^1/_2H_2O$), copper ($CuSO_4 \cdot 5H_2O$), lead ($PbCl_2$) and zinc ($ZnCl_2$). Two levels of contamination were applied. The first one corresponded to the maximum permissible concentrations of heavy metals contained in the Decree of the Minister for Environment of 9th September 2002 [8] and comprised, [mg · kg^{-1}], 4 Cd, 150 Cu, 100 Pb and 300 Zn. The other level was 3-fold higher than that and equalled, [mg · kg^{-1} soil], 12 Cd, 450 Cu, 300 Pb and 900 Zn. In addition to this, cadmium polluted objects received other heavy metals (Cu, Pb and Zn). The following objects contaminated with heavy metals were compared: Cd, Cu, Pb, Zn, CdCu, CdPb, CdZn, CdCuPb, CdCuZn, CdPbZn and CdCuPbZn. The effect of the heavy metals was verified according to the control (non-polluted) objects. The trials were conducted in two series: soil cropped with oats cv. Borowik (12 plants per pot) and uncropped soil.

Having been mixed with the fertilizers and heavy metals, the soil was brought to a moisture content of 60 % water capillary capacity and maintained at this level for two weeks. On day 14 samples for microbiological analyses were collected and oats was sown in the cropped soil series. The pots in the other series were maintained unsown for 61 days. In this period constant moisture (60 % of capillary water capacity) of soil was maintained. After that time, oats plants (in the inflorescence stage) were harvested, the yields were measured and the soil samples obtained from both series were assayed for counts of microorganisms. Thus, counts of microorganisms were assayed twice, on day 14 and day 61 of the experiment. The microbiological assays consisted of determinations of counts of oligotrophic bacteria on Onta and Hattori medium [9], actinomycetes – on Kuster and William medium supplemented with nystatin and actidione [10] and fungi – on Martin medium [11].

The results underwent statistical elaboration using Duncan's multiple range test and two-factor analysis of variance. All statistical calculations were performed with an aid of Statistica software [12].

Results and discussion

Counts of oligotrophic bacteria in soil sown with oats were 1.34-fold higher than in uncropped soil (Table 1). Independent of the soil use, heavy metals depressed rather

substantially the populations of oligotrophic bacteria. Higher rates of pollution produced particularly strong negative effects although these were not proportional to the increase in the pollution degree.

Oligotrophic bacteria proved to be very sensitive to copper. Their population decreased by 36 % in the soil polluted with the higher rate of copper and by 30 % when the lower dose of the contaminant was applied. A similar effect was produced by cadmium, which depressed populations of oligotrophic bacteria by 34 % and 20 %, respectively. Lead and zinc had a weaker influence on this group of microorganisms.

Soil contamination with cadmium in conjunction with copper, lead or zinc tended to be more toxic than the treatments involving single elements. However, less notable effects occurred after adding more pollutants, such as mixtures of three heavy metals. Larger modifications in the populations of oligotrophic bacteria were observed under the total contamination with all the four test elements, cadmium, copper, lead and zinc, especially in uncropped soil. The aggregated effect of these four heavy metals caused a 46 % decrease in the count of oligotrophic bacteria. This can be compared with a 36 % decline as a result of the contamination with three metals, 34 % – with two metals and 26 % – with a single metal.

Table 1

Number of oligotrophic bacteria [cfu $10^8 \cdot kg^{-1}$ d.m. of soil]

Object	Contamination level			
	1st		2nd	
	Manner of soil management			
	unsown	sown	unsown	sown
Control	137 ± 7	183 ± 5	137 ± 9	183 ± 6
Cd	115 ± 9	140 ± 6	90 ± 9	122 ± 7
Cu	106 ± 7	115 ± 5	90 ± 4	115 ± 5
Pb	112 ± 6	135 ± 5	115 ± 8	124 ± 7
Zn	104 ± 9	133 ± 7	119 ± 9	138 ± 6
CdCu	101 ± 6	121 ± 6	97 ± 6	104 ± 6
CdPb	88 ± 6	122 ± 6	96 ± 10	114 ± 9
CdZn	99 ± 9	111 ± 6	99 ± 10	113 ± 7
CdCuPb	88 ± 6	93 ± 6	104 ± 7	119 ± 7
CdCuZn	95 ± 9	117 ± 6	97 ± 6	106 ± 6
CdPbZn	104 ± 6	119 ± 6	85 ± 9	101 ± 6
CdCuPbZn	86 ± 6	104 ± 6	65 ± 5	94 ± 6
Average	103 ± 2	124 ± 2	99 ± 1	119 ± 2
LSD	a – 2.9; b – 1.2; c – 1.2; a · b – 4.0; a · c – 4.0; b · c – 1.6; a · b · c – 5.7			

LSD for: a – kind of contamination; b – contamination level; c – manner of soil management: 1st contamination level [mg · kg^{-1} d.m. of soil]: Cd – 4; Cu – 150; Pb – 100; Zn – 300; 2nd contamination level [mg · kg^{-1} d.m. of soil]: Cd – 12; Cu – 450; Pb – 300; Zn – 900.

Counts of actinomycetes (Table 2) was 2.15-fold higher in cropped than uncropped soil, which meant that the differences between the two types of soil use were much larger here than in the case of oligotrophic bacteria. On the other hand, toxic effect of particular heavy metals on actinomycetes was much weaker than that produced on oligotrophic bacteria. Actually, zinc was the only metal which depressed the count of actinomycetes (by 17–23 % in uncropped soil and 9 % in soil under oats), but this result was observed only when the lower rate of this metal had been introduced to soil. In the soil polluted with the triple doses of the metals relative their permissible levels, cadmium produced a negative effect on actinomycetes although its influence was much weaker than that of zinc. In the treatment consisting of unropped soil and the lower cadmium rate, the metal did not inhibit the growth of actinomycetes. Moreover, in the soil sown with oats, the same rate of cadmium stimulated the multiplication of these microorganisms. Cadmium added to soil together with copper had a significant negative effect on actinomycetes. The aggregated toxic effect produced by these two elements was much stronger when the lower doses of the metals were added to soil.

Table 2

Number of actinomycetes [cfu $10^8 \cdot kg^{-1}$ d.m. of soil]

Object	Contamination level*			
	1st		2nd	
	Manner of soil management			
	unsown	sown	unsown	sown
Control	48 ± 1	103 ± 7	48 ± 1	103 ± 7
Cd	48 ± 3	160 ± 14	46 ± 3	93 ± 8
Cu	63 ± 4	108 ± 8	44 ± 4	119 ± 11
Pb	47 ± 4	155 ± 12	59 ± 3	101 ± 5
Zn	40 ± 2	94 ± 7	37 ± 4	103 ± 5
CdCu	35 ± 2	89 ± 4	46 ± 4	101 ± 9
CdPb	36 ± 4	91 ± 6	58 ± 5	95 ± 9
CdZn	51 ± 4	88 ± 5	36 ± 3	62 ± 4
CdCuPb	49 ± 5	68 ± 6	55 ± 3	94 ± 7
CdCuZn	48 ± 3	105 ± 6	37 ± 2	80 ± 3
CdPbZn	49 ± 3	94 ± 6	40 ± 3	85 ± 8
CdCuPbZn	46 ± 3	126 ± 10	26 ± 2	76 ± 4
Average	47 ± 1	107 ± 2	44 ± 1	93 ± 1
LSD*	a – 3.8; b – 1.5; c – 1.5; a · b – 5.3; a · c – 5.3; b · c – 2.17; a · b · c – 7.5			

* Explantions are under Table 1.

Cadmium applied in conjunction with lead produced similar effects, except in uncropped soil with the triple doses of the contaminants. The strongest toxic effects on actinomycetes produced by a combination of these two elements occurred in uncropped soil treated with the stronger doses of cadmium and lead. Actinomycetes were the most sensitive to the joint effect produced by cadmium and zinc. When introduced at the

triple doses, these two heavy metals depressed the population of actinomycetes by 40 % in soil under oats. When lead was added to an analogous object, it significantly reduced the negative effect of soil contamination on actinomycetes, even though the total amount of contaminates had increased. Lead nearly completely alleviated the negative impact of soil contamination in combination with cadmium and copper, but this effect was observed only in uncropped soil at either of the levels of pollution. In turn, zinc applied together with cadmium and copper – at the lower dose – completely neutralised the toxic effect of copper and cadmium. In contrast, when applied at the higher dose it significantly rose the toxicity of a cadmium and copper mixture. The total contamination with all the four heavy metals, although the highest in terms of the quantity and quality of the pollution, did not cause any larger modifications in the population of actinomycetes that those generated by a mixture of cadmium and zinc.

Table 3

Number of fungi [cfu $10^6 \cdot kg^{-1}$ d.m. of soil]

Object	Contamination level*			
	1st		2nd	
	Manner of soil management			
	unsown	sown	unsown	sown
Control	30 ± 2	31 ± 3	30 ± 2	31 ± 3
Cd	34 ± 2	39 ± 2	32 ± 2	37 ± 3
Cu	42 ± 3	35 ± 1	33 ± 2	44 ± 3
Pb	33 ± 2	31 ± 2	34 ± 3	39 ± 4
Zn	30 ± 2	49 ± 3	36 ± 2	41 ± 2
CdCu	25 ± 2	32 ± 1	20 ± 2	25 ± 2
CdPb	33 ± 2	28 ± 2	30 ± 2	22 ± 2
CdZn	31 ± 2	48 ± 3	31 ± 2	30 ± 3
CdCuPb	37 ± 3	35 ± 3	34 ± 3	35 ± 3
CdCuZn	25 ± 2	34 ± 4	28 ± 1	30 ± 2
CdPbZn	29 ± 2	37 ± 2	35 ± 3	37 ± 3
CdCuPbZn	34 ± 2	37 ± 3	28 ± 3	36 ± 3
Average	32 ± 1	36 ± 1	31 ± 1	34 ±1
LSD*	a – 1.9; b – 0.8; c – 0.8; a · b – 2.7; a · c – 2.7; b · c – ns; a · b · c – 3.8			

* Explantions are under Table 1.

In contrast to oligotrophic bacteria and actinomycetes, counts of fungi were independent from the type of soil use and proved to be comparable in uncropped soil and soil under oats (Table 3). Fungi clearly differed from actinomycetes and, even more so, from oligotrophic bacteria, in their response to soil contamination with heavy metals. Each of the tested heavy metals, when applied individually, made fungal populations to grow. The highest increase in fungi was caused by zinc (on average, a 28 % increase), followed by copper (26 %), cadmium (16 %) and lead (12 %). In the soil under oats treated with the higher doses of the metals, a significant drop in the

count of fungi was noticed under the influence of a combination of cadmium and copper, cadmium and lead or cadmium and zinc. The number of fungi fell most substantially when cadmium was added together with lead; the smallest decline in fungal counts occurred under the joint effect of cadmium and zinc. The addition of lead to an object treated with cadmium and copper not only alleviated the negative influence of the two latter metals but contributed to a significant increment in the population of fungi. The count of fungi was also depressed when soil had received a mixture of cadmium, copper and zinc but an addition of a fourth element, lead, significantly reduced the adverse effect of the three former elements.

The present investigations have demonstrated that the mechanism of heavy metals affecting soil microorganisms is far from being completely recognised. Differences in the response to soil pollution observed between oligotrophic bacteria, actinomycetes and fungi could be attributed to some specific characteristics of these three groups of microogranisms, which in turn originate from the specific structure of their cells. The most sensitive cells die under the effect of heavy metals while other cells can adjust themselves to new conditions through certain physiological or genetic modifications. Some other cells, also tolerant to the presence of heavy metals, can be removed from microbial populations as a result of competition between microorganisms which can survive pollution with heavy metals [1]. Therefore, the growth of fungal populations observed under the effect of the four tested metals, when applied singly, cannot be considered as a positive outcome as long as we lack detailed knowledge of the structure of fungal cells. Some research [2] suggests that soil pollution with heavy metals can cause such large modifications in the species that the whole metabolism of soil can be disrupted [6].

The results on the response of microorganisms to heavy metals contained in this paper are further confirmed by several other reports [3, 4, 13, 14]. All these findings prove that the exact effect produced by heavy metal contamination of soil is conditioned not only by the amounts of heavy metals in soil environment but also by the presence of other metals, which can diminish toxicity of single metals [15–17].

Conclusions

1. Oligotrophic bacteria were most sensitive to soil pollution with heavy metals (cadmium, copper, lead and zinc). Acinomycetes were slightly less sensitive to such conditions and fungi proved to be the least sensitive microogranisms.

2. The adverse effect of heavy metals on microogrniams is not additive in character, ie it is not a total of the effects produced by single metals.

3. Lead, applied as $PbCl_2$, proved to mask most efficiently the effect produced by the other three heavy metals tested.

Acknowledgement

The research was conducted as part of a project No. N N305 2258 33 supported by the Polish Ministry of Science and Higher Education.

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REAKCJA DROBNOUSTROJÓW NA ZANIECZYSZCZENIE GLEBY KADMEM, MIEDZIĄ, CYNKIEM I OŁOWIEM

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Abstrakt: W doświadczeniu wazonowym badano wpływ zanieczyszczenia piasku gliniastego kadmem, miedzią, cynkiem i ołowiem oraz ich mieszaninami w różnych proporcjach na liczebność bakterii oligotroficznych, promieniowców i grzybów. Zastosowano 2 poziomy zanieczyszczenia, które wynosiły [mg · kg^{-1} gleby]: I poziom: Cd – 4; Cu – 150; Pb – 100; Zn – 300; II poziom: Cd – 12; Cu – 450; Pb – 300; Zn – 900. W wyniku badań stwierdzono, że bakterie oligotroficzne były najbardziej wrażliwe na

zanieczyszczenie gleby metalami ciężkimi (kadmem, miedzią, ołowiem i cynkiem), nieco mniej – promieniowce, a najmniej – grzyby. Negatywne oddziaływanie metali ciężkich na drobnoustroje nie miało charakteru addytywnego, tzn. nie było sumą skutków działania pojedynczych metali.

Słowa kluczowe: metale ciężkie, bakterie, grzyby, promieniowce

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 10 2010

Małgorzata KOSTECKA[1]

SEARCH FOR AN EFFICIENT COMPOUND WITH ANTIFUNGAL PROPERTIES INHIBITING *Fusarium* GENUS FUNGI

POSZUKIWANIE SKUTECZNEGO ZWIĄZKU O WŁAŚCIWOŚCIACH PRZECIWGRZYBICZYCH HAMUJĄCEGO ROZWÓJ GRZYBÓW Z RODZAJU *Fusarium* NA RÓŻNYCH GENOTYPACH ZBÓŻ

Abstract: A food quality finds considerable consumer's interests. Presence of contaminants in food is one of the principle criteria of foodstuff health safety assessment. Toxins of moulds, namely of *Fusarium* genus called mycotoxins, that are characterized by acute toxic action, are one of the most dangerous biological--origin substances found in food. Farm products may be contaminated with these metabolites beginning from the crop development in a field (mainly cereals), through their harvest, to storage and transport of final products. In order to reduce the food contamination due to mycotoxins, it is necessary to apply properly selected plant protection means. Own study upon achieving the active substances allowed for selecting compounds with promising biological action, that would become potential fungicides controlling moulds.

Keywords: mycotoxins, *Fusarium* species, antifungal activity, minimal inhibitory concentration – MIC

Fusarium genus fungi are the most isolated pathogens of worldwide crops [1, 2]. They are the reason of enormous economic loss resulting from their high pathogenecity and toxin-formation abilities, thus they are counted to the most dangerous filamentous fungi.

Fusarium infects crops of the basic economical importance for human [3], they occur on all cultivated winter and spring cereal species and can infect plants at their seedling, sprouting, and flowering stages [4–6]. Head blight is the most dangerous and economically significant cereal disease caused by *Fusarium* spp. [7]. It affects the lower ear weight and grain number per ear, plant growth inhibition, lower starch content in grains, and considerable worse baking quality of a flour achieved from infected grains [8].

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Under Polish climatic conditions, head blight is most frequently caused by a complex of diverse species such as *Fusarium culmorum*, *Fusarium avanaceum*, and *Fusarium graminearum* [9]. Quite large percentage of another pathogen has been lately observed – *Fusarium poae* that infects oats and other cereals panicles in Lublin region and eastern Poland [10, 11]. In addition, weather conditions (high air moisture and high temperatures, namely during flowering), as well as improper grain storage and transport conditions, favor mould development [12]. Deoxynivalenol (DON) is the most dangerous mycotoxin produced by *Fusarium* genus fungi. It is accumulated in infected plant cells and that way it penetrates the human's and animal's nutrition chain [13, 14]. Mycotoxins are low-molecular substances, against which an organism fails to produce any antibodies. They are lipophilic and hence they are prone to be deposited in fat cell fractions at plants and animals [15]. Prolonged exposure to mycotoxin action may result in endocrinological, neurological, and tumor diseases.

The problem of raw materials and foodstuff contamination with *Fusarium* genus fungi and their toxic metabolites cannot be eliminated applying traditional agrotechnical operations. An appropriate using properly selected fungicides, both in a view of fighting against diseases, existing weather conditions, and the rate applied are needed. However, quite great resistance of fungi against nowadays used antifungal means is a serious problem at controlling head blight. Due to fast mutation of strains towards some fungal species, up-to-date applied plant protection means became ineffective, while others retained their action, although at much increased doses. Furthermore, in the case of some mould strains, the active substance should be applied only once.

Therefore, studies upon achieving modern fungicides with systemic action that would have bilateral features, are extremely important at present. The own study conducted up-to-date included synthesis of diverse 2,4-dihydroxythiobenzamide derivatives in a view of their high activity towards phytopathogenic and yeast-like fungi, as well as dermatophytes [16–18]. Achieved results are promising and allow for searching for agents efficient to a narrow selected group of fungi – those of *Fusarium* genus.

Materials and methods

The study involved 13 different thiocarbonyl derivatives (Fig. 1) that were produced by means of organic synthesis at Chemistry Department, University of Life Sciences in Lublin, in accordance with the patented reaction mechanism [19]. Applying high--performance liquid chromatography (HPLC), the purity of achieved compounds could be confirmed.

In order to evaluate the biological activity of newly synthesized derivatives, *Fusarium* genus fungal species were tested; they were selected due to their presence in cereals and cereal-origin products.

Material for study comprised moulds. The material for inoculation was transferred onto Sabouraud substrate. Inoculum was made up of suspension containing 10^5 cfu per 1 cm^3 (milliliter). Aliquots of 20 mm^3 (µl) suspension were transferred onto Petri dishes with studied mediums containing corresponded concentrations of tested compounds.

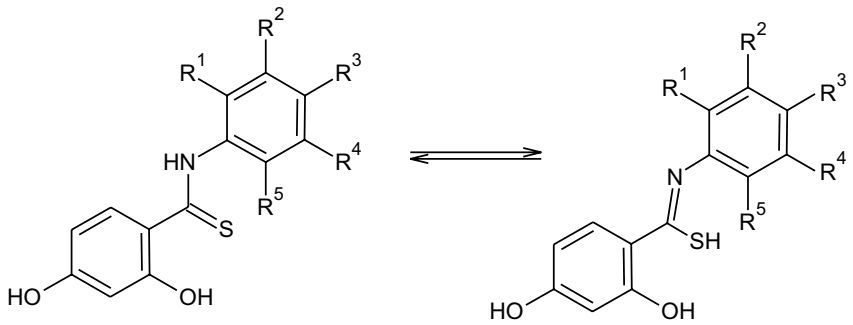

Fig. 1. Chemical structures of substituted 2,4-dihydroxythioamides

The control was made up of above inoculum on studied mediums that lack tested compounds or with 1 % DMSO. The incubation was performed at 27 ^{o}C, and *minimal inhibitory concentration* (MIC) reading was made after 5 days. Tests were conducted at Department of Pharmaceutical Microbiology, University of Medicine in Lublin.

Subsequent part of biological examinations was performed at Sub-department of Pesticide Applying and Formulation, Institute of Organic Industry in Warsaw (GLP certificate, Statement of GLP Compliance No. G013). Following standard fungicides were applied: carbendazyme (commercial name – Sarfun 500 S.C.) and precymidone (commercial name – Sumilex 500 S.C.). Assays were made in accordance with laboratory method for antifungal activity assessment (procedure SPR/FA_2/11) by estimating the influence of particular concentrations of tested compound present in a medium on growth of fungal colony that causes crop diseases.

Solution or suspension of tested compound was added to sterile and cooled PDA medium (3.9 g of DDA medium (Difco) + 0.2 g of agar in 100 cm^3 of distilled water) to achieve needed concentration in substrate. Tested compounds were dissolved in acetone and water mixture (1.5:1), while fungicides were dissolved in water.

Inoculum with mycelium of tested strains was put into solidified medium on 5 cm diameter Petri dishes. Dishes were incubated at 21 ^{o}C for 5 days. Then, the diameter of fungal colonies was measured. Based on Abbot's formula, it served for calculating the inhibition efficiency of tested compound or standard at particular concentration, the inhibition of mycelium linear growth was measured.

Following criteria of action efficiency were used:

Efficiency of linear growth [%]	Ranking of fungicidal control	
	score	action
Above 80 %	3	good
50–80 %	2	moderate
20–50 %	1	poor
20 % and less	0	no action

The assessment was made at 200 mg/dm^3 and 20 mg/dm^3 concentrations. The experiment was carried out in two replications for each combination.

Table 1

The MIC (*minimal inhibitory concentration*) values mg/dm^3 of itraconazole and fluconazole and thioamide compounds against *Fusarium* genus fungi, n = 11, on the Sabouraud's medium after 5 days of incubations

Comp. no.	Biological activity compounds	*Fusarium moniliforme* n = 2	*Fusarium graminearum* n = 1	*Fusarium poae* n = 1	*Fusarium culmorum* n = 2	*Fusarium oxysporum* n = 3	*Fusarium avanaceum* n = 2
1	*N*-5(2'methoxy,phenyl)-2,4-dihydroxythiobenzamid	100	100	100	100	100	100
2	*N*-1-(4'-benztrifluoromethyl)-2,4-dihydroxythiobenzamid	50	100	100	200	50	172.4
3	*N*-1-(3',5'-ditrifluoro methylbenz)-2,4-dihydroxythiobenzamid	50	50	50	100	50	50
4	*N*-1-(2'-izopropylo-4'-chlorobenz)-2,4-dihydroxythiobenzamid	50	25	50	100	50	100
5	*N*-1-(4'-fluoro,3'nitrobenz)-2,4-dihydroxythiobenzamid	25	50	100	200	100	100
6	*N*-2-(1'-phenylpirolo)2,4-dihydroxythiobenzamid	12.5	100	25	100	100	100
7	*N*-1-(5,6,7,8-tetrahydronaphthyl)-2,4-dihy droxythiobenzamid	100	100	200	200	200	100
8	*N*-1-(3',4'-benzmethyldiokso)-2,4-dihyroxythiobenzamid	200	200	200	200	200	200
9	*N*-1-(3,4-benzyldiokso)-2,4-dihydroxythiobenzamid	50	100	200	200	100	100
10	*N*-3(1,2,4-dithiazolo-5-thiono)-2,4-dihydroxythiobenzamid	25	78.5	25	200	46.8	62.4
11	*N*-(phenyl)-5-chloro-2,4-dihydroxythiobenzamid	25	100	100	200	180	187.5
12	*N,N*-[2-ethyl-3-(4'-chlorobenz)-crotoniano]-2,4-dihydroxythiobenzamid	25	50	25	100	50	25
13	*N,N*-[5-(1,2,3,4-tiatriazolo)-phenyl]-2,4-dihydroxythiobenzamid	25	62	25	50	23.5	29.2
14	Itraconazole	12.5	50	100	200	150	183.3
15	Fluconazole	100	100	200	200	200	100

Results and discusion

Evaluating the inhibition action of tested derivatives was expressed by means of determining the lowest inhibitive concentration MIC, ie the lowest concentration of subsequent dilutions of biologically active substance at which no microorganism growth can be observed. The MIC values are considered as a measure of compound effectiveness. MIC values were achieved for all tested preparations and for two reference substances towards *Fusarium* genus fungi (Table 1).

Fusarium culmorum appeared to be the most resistant – mean MIC value for applied derivatives amounted to 150 mg/dm^3, while *Fusarium moniliforme* was the least resistant – mean MIC was –56.73 mg/dm^3. Action of itraconasole and fluconasole also confirmed results achieved for tested compounds (*F. culmorum* – average MIC – 200 mg/dm^3, *F. moniliforme* – average MIC – 56.25 mg/dm^3).

The result analysis revealed that majority of tested compounds showed quite good biological activity, and their action was comparable with that of standard preparations. Derivatives No. 7, 8, and 9 were characterized by moderate activity – MIC values for them were within range of 125–200 mg/dm^3. Results for compounds No. 3, 4, 12, and 13 were much more promising – mean activities were 3-5-fold higher than for itraconasole and fluconasole. Particularly high action was recorded for *N,N*-[5-(1,2,3,4--thiatriasol)-phenyl]-2,4-dihydroxythiobenzamide (compound No. 13) with mean MIC – 35.78 mg/dm^3. It manifested 8-fold higher activity in relation to the most resistant *F. culmorum* than reference preparations and other compounds used in tests. Similar high action was observed towards other *Fusarium* genus fungi species.

Activity and functioning of tested compounds towards phytopathogenic fungi

During the laboratory tests (Table 2) at 200 mg/dm^3 concentration, all studied compounds revealed significant fungistatic activity (inhibition level remained at the level about 61–100 %). Compounds No. 3, 4, 5, 6, 10, 12 and 13 showed extremely strong inhibition action by complete inhibition of all three pathogens. Other derivatives also acted towards all three *Fusarium* genus fungi species although with 61–80 % efficiency. When compounds were applied at lower concentration (20 mg/dm^3), for majority of them, the inhibition activity decreased, or even complete lack of biological action was observed in some cases. However, derivatives No. 4, 6, 10, 12 and 13 showed quite good inhibition activity – depending on the pathogen type, their action was within 41–100 % range. On a base of earlier own studies [17, 20] and along with analysis of here achieved results, it can be confirmed that chemical structure of a molecule determines the biological action of these compounds. At the most active derivatives No. 6, 10 and 13, presence of heterocyclic substituents (pyrole, dithiasole, and tiatriasole) apparently affected the elevated activity. Presence of sulfur and nitrogen atoms having free electron pairs favors the compound's conformation during penetrating the barrier of biological membrane, thus has positive effects of derivative's biological action.

Table 2

Antifungal efficiency of 13 compounds and 2 standard fungicides against *Fusarium* genus fungi

Compound	*Fusarium culmorum*		*Fusarium graminearum*		*Fusarium poae*	
	200 mg/dm^3	20 mg/dm^3	200 mg/dm^3	20 mg/dm^3	200 mg/dm^3	20 mg/dm^3
1	3	1	3	1	3	0
2	3	1	3	1	3	1
3	4	2	4	1	4	1
4	4	3	4	4	4	2
5	4	2	4	3	4	3
6	4	3	4	4	4	3
7	3	2	3	2	3	1
8	3	1	3	2	3	1
9	3	0	3	1	3	0
10	4	2	4	4	4	4
11	3	1	3	3	3	2
12	4	3	4	4	4	2
13	4	2	4	4	4	4
Carbendazyme	4	4	4	4	4	4
Precymidone	—	—	4	4	4	4

Efficiency of linear growth in %:
[0] – 0–20 %; [1] – 21–40 %; [2] – 41–60 %; [3] – 61–80 %; [4] – 81–100 %.

Among tested fungi, *Fusarium culmorum* was the most resistant, while *Fusarium graminearum* the least resistant; for the latter, values of mycelium growth inhibition were the highest, both at 200 mg/dm^3 and 20 mg/dm^3 concentrations of tested compounds.

Having taken into account the results from studies involving standards being biologically active substances (procymidone and karbendazyme), achieved derivatives may be interesting research objects. It refers in particular to compounds, the inhibition action of which is at the same level as that of applied standard fungicides. They can be considered as new leading systems and use them in further *in vitro* tests towards other types of pathogens as well as at *in vivo* studies.

Conclusions

Conducting more detailed and wider *in vitro* and *in vivo* experiments seems to be purposeful, because it would allow for more accurate learning the antifungal action scope of high-activity compounds.

Modifying the molecule structure, mainly by introducing the heterocyclic substituents containing nitrogen and sulfur atoms might be also efficient, which seems to have positive influence of increased biological activity of synthesized derivatives.

Performed experiments and tests give the hope for isolating the compound that would be highly effective in controlling the moulds responsible for introducing mycotoxins into the food and cereal products.

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POSZUKIWANIE SKUTECZNEGO ZWIĄZKU O WŁAŚCIWOŚCIACH PRZECIWGRZYBICZYCH HAMUJĄCEGO ROZWÓJ GRZYBÓW Z RODZAJU *Fusarium* NA RÓŻNYCH GENOTYPACH ZBÓŻ

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Abstrakt: Jakość żywności wzbudza szczególne zainteresowanie konsumentów. Obecność zanieczyszczeń w żywności jest jednym z podstawowych kryteriów oceny bezpieczeństwa produktów żywnościowych. Do niebezpiecznych substancji pochodzenia biologicznego coraz częściej spotykanych w żywności należą toksyny grzybów pleśniowych, szczególnie z rodzaju *Fusarium* zwane mikotoksynami, charakteryzujące się ostrym działaniem toksycznym. Produkty rolne mogą ulec zanieczyszczeniu tymi metabolitami począwszy od rozwoju roślin na polu (głównie zboża), poprzez zbiór, po przechowywanie i transport gotowych produktów. W celu ograniczenia skażenia żywności mikotoksynami konieczne jest stosowanie odpowiednio dobranych środków ochrony roślin. Badania własne nad otrzymywaniem substancji aktywnych pozwoliły wybrać związki o obiecującym działaniu biologicznym, które mogą stać się potencjalnymi fungicydami, zwalczającymi grzyby pleśniowe.

Słowa kluczowe: mikotoksyny, grzyby z rodzaju *Fusarium*, aktywność przeciwgrzybicza, MIC, 2,4-dihydroxytiobenzamidy

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 10 2010

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INVESTIGATIONS ON FUNGICIDAL PROPERTIES OF 1,3,4-THIADIAZOLE DERIVATIVES

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Abstract: The results of the research concerning the effect of 1,3,4-thiadiazole derivatives on fungicidal *Candida albicans* are discussed. In all the conducted tests the reference strain of *Candida albicans* ATCC 10231 was used. To obtain the desired results, the following standard and synthesized thiadiazole derivatives were tested: 2-acetylamino-1,3,4-thiadiazolo-5-sulfonamide; 2-acetylamino-5-chloro-1,3,4-thiadiazole; 2-amino--1,3,4-thiadiazole; 2-acetylamino-1,3,4-thiadiazole; 2-acetylamino-1,3,4-thiadiazolo-5-sulfonic acid and bis(2--acetylamino-1,3,4-thiadiazolo)-5,5'-disulfonamide. In all the examined compounds the increase in the *Candida albicans* inhibition zone proportional to the increase of the compound concentration was observed. The only exception is 2-acetylamino-1,3,4-thiadiazole demonstrating the opposite tendency. While carrying out the research, it was found that 2-amino-1,3,4-thiadiazole proved to be the most effective of all the compounds within the tested group. It was also found that the higher the concentration of the compound, the higher the growth control zone (y) of *Candida albicans.* This phenomenon can be described by means of the equation: $y = 0.9167x^3 - 7.4286x^2 + 10.655x + 23.1$ (where: x – concentration of 2-amino-1,3,4-thiadiazole).

Keywords: *Candida albicans*, antifungicidal activity, 1,3,4-thiadiazole derivatives, MIC

It is known that 1,3,4-thiadiazole derivatives have biological activity with their antifungal action [1, 2]. Also, metal complexes with 1,3,4-thiadiazole derivatives as ligands showed *in vitro* antifungal activity against *Candida* spp [3]. The published literature discusses the fact that such substance as β-amino 21 acid (BAY 10--8888/PLD-118) demonstrates a high antifungal activity against *Candida albicans* [4]. Additionally, the strains of the bacteria *Candida albicans* are susceptible to such triazoles as: fluconazole 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazole-1-ilo)-propane--2-ol [5]. Frequent infections, occurring as a result of *Candida albicans*'s opportunistic pathogen (attacking weakened organism), are widely treated by antifungal preparation –

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fluconazole, which is an ergosterol biosynthesis inhibitor, the basic sterole compound existing in the fungi cell membrane [6–8].

It is known that the protection against *Candida albicans* was observed in the glucan-treated groups of patients. These observations suggest that the biological response modifiers (BMRs) such as glucan may be effectively applied in case of the patients in risk of the post-operative infection [9–11].

The characteristic features of a *Candida albicans* bacterial colony are as follows: cream-yellow colour, slightly convex above agar, not growing into the substrate, smooth, glossy surface, which becomes plicate while aging; usually smooth edges, (the fuzzy one exist only for a few bacterial strains); it releases characteristic yeast odor. The maximum growth temperature is 43–46 ^{o}C; the growth is stimulated by biotin and some of the bacterial strains are stimulated by thiamine; cells form bacterial culture tolerate the osmotic pressure of 8–12 % of sodium chloride solution [12].

Pathogenesis. The species is regarded as the most frequent etiological agent of generalized human and organ candidiasis. *Candida albicans* invasions may concern all the tissues, organs and human body systems in various stages of their development. The perfect stage is not known [12].

Yeast and fungi are also solid components of the soil microflora. Microflora complex is governed by the natural ecosystem; fungus population is dynamic which is a result of the sanitary condition connected with the fertilization of the soil polluted with sewage. Liquid animal excrements used as an organic fertilizer highly pollute cultivable soil with yeast fungi and mould. However, the fungi settled only in connection with 15 species from *Cryptococcaceae* family (including *Candida albicans* and others).

Fertilizations by the use of the following: the fermented liquid manure in a form of agricultural waste, sewage from food production and urban wastewater are potentially dangerous for animals and human beings [13].

The amount of the microorganisms in sewage is assessed on the basis of the bacteria detection regarded as the sanitary indicators. In western European countries, *Salmonella* is such an indicator; in Poland – *Escherichia coli,* the presence of which makes it possible to determine the *Coli* titre. In the USA, the fungus *Candida albicans* was accepted a relevant indicator for water and sewage assessment [14–16].

The aim of this study was to test the standard and synthesized derivatives of 1,3,4-thiadiazoles against the *Candida albicans* mycelium growth inhibition.

Experimental part

The following synthesized and standard compounds (Pharmaceutical Plants Polpharma, Starogard Gdanski, Poland) were used in the biological research for the test fungicidal properties: 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (AcATS); 2-amino--1,3,4-thiadiazole (AT); 2-acetylamino-1,3,4-thiadiazole (AcAT); 2-acetylamino-5-chloro--1,3,4-thiadiazole (AcATCl); 2-acetylamino-1,3,4-thiadiazolo-5-sulfonic acid ($AcATSO_3H$) and bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide (bAcATDS). Reagents of analytically pure class produced by Sigma-Aldrich and POCh Gliwice (Poland) were used for the compounds preparation.

Chemical syntheses of 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (AcATS), of 2-amino-1,3,4-thiadiazole (AT), and of bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-di-sulfonamide (BAcATDS) were discussed in the other article concerning the chemical characteristics of ^{13}C NMR of above mentioned compounds [17].

Preparing 2-acetylamino-1,3,4-thiadiazole (AcAT)

2-acetylamino-1,3,4-thiadiazole (AcAT) was obtained with the method applied by Kanaoka [18], according to which 1-ethoxymethylenothiosemicarbazide was obtained from the reaction products such as thiosemicarbazide and ethyl orthoformate. 2-acetyl-amino-1,3,4-thiadiazole (AcAT) was obtained as a result of the ring formation of 1-ethoxymethylenothiosemicarbazide with acetic anhydride. 9.0 g (0.10 mole) of thiosemicarbazide and 16.4 cm^3 (0.10 mole) of ethyl orthoformate (d = 0.8910 g/cm^3) were introduced to the reactor of volume 350.0 cm^3, equipped with a mechanic mixer and a reflux condenser. Next, the mixture was heated in a water bath for 2 hours. After this period of time, 250.0 cm^3 (4.70 mole) of acetonitrile (d = 0.7830 g/cm^3) was introduced to the mixture and the whole mixture was heated to boiling. The mixture was dried thermally. After cooling, the product was dried until the dry mass was attained, the total yield was 3.48 g (24 %) of ethyl orthoformate thiosemicarbazide. 14.0 cm^3 (0.15 mole) of acetic anhydride (d = 1.0840 g/cm^3) was added to 3.0 g (0.02 mole) of ethyl orthoformate thiosemicarbazide. Then, the mixture was being heated at the temperature of 90 ^oC for one hour. The obtained precipitate was filtered, recrystallized from hot water and dried until dry mass was obtained, (yield 90 %) of white crystals of 2-acetylamino-1,3,4-thiadiazole with melting point at the temperature of 265–267 ^oC (literature temperature of 268–269 ^oC [19–21]).

Preparing 2-acetylamino-5-chloro-1,3,4-thiadiazole (AcATCl)

First of all 2.59 mole (80.0 cm^3) of 36 % hydrochloric acid (d = 1.1830 g/cm^3) and 0.33 mole (10.0 cm^3) of 30 % hydrogen peroxide (d = 1.1110 g/cm^3) were introduced to the 350.0 cm^3 volume reactor, equipped with a mechanic stirrer and a thermometer. Reagents were introduced in batches so that the temperature of the mixture should not exceed 10 ^oC. Next, 0.05 mole (8.04 g) of 2-acetylamino-5-mercapto-1,3,4-thiadiazole and 1.32 mole (75.0 cm^3) of 96 % acetic acid (d = 1.0610 g/cm^3) were introduced. The reaction was being carried out for 8 hours at the temperature ranging from 10 to 15 ^oC. The obtained yellow solution was filtered on Buchner's set. White, crystalline substance of 2-acetylamino-5-chloro-1,3,4-thiadiazole was precipitated from the filtrate. Next, it was separated and dried at the ambient temperature until the dry mass was attained. Consequently, 4.32 g of the product (yield 53 %) was obtained with the melting point at the temperature of 245 ^oC (literature temperature 245–246 ^oC) [22].

Preparing 2-acetylamino-1,3,4-thiadiazolo-5-sulfonic acid ($AcATSO_3H$)

First, 24.8 cm^3 (0.435 mole) of 99.5 % CH_3COOH (d = 1.0520 g/cm^3), 28.7 cm^3 (0.93 mole) of 36 % HCl (d = 1.1820 g/cm^3), 8.06 g (0.046 mole) of 2-acetyl-

amino-5-mercapto-1,3,4-thiadiazole and 24.1 cm^3 (0. 786 mole) of 30 % H_2O_2 (d = = 1.1110 g/cm^3) were introduced in turn into the 350.0 cm^3 volume reactor, equipped with a thermometer and the stirrer, and placed in the water bath filled with ice. The reaction time was 2 hours from the moment of introducing the oxidizer at the temperature between 5 and 10 ^oC. The reaction precipitate was filtered and washed with a great amount of ice cold distilled water in order to remove the residue of acids and the oxidizer. The product was stored at the temperature from 7 to 10 ^oC. As a result 5.4 g of 2-acetylamino-1,3,4-thiadiazolo-5-sulfonic acid was obtained (yield 53 %), and melting point of substance was determined at the temperature of 282 ^oC.

Characterized by the concentration ranging from 0.5 to 50.0 mg/cm^3, the solutions of the synthesized compounds presented above were prepared in dimethyl sulfoxide (DMSO). A control sample with fungicidal properties was also made. The effect of the tested heterocyclic compounds on the sensitivity of *Candida albicans* fungi was then determined [23]. The ATCC 10231 fungi strains were received from Hohenheim University in Stuttgart, Germany. They were multiplied within 24 hours, diluted in the Standard-I Broth liquid substratum and added to Standard-I Agar (Standard I-Nhragar, Merck No. 7881) solid medium with 0.5 cm^3 of suspended fungi per 250.0 cm^3 of agar. The agar with the inoculated fungi was spilled on the Petri dishes and left to solidify. Then, in the solidified bases, cylindrical wells with the radius of 4 mm were cut and filled with the solutions of the tested compounds. This activity was performed twice each time: 25.0 and 50.0 mg/cm^3; 1.0 and 12.5 mg/cm^3; 0.25 and 0.5 mg/cm^3. The diluent itself was acting as the control. Afterwards, samples were being incubated for 24 hours at the temperature of 37 ^oC [23–25]. After the incubation, the areas of the fungi growth inhibition were measured (in mm) and the minimal inhibition concentrations (MIC) were defined [25].

Results and discussion

The samples with the lowest concentration of the tested chemical compounds (0.25 mg/cm^3) were biologically inactive. Concentrations of 0.5 mg/cm^3 and 1.0 mg/cm^3 were also rather unreliable for most of the chemical compounds, except for 2-amino-1,3,4--thiadiazole (AT). MIC parameter for AT is 0.5 mg/cm^3, and for AcATS, bAcATDS, $AcATSO_3H$, AcATCl and AcAT MIC = 12.5 mg/cm^3.

Figure 1 presents the relationship determined for all the tested chemical compounds in form of concentrations. These compounds demonstrated, respectively: 12.5, 25.0 and 50.0 mg/cm^3 and the size of the *Candida albicans* inhibition growth zone [mm]. It was found that *Candida albicans* was sensitive only to the concentration of 50.0 mg/cm^3 for all the tested 1,3,4-thiadiazole derivatives. Other lower concentrations of 2-acetyl-amino-1,3,4-thiadiazolo-5-sulfonamide (AcATS) did not demonstrate any fungicidal activity.

For most of the heterocyclic compounds, the size of the growth inhibition zone of *Candida albicans* ranged from 5 to 8 mm. Only the 2-amino-1,3,4-thiadiazole the growth inhibition zone exceeded the average limits ranging from about 12 to 27 mm.

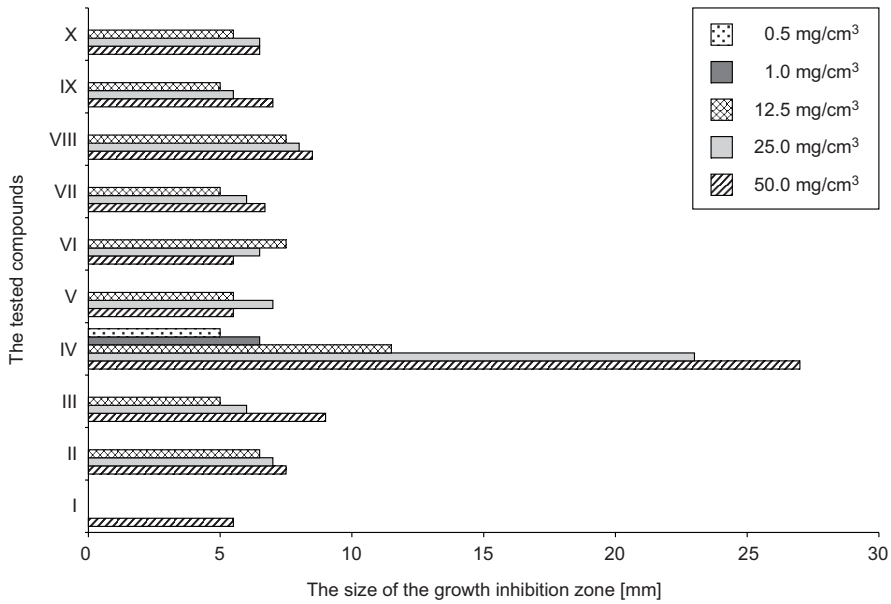

Fig. 1. The size of the growth inhibition zone as a function of 1,3,4-thiadiazole derivative concentration. The tested concentrations were: a) 0.5 mg/cm^3, b) 1.0 mg/cm^3, c) 12.5 mg/cm^3, d) 25.0 mg/cm^3, e) 50.0 mg/cm^3. Markings of the compounds: I – AcATS standard, II – AcATCl standard, III – AcATCl, IV – AT, V – AcAT standard, VI – AcAT, VII – $AcATSO_3H$ standard, VIII – $AcATSO_3H$, IX – bAcATDS standard, X – bAcATDS

For most tested compounds (II, III, IV, VII, VIII, IX), a linear dependence of the growth inhibition zone on *C. albicans* as a function of concentration was observed. Taking into the account the data in Fig. 1, one could notice that two derivatives of thiadiazoles: AcAT and bAcATDS were an exception. For AcAT the lowest concentration 12.5 mg/cm^3 of the standard (V) induced the inhibition equal to 5.5 mm and synthesized (VI) – 7.5 mm (±2.0). The standard (V) of AcAT and the synthesized (VI) of concentration 25.0 mg/cm^3 inhibited the growth of *Candida albicans,* respectively, by 7.0 and 6.5 mm (±0.5). These compounds characterized by the concentration of 50.0 mg/cm^3 inhibited the growth equally by 5.5 mm. For the synthesized AcAT (VI), there was an inverse relationship of the growth inhibition zone from the used concentration of the compound. Standard AcAT did not demonstrate the explicit dependence, which might be a result of the measuring error.

BAcATDS (X) was the other compound which, with the concentration of 12.5 mg/cm^3, inhibited the growth of *C. albicans* by 5.5 mm. But in case of synthesized bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulphonamid, the same inhibition was observed for the concentrations of 25.0 and 50.0 mg/cm^3. It was also found out that the standard bAcATDS (IX) has a tendency towards a decrease in the growth inhibition zone along with a decrease in the tested compound concentration. Therefore, the synthesized derivative X probably indicates a similar tendency, as the results of zone measurements were within the limits of permissible error.

Table 1

Activity of 1,3,4-thiadiazole compounds for *Candida albicans*

Compoud	Concentration [mg/cm^3]														
	0.5			1.0			12.5			25.0			50.0		
	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity
I	—	—	—	—	—	—	—	—	—	—	—	—	3.0	0.8	+
II	—	—	—	—	—	—	3.5	0.9	+	3.5	0.9	+	4.5	1.1	++
III	—	—	—	—	—	—	2.5	0.6	+	3.0	0.8	+	4.5	1.2	++
IV	2.5	0.6	+	4.0	1.0	++	6.0	1.5	++	14.0	3.5	+++	14.5	3.6	+++
V	—	—	—	—	—	—	3.0	0.8	+	3.5	0.9	+	3.0	0.8	+
VI	—	—	—	—	—	—	3.5	0.9	+	3.5	0.9	+	3.0	0.8	+
VII	—	—	—	—	—	—	2.5	0.6	+	3.0	0.8	+	3.5	0.9	+
VIII	—	—	—	—	—	—	4.0	1.0	++	4.0	1.0	++	4.5	1.1	++
IX	—	—	—	—	—	—	2.5	0.6	+	3.0	0.8	+	3.5	0.9	+
X	—	—	—	—	—	—	3.0	0.8	+	3.5	0.9	+	3.5	0.9	+

Where: +++ – high activity; ++ – medium activity; + – low activity; — – deficiency in activity; indications of the compounds as in Fig. 1.

Table 1 presents the determined activities of the tested chemical compounds for different concentrations reacting on the fungi and the values necessary to determine biological activity. The values presented in the table describe, respectively: R – half of the zone of the fungi growth inhibition, r – radius of the well. Basing on R/r, the activity of a given compound was determined for tested fungi strains within the range of 1,3,4-thiadiazole concentration. Interpreting the results: the low activity is found when the ratio of the studied chemical substance $R/r < 1$. The medium activity is for $1 \leq R/r < 2$, and the ratio $R/r \geq 2$ indicates high activity [23]. As it results from the data, after 24 hours of incubation at 37 ^{o}C, the zone of fungi growth inhibition, being a minimum inhibitory concentration (MIC), depended on a tested compound type and the fungi strain.

Among all the tested heterocyclic compounds AcATS showed the lowest activity against *Candida albicans*. It demonstrated the low activity only for the concentration of 50.0 mg/cm^3. The next chemical compound demonstrating low activity against *Candida albicans* was bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide (bAcATDS), both as a standard and a synthesized substance. However, the compound differed from AcATS because it demonstrated low activity not only for the concentration of 50.0 mg/cm^3 but also for 25.0 and 12.5 mg/cm^3. This situation might be perceived as a result of a similar composition of these substances. Another heterocyclic compound, showing the low activity against *Candida albicans* in the same range of the tested concentrations as for bAcATDS was the standard and the synthesized 2-acetyl-amino-1,3,4-thiadiazole. All of the presented chemical compounds have in their structure: acetylamine (CH_3CONH-), sulfonamide (NH_2SO_2-) or just hydrogen (H-) as the final group at the heterocyclic ring. Such chemical composition may result in the low activity of these compounds. Both the standard and the synthesized compound of 2-acetylamino-5-chloro-1,3,4-thiadiazole showed medium and low activity against *Candida albicans*. For concentrations 12.5 and 25.0 mg/cm^3 it showed the low fungicidal activity, but for the concentration of 50.0 mg/cm^3 – the medium activity. The concentration growth of 2-acetylamino-5-chloro-1,3,4-thiadiazole resulted in the activity growth of this compound. Such behavior with respect to *Candida albicans* may be interpreted by the presence of chlorine as the final function group in position 5 of 1,3,4-thiadiazole ring. Interesting results were obtained for 2-acetylamino-1,3,4-thia-diazolo-5-sulfonic acid. The sulfonic acid standard showed a low activity against *Candida albicans* for the concentrations ranging from 12.5 to 50.0 mg/cm^3. However, the same chemically obtained substance showed the medium activity. This difference can be attributed to non-perfect purity of the obtained substance the separated 2-acetylamino-1,3,4-thiadiazolo-5-sulfonic acid ($AcATSO_3H$) and the presence of the compounds occurring in equilibrium:

2-acetylamino-1,3,4-thiadiazolo-5-sulfonic acid $\rightleftarrows$
$\rightleftarrows$ 2-acetylamino-1,3,4-thiadiazolo-5-sulfochloride,

$$R\text{-}SO_3H \rightleftarrows R\text{-}SO_2Cl,$$

which may consequently result in the fungicidal activity growth. The highest and the most diversified activity with *Candida albicans* was found for 2-amino-1,3,4-thiadiazole (AT). This compound has the simplest chemical structure and is the most toxic compound among the tested heterocyclic substances [25]. It was characterized by the low activity for the concentrations of 0.5 mg/cm^3, the medium activity for concentrations of 1.0 and 12.5 mg/cm^3, and the high activity for the concentrations of 25.0 and 50.0 mg/cm^3. This state of being permits the selective elimination or has an influence on *Candida albicans* for 2-amino-1,3,4-thiadiazole. This fact is better illustrated by Figure 2, presenting the *Candida albicans* inhibition growth zone [mm] against the used 2-amino-1,3,4-thiadiazole concentration. The growth tendency was determined by the following cubic polynomial equation: $y = 0.9167x^3 - 7.4286x^2 + 10.655x + 23.1$ (where: y – size of the inhibition growth zone, x – AT concentration) with the high correlation coefficient $R^2 = 0.9895$.

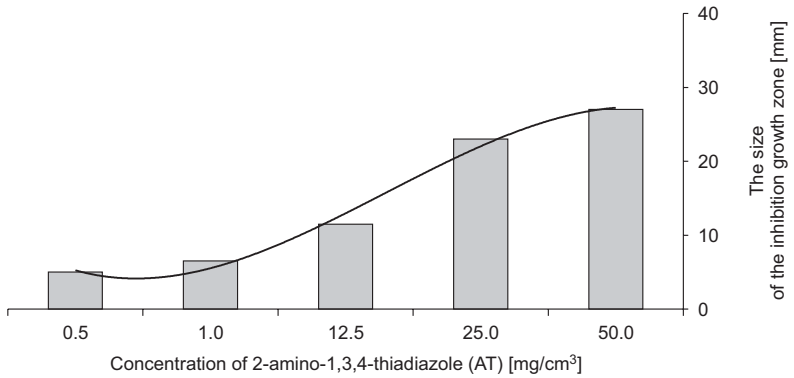

Fig. 2. Fungicidal activity of 2-amino-1,3,4-thiadiazole against *Candida albicans*

Conclusion

The fungicidal activity of 1,3,4-thiadiazole derivatives was tested within the concentration ranging from 0.25 to 50.0 mg/cm^3. The tested compounds causes the increase in the growth inhibition zone of fungus *Candida albicans* along with an increase in their concentrations. Additionally, 2-acetylamino-1,3,4-thiadiazole (AcAT) is an exception and shows an opposite tendency. 2-Amino-1,3,4-thiadiazole (AT) of MIC = 0.5 mg/cm^3 was characterized by the highest and the most diversified activity against *Candida albicans*. This compound may be potentially applied to treat *Candida albicans* mycosis and to reduce the sanitary environment pollution. The highest MIC value (50.0 mg/cm^3) was obtained for 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (AcATS), which showed the low activity against *Candida albicans*.

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Abstrakt: W prezentowanej pracy określano działanie grzybobójcze pochodnych 1,3,4-tiadiazoli. We wszystkich testach używano referencyjnego szczepu *Candida albicans* ATCC 10231. W tym celu przetestowano następujące syntetyzowane i wzorcowe związki heterocykliczne: 2-acetyloamino-1,3,4-tiadiazolo-5-sulfonamid; 2-acetyloamino-5-chloro-1,3,4-tiadiazol; 2-amino-1,3,4-tiadiazol; 2-acetyloamino-1,3,4-tiadiazol; kwas 2-acetyloamino-1,3,4-tiadiazolo-5-sulfonowy oraz bis(2-acetyloamino-1,3,4-tiadiazolo)-5,5'-disulfonamid. Badane związki proporcjonalnie ze zwiększeniem stężenia zwiększają strefę zahamowania wzrostu grzyba *Candida albicans*. Wyjątkiem jest 2-acetyloamino-1,3,4-tiadiazol, który wykazuje odwrotną tendencję. Najskuteczniejszym z badanej grupy związków okazał się 2-amino-1,3,4-tiadiazol. Wykazano, że ze zwiększeniem stężenia tego związku rośnie rozmiar strefy zahamowania wzrostu (y) *Candida albicans* zgodnie z równaniem $y = 0{,}9167x^3 - 7{,}4286x^2 + 10{,}655x + 23{,}1$ (gdzie: x – stężenie 2-amino-1,3,4-tiadiazolu).

Słowa kluczowe: *Candida albicans*, aktywność fungistatyczna, pochodne 1,3,4-tiadiazoli

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Małgorzata KŁYŚ[1]

EFFECTS OF LOWERED TEMPERATURES ON THE MIGRATION ACTIVITES OF THE GRAIN WEEVIL *Sitophilus granarius* L. (*Coleoptera*, *Curculionidae*)

WPŁYW OBNIŻONEJ TEMPERATURY NA AKTYWNOŚĆ MIGRACYJNĄ WOŁKA ZBOŻOWEGO *Sitophilus granarius* L. (*Coleoptera*, *Curculionidae*)

Abstract: The subject of our study was the grain weevil *Sitophilus granarius* L. In this work based on laboratory research, it has been presumed that lowering environment temperatures can have an effect on emigration and immigration processes, and thus on the spread of this pest. The control culture was conducted at a temperature of 28 ^{o}C. The environment temperature of the experimental cultures was changed weekly, and consecutively set at: 25 ^{o}C, 10 ^{o}C, 21 ^{o}C and 25 ^{o}C. In conditions facilitating the migration of adult *S. granarius* specimens, the lowered temperature of the surroundings reduced the population's migration activity, and a particular drop in female migration activity was observed. Females are more sensitive to the effects of lower temperatures.

Keywords: *Sitophilus granarius* L., migration activity, population dynamics, mortality, sex ratio

Granary insects live in enclosed spaces. This is why they are not sensitive to violent temperature changes and long periods of unfavourable thermal conditions. A constant temperature of between 18 to 30 ^{o}C in storehouses around the world explains the swift development of these pests. Yet the temperature in granaries can be altered within a certain range in order to create less favourable conditions for the life and development of these harmful insects [1, 2]. Furthermore, the population of granary cockchafers demonstrates a very large tendency to spread via migration. Restricting the migration processes may provide the chance to prevent new habitats from being attacked by these pests, or reduce their harmfulness [3, 4].

Our research subject is the grain weevil *Sitophilus granarius* – a cosmopolitan species. In Poland it is a common pest of stored grain in all types of storehouses. The

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aim of the following research was to establish the impact of reduced temperatures on the migration activities of the *S. granarius*.

Materials and methods

The research was performed in a laboratory, in thermostats. Emigration was taken into consideration in the experiments on the migration activities of the *S. granarius* populations, ie the departure of specimens from the initial population, as well as two-way migration, ie the departure of specimens from the initial population with the option of return. Two sets of experimental breeding vessels were set up, allowing the adult *S. granarius* specimens to go beyond the bounds of the habitat. Each set was composed of two plastic vessels differing in size. 40 g of wheat was poured into the smaller vessel (bottom surface area: 28 cm^2), into which 40 adult insects of uniform age were introduced, which were extracted from the basic general cultures. Two experimental variants were conducted.

Variant 1. Emigration. The bottom of the larger vessel (surface area: 50 cm^2) was covered with a layer of glycerine; inside it was placed a small container containing wheat with the initial population. The external vessel was closed off with a perforated cover that ensured air-flow. The internal vessel was left open, allowing the specimens free access to the external vessel. The glycerine at the bottom of the external vessel was a trap that kept the emigrants from returning to the wheat. The experiment lasted four weeks. The temperature environment was changed at weekly intervals. The following environment temperature variants were applied in the following order: 25 ^oC, 10 ^oC, 21 ^oC and 25 ^oC, with a constant relative air humidity of 70 %. The control experiment was analogous, except that it was conducted at a constant temperature of 28 ^oC, which is optimal for this insect species.

Variant 2. Two-directional migrations. A set of vessels analogous to that of the prior experiment was applied. Here, however, 40 g of wheat was poured into both vessels. 40 adult insects of uniform age were introduced to the smaller vessel. Then the smaller vessel was placed directly onto the wheat in the external vessel. This gave the specimens the option of either emigrating, or returning to the initial population. The control experiment was analogous, but conducted at a constant temperature of 28 ^oC. All the experiment variants were repeated six times. The impact of changeable, reduced temperatures on migration activities has been evaluated on the basis of population sizes analyses and rates of migration, sex structure and mortality. The results were statistically analysed. The Chi-square test from the Statistica v. 5.5 s. 999 – FGPJI-N4 – program was used.

Results and discussion

The results supported the claim that temperature affects the emigration activities of the *S. granarius*. When the temperature was reduced to 10 ^oC the emigration activity fell to 30 %, and at 21 ^oC emigration remains 40 %. On the other hand, in the control

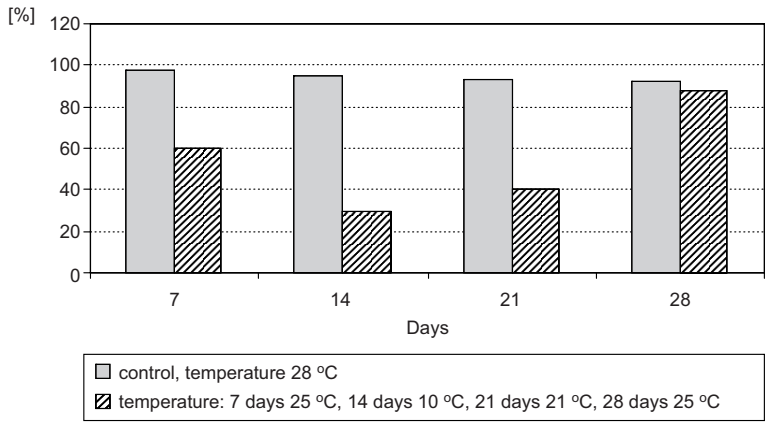

Fig. 1. Emigrational activity of the population of *S. granarius*

culture, it remained very high throughout the experiment, reaching above 90 % (Fig. 1). The number of emigrants under the constant temperature conditions remained high during the whole research period. It was higher than the initial population size (Table 1). An analysis of the Chi-square test demonstrated that the differences between the size of the emigrants in the constant temperature and the emigrants size in the lowered temperature conditions were statistically significant. The analysis of the sex ratio showed that lowering the temperature leads to equal numbers of male and female emigrants. With the constant temperature, however, there were more females among the emigrants, and the sex ratio was lower than 1 (Table 2).

Table 1

Numbers of individuals in initial populations and in emigrating groups of *S. granarius*

Days	Tempera-ture [°C]	Initial population	SD	Emigration groups	SD	Tempera-ture [°C]	Initial population	SD	Emigration groups	SD
7	28	2	0.8	38	1.5	25	16	2.3	24	3.3
14	28	1	0.8	39	2	10	28	2.8	12	1.6
21	28	3	1.2	37	1.9	21	18	2.4	12	1.8
28	28	4	1.2	45	3.1	25	5	1.5	35	2.9

Table 2

Sex ratios in *S. granarius* in emigrating condition

Days	Temperature [°C]	Initial population	Emigration groups	Temperature [°C]	Initial population	Emigration groups
7	28	1	0.7	25	1	1
14	28	1.1	0.8	10	0.9	1
21	28	1	0.9	21	1.1	1
28	28	1	0.9	25	1.1	1

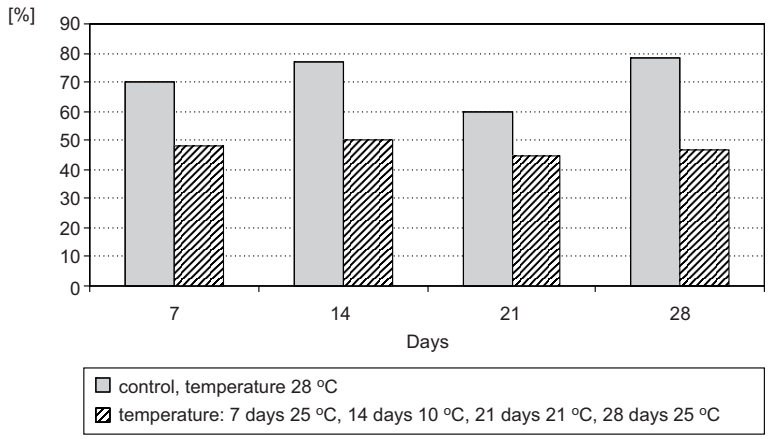

Fig. 2. Migrational activity of the population *S. granarius*

In the case of two-directional migrations, the lowering of the temperature also caused reduced migration activity in *S. granarius*. The value of the migration ratio fluctuated between 45 and 50 %. In the control cultures, meanwhile, the migration ratio was higher and reaches from 60 to 78 % (Fig. 2). At the optimal temperature the migration activity of females and males was generally similar. At lower than optimal temperatures, males showed higher migration activity (Table 3).

Table 3

Sex ratios in *S. granarius* in migrating condition

Days	Temperature [^{o}C]	Initial population	Migration groups	Temperature [^{o}C]	Initial population	Migration groups
7	28	0.9	1	25	1	1.1
14	28	1.1	0.9	10	0.9	1.2
21	28	0.8	1	21	1	1.5
28	28	1	1	25	0.8	1.3

At the lowered temperature the death rate of the initial population is very low, oscillating between 5 and 10 %. In the migrating groups the mortality rate is higher, oscillating between 13 and 36 %. At a constant temperature of 28 ^{o}C, the mortality in the migrating groups is higher than that of the initial population (Fig. 3).

Temperature and humidity are the main factors limiting the appearance of granary pests [5, 6]. This study has dealt with the problem of the effect of lowered temperatures on migration. Limiting migration processes can provide the chance to prevent these pests from attacking new habitats, thus reducing their harmfulness. The research conducted on populations of other pest species *Rhyzopertha dominica, Oryzaephilus surinamensis* or *Sitophilus oryzae* indicate these insects' considerable migration activity

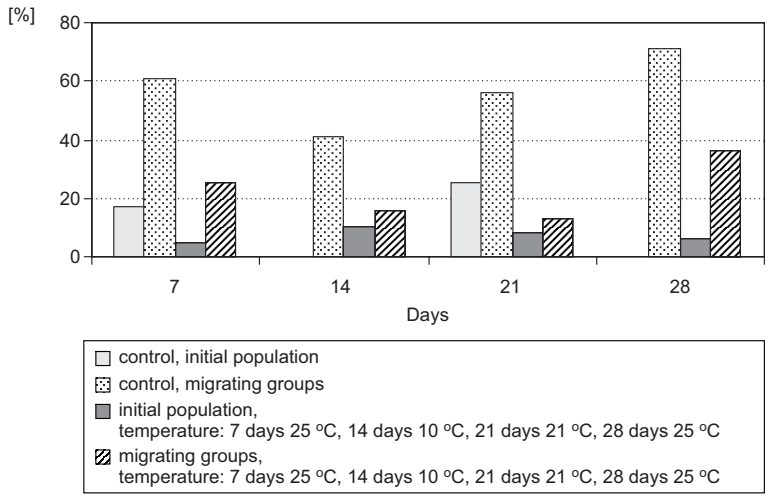

Fig. 3. Percent of dead individuals in population *S. granarius* in migration condition

amongst grain heaps, as well as outside them. This leads to their constant spread over substantial distances, and their attacking of new grain stores [7–9].

The *S. granarius* population, much like that of other storehouse pest species, shows a great deal of migration activity. This species' tendency to migrate explains its high degree of harmfulness and spread in various climate zones.

Conclusions

1. In changeable conditions, lowered temperatures resulted in the reduced emigration activity of the *S. granarius*.

2. Lowered temperatures cause the reduction of female emigration activity. Females are more sensitive to effects of low temperatures.

3. Lowered temperatures do not increase the mortality of the *S. granarius* population.

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WPŁYW OBNIŻONEJ TEMPERATURY NA AKTYWNOŚĆ MIGRACYJNĄ WOŁKA ZBOŻOWEGO *Sitophilus granarius* L. (*Coleoptera*, *Curculionidae*)

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Abstrakt: Obiektem badań był wołek zbożowy *Sitophilus granarius* L. W badaniach laboratoryjnych przyjęto założenie, że obniżenie temperatury środowiska może wywierać wpływ na procesy emigracji i imigracji, a tym samym na rozprzestrzenianie się tego szkodnika. Hodowle kontrolne prowadzono w temperaturze 28 ^{o}C. W hodowlach eksperymentalnych co tydzień zmieniano temperaturę środowiska, stosując kolejno: 25 ^{o}C, 10 ^{o}C, 21 ^{o}C i 25 ^{o}C. W warunkach umożliwiających migracje osobnikom dorosłym *S. granarius* obniżenie temperatury otoczenia spowodowało zmniejszenie aktywności migracyjnej populacji, a szczególnie spadek aktywności migracyjnej samic. Samice są bardziej wrażliwe na oddziaływanie niskich temperatur.

Słowa kluczowe: *Sitophilus granarius* L., aktywność migracyjna, liczebność populacji, śmiertelność, wskaźnik płci

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EFFECT OF APPLICATION OF CAPSAICIN AND PYRETHROID ON METABOLIC RATE IN MEALWORM *Tenebrio molitor*

WPŁYW ZASTOSOWANIA KAPSAICYNY I PYRETROIDU NA TEMPO METABOLIZMU U LARW MĄCZNIKA MŁYNARKA *Tenebrio molitor*

Abstract: In the research presented the effect of Bulldock insecticide and capsaicin on CO_2 release in mealworm *Tenebrio molitor* was assessed. Metabolic rates of insects intoxicated with tested substances were measured using flow-through respirometry. The results obtained showed considerable increase in CO_2 release after application both pyrethroid and capsaicin. The highest metabolic rates were observed after simultaneous intoxication with capsaicin and insecticide. These results suggest that capsaicin enhances the toxic effect of Bulldock and may be used as this insecticide's synergist against mealworms.

Keywords: capsaicin, mealworms, metabolic rate, pyrethroids.

Environment contamination caused by excessive use of insecticides, as well as growing problems connected with resistance force us to look for new directions in crop protection. One of the ideas is to use synergists that will increase insecticidal properties of pesticides. Synergist is safe to environment and people chemical substance, which added to insecticides will increase their toxicity. Our research focuses on the use of capsaicin, an alkaloid responsible for the spicy taste of pepper, as a synergist to pyrethroids.

Pyrethroids are a class of insecticides derived from *Chrysantenum* plant extracts. This natural insecticide served as a base for synthetic pyrethroids production [1]. For almost forty years these insecticides have been commonly used in crop protection, and by the mid-1990's they amounted to 23 % of the world insecticide market and occupied the second position just after organophosphate pesticides [2]. The mode of action of pyrethroids is connected with action of these substances on insects' nervous system. They open the sodium channels, cause membrane depolarization and disturb nerve

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impulse conduction, which leads to insect's paralysis [3]. Unfortunately the extensive use of pyrethroids caused resistance occurence in many species, which is especially disadvantageous in the case of such species as *Anopheles gambiae*, main malaria vector in Africa [4]. Resistance may come into existence as a result of many different molecular mechanisms, mainly due to the increased concentration of detoxification enzymes or by reduction of sensitivity of insects sodium channels to pyrethroids, called 'knockdown' resistance [5]. Despite resistance pyrethroids are profitable to use because of low toxicity to mammals and impermanence in the environment. For that reasons pyrethroids are extensively used all the time, so it is very important to find substances which will increase their toxicity without any harm to living organisms other than pests.

The aim of the presented studies was to assess the effect of capsaicin and pyrethroid insecticide on metabolic rate of *Tenebrio molitor* larvae.

Materials and methods

The experiments were performed on mealworms (*Tenebrio molitor*) reared in 25 ^{o}C and under 12 h/12 h light-dark cycle.

Mealworms were intoxicated with the following substances:

No.	Quantity	Substance
1	10 mm^3	Water (control group)
2	10 mm^3	Insecticide Bulldock 025 EC solution (182 mm^3 dissolved in 1 l of water) – this pesticide belongs to pyrethroids, the active substance is β-cyfluthrin
3	10 mm^3	Capsaicin dissolved in alcohol solution (10^{-4} M)
4	10 mm^3	Ethyl alcohol in concentration 0.1 % – capsaicin does not dissolve in water, but in alcohol. (To make sure that the observed results are real effects of action of capsaicin)
5	10 mm^3	Mixture of alcohol and insecticide in the same concentrations
6	10 mm^3	Mixture of capsaicin (dissolved in alcohol) and Bulldock in the same concentrations

The metabolic rates for each mealworm were measured using flow-through respirometry. After intoxication with the tested substances the mealworms were placed in 2 cm diameter glass-aluminium chambers in 25 ^{o}C. Dry, CO_2 – free air was pumped through the chambers at 50 cm^3/min to a infrared CO_2 analyzer (Qubit Systems Inc., Kingston, Canada). CO_2 release of insects was measured for four hours from intoxication.

Mean metabolic rate of 6–8 mealworms intoxicated with particular substance was subjected to statistical analysis. t-Student test was used to compare the difference between control and intoxicated groups. Significant difference was achieved when $p < 0.05$.

Results and discussion

Many toxic compounds may have an influence on physiological processes in insects, which results very often in organisms dysfunction. Under stress conditions, such as toxicant application, insect may increase their metabolism to cope with changed energy demands [6].

The study presented clearly showed that both capsaicin, as well as pyrethroid increase CO_2 release in *Tenebrio molitor* larvae (results are shown in Fig. 1 and Fig. 2). In study presented, application of a natural alkaloid, capsaicin, resulted in a shift in carbon dioxide release (on average 1.33 times higher in comparison with control group) during all four hours of the experiment. The experiments performed on mice and rats revealed that capsaicin promotes secretion of adrenaline and increase in oxygen consumption after single administration [7, 8].

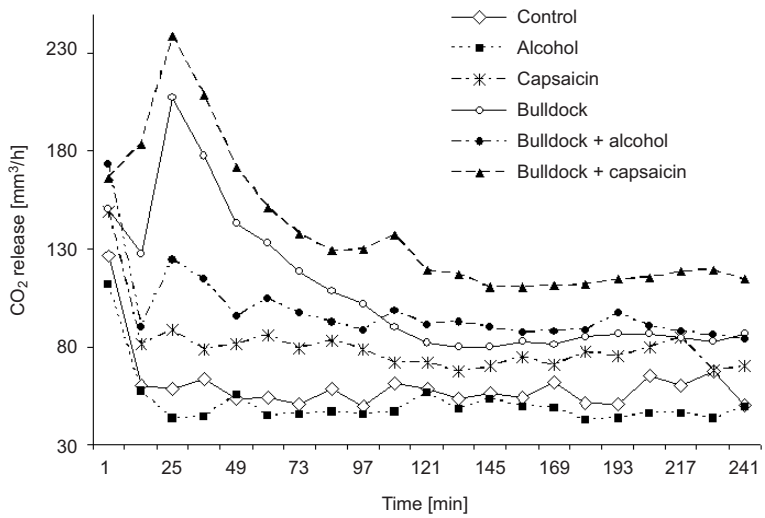

Fig. 1. Changes in CO_2 release in *Tenebrio molitor* larvae after intoxication with the tested substances during four hours of experiment

Mealworms intoxicated with Bulldock demonstrated significant increase in CO_2 release (the intoxicated insects released about 1.8 of CO_2 times more than control ones). Similar results were obtained by Zafeiridou and Theophilidis [9], where application of 50 and 100 ng of deltamethrin (pyrethroid) per mealworm beetle caused concentration-dependent increase of respiratory rate. Enhanced metabolic rate in insects may be a result of detoxication processes initiation, convulsion caused by poisoning or excitation of respiratory motoneurons. Pyrethroids increase the probability of the sodium channels' opening and cause gradual membrane depolarization, which triggers excitation of the cells. This leads to increased work of respiratory motoneurons and enhanced CO_2 release in insects. Another study showed that application of insecticides has a great influence on glycogen metabolism in intoxicated insects body. Increased levels of trehalose and glucose in the haemolymph are suggested to be a result of raised transportation of these compounds from fat body to haemolymph to supply energy for enhanced detoxification processes [6]. Similar processes may be observed after application of other toxins, such as capsaicin.

Combination of insecticide and alcohol did not change significantly the metabolic rate of the examined insects in comparison with Bulldock alone. In that case we can

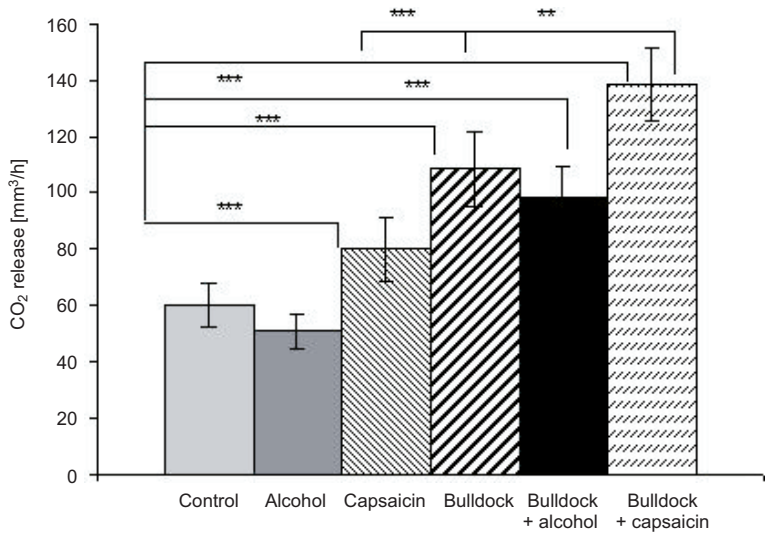

Fig. 2. CO_2 release in mealworms after intoxication with the tested substances (mean ± SE). Statistical significant difference is shown as * (** – $p < 0.01$;*** – $p < 0.001$)

exclude the effect of alcohol on the metabolism of both capsaicin (because alcohol alone did not have any significant influence either), as well as insecticide.

Simultaneous application of capsaicin and pyrethroid resulted in the highest growth of CO_2 release. We can observe synergism between capsaicin and insecticide, appearing as increased metabolic rate in intoxicated mealworms. Simultaneous effect of these two compounds probably resulted in activation of intensive detoxification processes. What is more, recent researches showed that pyrethroids may also act on voltage-dependent calcium channels and increase calcium influx [10]. The main target for capsaicin in mammals is vanilloid receptor subtype 1 (TRPV1), which is calcium-permeable cation channel. Capsaicin binding to this receptor results in channel opening and calcium influx. It may be possible that capsaicin exerts an influence on calcium homeostasis in insects as well. Simultaneous action of capsaicin (possible calcium influx) and pyrethroids (sodium and calcium influx) would result in strong response of insects. This would explain why one hour after application the intoxicated insects were paralysed. Capsaicin intensified the effect of pyrethroid action. This result suggest that capsaicin may be used as pyrethroid insecticides synergist against mealworms. Because of increased metabolic rate insects have higher energy demands. Combination of capsaicin and pyrethroid may not cause death of pests, but after intoxication insects will have higher metabolic rate and energy consumption, so it may not be enough energy supplies to reproduce.

Acknowledgements

This research was funded by the Ministry of Science and Higher Education grant number 3039/B/P01/2008/34. Additional funding by the European Social Fund and National Budget Through "Zintegrowany

Program Operacyjny Rozwoju Regionalnego, Działania 2.6 – Regionalne Strategie Innowacyjne i Transfer Wiedzy" of the Kujawsko-Pomorskie province stipends for doctoral candidates 2008/2009 – ZPORR.


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WPŁYW ZASTOSOWANIA KAPSAICYNY I PYRETROIDU NA TEMPO METABOLIZMU U LARW MĄCZNIKA MŁYNARKA *Tenebrio molitor*

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Abstrakt: W prezentowanych badaniach określano wpływ insektycydu Bulldock i kapsaicyny na tempo uwalniania CO_2u larw mącznika młynarka *Tenebrio molitor*. Tempo metabolizmu owadów intoksykowanych badanymi substancjami mierzono przy użyciu układu respirometrycznego, służącego do pomiarów w układzie przepływowym. Uzyskane wyniki wykazały znaczący wzrost ilości uwalnianego CO_2 po aplikacji zarówno pyretroidu, jak i kapsaicyny. Najwyższe tempo metabolizmu odnotowano po jednoczesnej intoksykacji kapsaicyną i insektycydem. Przedstawione wyniki sugerują, że kapsaicyna zwiększa toksyczny wpływ pestycydu Bulldock i prawdopodobnie może być używana jako synergetyk dla tego insektycydu przeciwko larwom mącznika młynarka.

Słowa kluczowe: kapsaicyna, larwy mącznika młynarka, tempo metabolizmu, pyretroidy

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THE QUALITY OF PLUM FRUITS AFTER STORAGE DEPENDEND OF QUALITY PARAMETERS DURING HARVEST

JAKOŚĆ OWOCÓW ŚLIWY PO PRZECHOWYWANIU W ZALEŻNOŚCI OD PARAMETRÓW JAKOŚCOWYCH W CZASIE ZBIORU

Abstract: European plums 'Bluefre', 'Valor', 'Valjevka' and 'Elena' cvs. were harvested and graded according to colour and firmness, and on this basis divided into three maturity fractions. The fruits were analysed for mass loss, flesh firmness, total soluble solids (TSS), skin ground colour as well as incidence of fungal diseases and physiological disorders after 4 weeks of cold storage at 1 ^{o}C and 2 days of ripening at 20 ^{o}C.

Significant differences were found in fruit weight loss, skin colour, firmness and TSS among maturity fraction during and after storage. A decrease in firmness, fruit mass, and skin colour as well as an increase in TSS were observed, however all changes depended on the maturity stage during harvest. Less mature fruits withstood better cold storage than more mature. All fruits showed flesh browning symptoms after 4 weeks of storage and the least browning symptoms were found for 'Valor', regardless of the fruit maturity during harvest. The most browning symptoms were observed for 'Valjevka', which indicates the low suitability of this cultivar for 4-week storage. The best storability was observed for 'Valor' due to the best after-storage quality. Both firmness and skin colour can be applied to the assessment of the optimum harvest date of fruits intended for storage.

Keywords: plum, cold storage, TSS, firmness, optimum harvest date, colour

Plums are highly perishable and, depending on the cultivar, may only have a commercial life of 2–6 weeks even when stored at 0 ^{o}C, due to the appearance of physiological disorders such as internal browning and gel formation [1]. Maturity at harvest is the most important factor that determines storage-life and final fruit quality. Immature fruits are more subject to shrivelling and mechanical damage, and are of inferior flavour quality when ripe. Overripe fruits are likely to become soft and mealy with insipid flavour soon after harvest. Fruits picked either too early or too late in their

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season are more susceptible to postharvest physiological disorders than fruits picked at the proper maturity [2]. An immature fruit may ripen off the tree but it will be of poor quality [3]. Ripening involves changes that transform the mature fruit into one ready to eat. Changes associated with ripening include loss of green colour and development of yellow, red and other colour characteristics of the cultivar [4]. Once the fruit ripens, senescence begins; physical and chemical changes continue after "optimum" ripeness is reached, including further softening, loss of desirable flavour and complete breakdown [5]. Fruits harvested at an earlier maturity withstand cold storage better than more mature fruit. However, less mature fruits initially have poorer quality than those which are more mature [6].

Flesh firmness can be used as a maximum maturity index to determine how late fruits can be harvested and still ensure good quality after transport, shipping and marketing, and as the minimum firmness that plums can be harvested in order to avoid bruising during standard postharvest handling [4]. The use of only firmness or TSS as a maturity index alone is limited by variation among cultivars, production area and season [7, 8]. Parameters such as fruit size, skin colour, flesh firmness, soluble solids or acidity are used to determine the maturity of fruit at harvest [4, 9]. However, the decision when to harvest should also take into account other factors, such as fruit drop, environmental conditions, hand labour availability, market prices, distance to market, potential transportation damage and storage temperature [10].

This study was undertaken to establish the influence of harvest maturity on cold-storage life and quality, and to evaluate the maturity parameters of four important cultivars in Poland.

Materials and methods

The four autumn plum cultivars used in this study were of the European type (*Prunus domestica* Lindl.). The experiments were performed during the 2008 season, with fruit harvested according to visual symptoms of maturity (colour and firmness) at the following dates: August 25th – 'Valor', September 1st – 'Bluefre' and 'Valjevka', and September 8th – 'Elena'. Fruits were selected from 12-year-old trees on a 'Wegierka Wangenheima' rootstock grown in an experimental farm 25 km west from Poznan. All fruits were handpicked and transported on the day of harvest to the Postharvest Laboratory at the Poznan University of Life Sciences.

For each cultivar a sample of around 60 kg of fruits (5 boxes) was harvested. The fruits within each sample were graded according to colour and firmness and on this basis divided into three fractions: first – hard fruits not fully coloured, described as *harvest maturity* fruits (HM1), second – fruits fully coloured, softer than the first fraction but not in *edible maturity* yet (HM2), and third – fruits close to consumption maturity (CM). From the afore-mentioned fractions samples of fruits for preliminary measurements were taken and the rest of fruits were stored immediately at 1 ^{o}C, RH 90 % for 4 weeks.

Twenty-five fruits from each fraction were numbered for identification on the peel surface, in order to make non-destructive mass loss measurements during and after storage. The fruits were assessed for mass loss, skin ground colour, firmness and TSS

content. In addition, fruit quality was also evaluated by determining levels of shrivelling and rot incidence by examining all boxes after storage. Mass loss and skin ground colour after the 2-days shelf-life period was evaluated by weighing samples at room temperature, just before the fruits were destructively sampled for physicochemical assessments. Skin ground colour was measured on each fruit with a colorimeter (Minolta, CR-200) as *L**, *a**, *b** values. Three determinations of the colour parameters were made along the equatorial axis of each fruit. Flesh firmness was determined after peeling, using an Effegi penetrometer mounted on a hand-operated press, to minimize loading rate variations associated with the operator, and fitted with an 7.9 mm diameter plunger. Measurements were taken at three equatorial positions on each fruit at 120^{o} with the 8 mm deep and the results were expressed in kilogram force (kG).

After the assessment of fruit firmness, fruit juice was extracted by homogenising fruit flesh in a blender, making 4 unified sample. The TSS of the juice was measured for each sample with a digital refractometer.

The data were subjected to analysis of variance. The mean comparisons were performed using the Duncan test to examine differences ($p < 0.05$) among the harvest dates.

Results and discussion

All fractions of fruits were significantly different with regard to firmness and skin colour a* value during harvest (Table 1). The skin colour a* value is the most interesting and representative colour attribute of plums because it describes the colour changes from green to red which are typical of the maturation of fruits, so it could be used to select different ripeness stages of these plums, which was proved as well for the plum fruits 'Green Gage' by Guerra and Casquero [6].

Table 1

Quality parameters during harvest

Cultivar	Maturity at harvest	Colour of fruit			Firmness [kG]	TSS**
		L	a	b		
'Bluefre'	HM1	35.1 a*	2.2 b	–7.4 a	5.85 c	13.6 a
	HM2	34.1 a	1.6 ab	–7.7 a	4.03 b	14.2 a
	CM	33.9 a	1.2 a	–7.5 a	2.75 a	16.3 b
'Valjevka'	HM1	35.3 a	1.0 b	–7.3 a	4.40 a	13.0 a
	HM2	35.1 a	0.9 b	–7.1 a	3.55 a	14.1 b
	CM	34.3 a	–0.1 a	–7.1 a	2.95 a	15.9 c
'Valor'	HM1	32.5 a	3.6 b	–5.1 a	5.20 c	18.0 a
	HM2	31.8 a	3.2 b	–5.2 a	3.60 b	17.9 a
	CM	31.6 a	2.3 a	–5.4 a	2.10 a	21.2 b
'Elena'	HM1	33.4 b	5.3 b	–2.9 a	3.90 b	12.7 a
	HM2	31.5 ab	4.4 b	–4.7 a	3.30 ab	13.1 a
	CM	30.1 a	1.0 a	–6.4 b	2.75 a	14.1 b

Explanation: * Different letters indicate significant differences between maturity stages, according to Duncan's test (p 0.05); ** Total Soluble Solids; 1 kG = 9.807 N; 1 N = 1 kg · m · s^{-2}

Differences of TSS content were observed for each maturity group of 'Valjevka', but for other cultivars there were no significant differences between HM1 and HM2.

TSS increased significantly during the ripening period from 2 weeks of cold storage onwards (Table 2).

Table 2

Quality parameters after 2 weeks of cold storage

Cultivar	Maturity at harvest	Colour of fruit			Firmness [kG]	TSS**
		L	a	b		
'Bluefre'	HM1	35.5 a*	0.3 a	–8.6 a	1.5 a	16.0 a
	HM2	36.2 a	0.1 a	–7.5 a	1.7 a	15.5 a
	CM	38.0 b	–0.5 a	–10.4 a	1.6 a	16.1 a
'Valjevka'	HM1	33.5 a	0.8 b	–7.9 a	3.75 b	12.8 a
	HM2	34.0 a	0.5 b	–6.9 a	2.6 a	15.3 b
	CM	34.5 a	–1.6 a	–8.1 a	2.6 a	15.4 b
'Valor'	HM1	32.8 a	4.8 c	–6.9 a	4.5 c	17.9 a
	HM2	32.9 a	3.0 b	–6.1 a	2.8 b	18.5 a
	CM	32.8 a	0.3 a	–8.9 a	1.4 a	20.7 b
'Elena'	HM1	36.9 b	2.2 a	–5.5 a	3.4 b	13.2 a
	HM2	33.5 a	3.1 a	–5.5 a	2.65 a	15.3 c
	CM	33.7 a	1.4 a	–5.1 a	2.55 a	14.4 b

Explanation: See Table 1.

The change in TSS during harvesting and cold storage resembles that found for 'Songold' plums by Taylor et al [11] and by Guerra and Casquero [6]. No difference of TSS between HM2 and CM for 'Bluefre' and 'Valjevka' suggests quicker ripening at cold storage as compared with the two other cultivars. The low and equal level of fruit firmness, as well as no differences of skin colour a* value for 'Bluefre' fruits confirm the above conclusion, too.

TSS content in HM1 and HM2 of all plum cultivars increased during 4 weeks of cold storage (Table 3). Except 'Bluefre', where there were no differences in the sugar level, the lowest sugar level was found in fruits which where the most mature during harvest (CM). TSS of 'Elena' fruits neither at harvest nor after storage reached the values described in other papers [12]. Only the last measurement after 4 weeks of storage showed values over 17 % for HM1. These low values could be influenced by a high crop load [6].

Firmness after the whole period of storage depended on the maturity stage during harvest, but in this study the differences in firmness were not big and significant differences were observed only between the HM1 and the remaining two fractions of 'Valor' and 'Elena'. There were no differences of skin colour after four week of storage.

Mass loss of fruits during storage was highly influenced by the date of harvest (Fig. 1).

Table 3

Quality parameters after 4 weeks of cold storage

Cultivar	Maturity at harvest	Colour of fruit			Firmness kG]	TSS**
		L	a	b		
'Bluefre'	HM1	35.8 a*	–0.7 b	–7.4 a	0.6 a	17.1 a
	HM2	35.4 a	–0.9 b	–7.8 a	0.5 a	16.9 a
	CM	34.7 a	–1.5 a	–8.9 a	0.5 a	16.6 a
'Valjevka'	HM1	31.8 a	0.2 a	–7.3 a	1.6 a	20.7 b
	HM2	32.2 a	0.1 a	–7.2 a	1.3 a	16.0 a
	CM	32.6 a	–0.8 a	–6.9 a	1.4 a	17.3 a
'Valor'	HM1	34.7 b	0.4 a	–7.1 a	1.6 b	17.9 a
	HM2	34.2 b	1.2 a	–7.3 a	1.2 a	20.2 b
	CM	32.6 a	0.0 a	–6.7 a	0.9 a	16.4 a
'Elena'	HM1	36.6 b	1.8 a	–5.9 a	3.1 b	17.7 c
	HM2	33.8 a	1.9 a	–5.1 a	2.1 a	16.7 b
	CM	32.2 a	1.6 a	–5.7 a	2.3 a	14.8 a

Explanation: See Table 1.

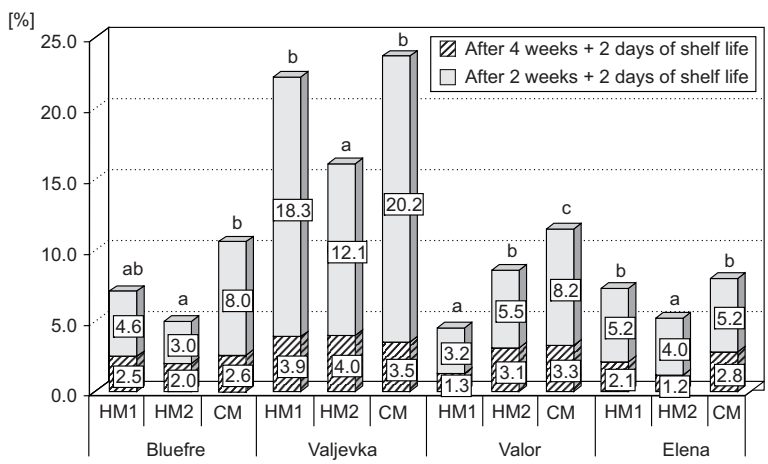

Fig. 1. Mass loss of plums fruits during and after cold storage

Fruits of the 'Valor' cultivar which were softer during harvest lost more mass, with mass loss being the biggest for CM and the lowest for HM1. This was not found for other cultivars, in which the HM2 fraction had the lowest mass loss. The ripest fruits usually start shriveling sooner, which causes higher transpiration [6], but unripe fruits often lose more weight, too, because of a thinner wax cover on the skin [13].

Storability of fruits could be measured by loss caused by infection with fungal diseases (rotten fruits) and incidence of physiological disorder (flesh browning) (Fig. 2).

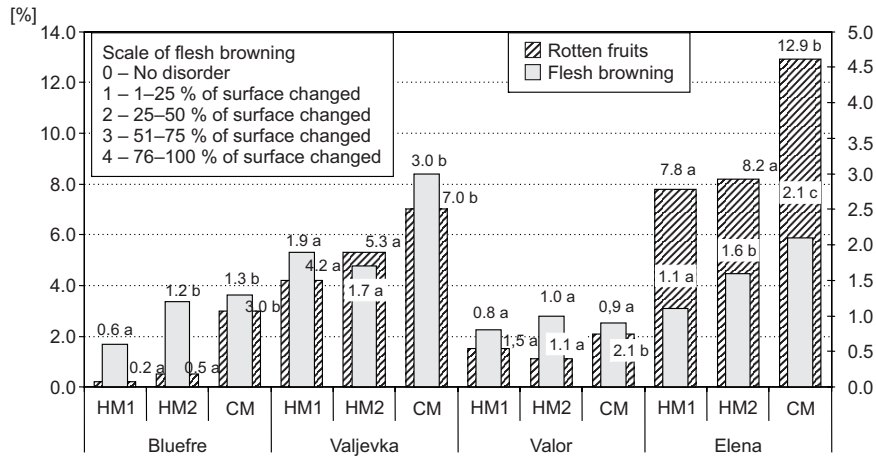

Fig. 2. Loss of fruits after storage caused by rotting and physiological browning

The percentage of rotten fruits varied from 0.2 % for HM1 of 'Bluefre' fruits to 12.9 % for CM of 'Elena' fruits, nevertheless the biggest losses among cultivar were observed for CM of all cultivars. Only incidence of flesh browning of 'Valor' fruits did not show any influence of treatment. All fruits showed flesh browning symptoms after 4 weeks of storage. The least browning symptoms were found for 'Valor', regardless of the fruit maturity during harvest, which suggests the highest storability of 'Valor' of all examined cultivars. In the remaining cultivars the CM fraction showed the most browning symptoms and the browning symptoms in most of fruits of 'Valjevka' indicate the low suitability of this cultivar for 4-week storage. Because of high rotting of each 'Elena' fraction seems to be as well not good cultivar for longer storage at NA condition.

Conclusions

1. The best storability was observed for 'Valor' due to the smallest losses of fruits and the best after-storage quality.

2. The minimum firmness of large fruit cultivars ('Valor' and 'Bluefre') required for cold storage should be approx. 5 kG, whereas the firmness of small fruit cultivars ('Valjevka', 'Elena') should be 4 kG.

3. Both firmness and skin colour can be applied to the assessment of the optimum harvest date of fruits intended for storage.

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JAKOŚĆ OWOCÓW ŚLIWY PO PRZECHOWYWANIU W ZALEŻNOŚCI OD PARAMETRÓW JAKOŚCOWYCH W CZASIE ZBIORU

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Abstrakt: Odmiany śliwy ‘Bluefre’, ‘Valor’, ‘Valjevka’ i ‘Elena’ po zbiorze zostały rozsortowane w zależności od wybarwienia i jędrności na trzy grupy różniące się stadium dojrzałości. Po 4 tygodniach przechowywania w chłodni zwykłej w temperaturze 1 ^{o}C i 2 dniach dojrzewania w temperaturze 20 ^{o}C analizowano stratę masy owoców, jędrność, zawartość ekstraktu, barwę skórki oraz występowanie chorób grzybowych i fizjologicznych.

Stopień dojrzałości owoców w czasie zbioru wpływał na utratę masy na skutek transpiracji, na zabarwienie owoców, jędrność i zawartość ekstraktu po 2 i 4 tygodniach przechowywania. Jędrność i masa owoców malała, a zwiększała się zawartość ekstraktu, jednak tempo i stopień tych zmian były ściśle uzależnione od stadium dojrzałości w czasie zbioru. Mniej dojrzałe owce wykazywały mniejsze straty spowodowane chorobami grzybowymi, jednak u wszystkich odmian po 4 tygodniach stwierdzono fizjologiczne przebarwienia miąższu. Najmniejsze zbrunatnienie stwierdzono u owoców odmiany ‘Valor’, a ponadto nie było ono uzależnione od początkowej dojrzałości. Największe zbrunatnienie miąższu stwierdzono po przechowywaniu owoców odmiany ‘Valjevka’, natomiast największe straty powodowane chorobami grzybowymi u owoców odmiany ‘Elena’, co pozwala uznać je za mało przydatne do 4-tygodniowego przechowywania w chłodni.

Słowa kluczowe: śliwa, przechowywanie, data zbioru, ekstrakt, jędrność, barwa

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 17, No. 10 2010

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SEASONAL CHANGES OF HYDROCHEMICAL CONDITIONS IN SELECTED LAKES OF THE DRAWA NATIONAL PARK (POLAND)

SEZONOWE ZMIANY WARUNKÓW HYDROCHEMICZNYCH WYBRANYCH JEZIOR DRAWIEŃSKIEGO PARKU NARODOWEGO

Abstract: The aim of this study was to determine seasonal changes of hydrochemical conditions in subsurface and near-bottom water in lakes Marta and Sitno located in the Drawa National Park (Poland). Hydrochemical monitoring was conducted in years 2005–2007 and comprised determination of temperature, dissolved oxygen, pH, phosphates, nitrates(III), nitrates(V), iron(II) and total iron. The study revealed that in both lakes oxygen saturation of subsurface and near-bottom water within the littoral zone reached maximum values in spring and minimum values in autumn. In deeper layers, hypoxia occurred in near-bottom water throughout the whole study period. Average temperature of both subsurface and near-bottom water was significantly higher in lake Marta. Concentrations of nitrates (III and V) were higher in lake Sitno, and the difference was statistically significant for near-bottom layer. Elevated concentrations of nutrients observed in both lakes indicate their eutrophic character. Lower nitrate(III) concentrations in lake Marta may evidence occurrence of denitrification processes.

Keywords: hydrochemical conditions, lakes, the Drawa National Park

Water chemistry in lakes and reservoirs is determined by numerous factors of non-monotonous dynamics of changes. Local water chemistry depends on factors such as climate, hydrology, hydrogeology and soil conditions within the catchment area, that can decrease or increase the rate of matter delivery to the lake [1]. Morphometrical and hydrological features of the lake also affect its natural susceptibility to degradation [2], therefore each water body has individual features which differentiate and model its hydrochemical conditions [3]. However, the dynamics of hydrochemical changes in lakes are influenced most significantly by human economic activity, resulting not only in transformation but also often in degradation of the aquatic environment. Human pressure, transforming primary landscape into agricultural one, contributes to disturb-

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ances in water conditions and makes local water cycles shorter. Due to insufficient infiltration of water from precipitation, surface runoff enriches surface waters with chemical elements inflowing from the catchment basin [4], increasing their trophy. Reduction of lake eutrophication rate depends on many factors that affect complicated biogeochemical cycles and determine cycling between biotic and abiotic elements of aquatic systems [5]. These relationships are also conditioned by the dynamics of water masses divided into separate layers of totally different properties [6]. Processes running in particular layers depend on the season of the year. Knowledge on seasonal changes of hydrochemical conditions is especially important in regard to lakes located in protected areas. For such lakes, long-term analysis of physicochemical factors enables detection of possible threats to their homeostasis, which is important for controlled fishery exploitation.

Therefore, the aim of this study was to determine seasonal changes of hydrochemical conditions in two lakes of different kind in the Drawa National Park (Poland). Both lakes have been monitored for the number and health condition of economically valuable fish: whitefish (*Coregonus lavaretus*) and vendace (*Coregonus albula*) in Lake Marta, and lake trout (*Salmo trutta* m. *lacustris*) in Lake Sitno.

Material and methods

Study area

Water bodies (≥ 1 ha) in north-western Poland amount to 1575 lakes of which 21 (total area 937 ha) are located in the Drawa National Park [7]. This study comprised two lakes of similar area situated in the Drawa National Park. The first one – Lake Marta – is a non flow-through, mesotrophic, dimictic lake with a maximum depth of 25 m, average depth of 7.7 m and area of 66.1 ha, inhabited by *Chara spp*. The second one – Lake Sitno – is a flow-through, eutrophic, polymictic lake with a maximum depth of 7 m, average depth of 4 m and area of 67.2 ha. Regarding susceptibility to degradation, these lakes have been classified into categories 2nd and 3rd, respectively [8]. Hydrochemical monitoring of these lakes was conducted in years 2005–2007, in four seasons, in subsurface and near-bottom water layers. Sampling sites were selected within the littoral zone and in the deepest places of the lakes, their location being identified by GPS. Seven sampling sites were located in Lake Marta, while six sites in Lake Sitno. To examine hydrochemical conditions of the flow-through Lake Sitno, water samples were collected in places of inflow and outflow of the Plociczna River.

Spring samples were collected in April or May, summer samples were collected in July or August, autumn samples were collected in September or October, while winter samples were collected in December. The samples were collected with standard hydrochemical methods. Temperature, dissolved oxygen and pH were measured *in situ*, while concentrations of phosphates, nitrates(III), nitrates(V), iron(II) and total iron were determined in the laboratory. Laboratory measurements were based on colorimetric methods and conducted with a HACH DR 890 spectrophotometer.

Confidence semi-intervals for the parameters examined (average values for the whole study period and for particulate seasons) were calculated with the non-parametric

Mann-Whitney U test at the significance level $p < 0.05$. The calculations were made with the aid of the StatSoft Statistica 7.1 software.

Results and discussion

In every season during the study period, water in both subsurface and near-bottom layers of lakes Marta and Sitno had slightly alkaline reaction. In Lake Marta pH varied from 7.73 (near-bottom layer, spring) to 8.31 (subsurface layer, autumn), while in Lake Sitno pH ranged from 7.73 (near-bottom layer, spring) to 8.08 (subsurface layer, winter) (Table 1). Statistical analysis revealed that water pH was significantly higher in Lake Marta during the whole study period (Table 2). In both lakes subsurface pH values were slightly higher than near-bottom ones during the whole study period, except for autumn in Lake Sitno. The detected values of water pH were advantageous, as such conditions favour intensification of nitrate(III) reduction [9]. This process occurs mainly in the near-bottom layer containing anaerobic zones where heterotrophic and autotrophic strains of bacteria contribute to denitrification and reduction of nitrate(III) to gaseous nitrogen [10].

The average water temperature in both subsurface and near-bottom layer was significantly higher in Lake Marta (Table 2), despite the lake being deeper than Lake Sitno. This illustrates a significant cooling influence of the Plociczna River on Lake Sitno. Moreover, polymictic character of Lake Sitno contributes to constant mixing of warmer epilimnetic and cooler hypolimnetic water, as evidenced by lower, than in Lake Marta, differences between average temperatures of subsurface and near-bottom layers. Morphometric features of Lake Marta (especially high depth, shape of the lake's basin, clearly distinguished epi- and hypolimnion) create optimal conditions for salmonid fish [11]. On the contrary, a flow-through character of Lake Sitno combined with intensified primary production creates optimal conditions for cyprinid fish.

Another factor crucial for fish is dissolved oxygen. In the summer and autumn periods, values of this parameter were similar for both lakes (Table 2), while in the winter and spring periods water in Lake Sitno contained significantly more dissolved oxygen than water in Lake Marta. Low dissolved oxygen content occurred in Lake Sitno, however it has never been totally depleted [12]. In both lakes, maximum dissolved oxygen in subsurface and near-bottom waters was detected in spring, and minimum – in autumn (Table 1). This tendency might have originated from vegetation zone developed around the lakeshore. In a consequence of an extended littoral zone, organic matter accumulated in autumn after vegetation period and its decomposition temporarily diminished dissolved oxygen content. Higher oxygen saturation of subsurface water might increase pH in the epilimnion [13]. Favourable conditions for brood-stocks of vendace (*Coregonus albula*) and whitefish (*Coregonus lavaretus*) in Lake Marta and lake trout (*Salmo trutta* m. *lacustris*) in Lake Sitno occurre in spring, when the lakes are the richest in dissolved oxygen. Near-bottom water in Lake Marta has the most stable oxygen conditions whole year round, and this favours occurrence of salmonids, however, autumnal hypoxia in the littoral zone may impair reproduction in the fish, as they spawn at that time [14]. This has been reflected by a decrease in

Table 1

Ranges and average values of hydrochemical parameters for 6 sampling sites in Lake Marta and 7 sampling sites in Lake Sitno for years 2005–2007

Parameters	Subsurface layer				Near-bottom layer			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Lake Marta								
pH	7.76 (7.67–7.87)	8.06 (7.62–8.5)	8.31 (8.15–8.56)	8.03 (8.00–8.05)	7.73 (7.61–7.80)	7.94 (7.42–8.51)	8.14 (8.01–8.36)	7.94 (7.92–7.96)
Temperature [°C]	14.6 (13.8–15.6)	22.2 (19.9–26.4)	15.8 (10.2–19.1)	5.1 (4.0–6.5)	14.0 (12.4–15.3)	20.1 (17.1–23.0)	14.1 (9.5–17.1)	5.0 (4.0–6.4)
O_2 [mg · dm^{-3}]	11.1 (10.5–11.9)	9.1 (8.3–10.5)	9.3 (7.8–10.5)	9.9 (9.5–10.1)	10.5 (10.3–10.8)	8.3 (7.0–10.4)	8.4 (6.9–10.2)	8.4 (8.1–8.7)
NO_2-N [mg · dm^{-3}]	0.011 (0.010–0.012)	0.011 (0.008–0.014)	0.017 (0.012–0.023)	0.011 (0.010–0.013)	0.010 (0.010–0.011)	0.008 (0.005–0.010)	0.017 (0.014–0.018)	0.013 (0.012–0.013)
NO_3-N [mg · dm^{-3}]	8.6 (8.5–8.8)	6.7 (5.3–10.6)	8.6 (6.2–10.6)	7.2 (5.0–8.9)	8.8 (8.8–8.9)	6.1 (5.4–7.0)	6.4 (5.9–6.9)	6.7 (4.6–8.9)
PO_4^{-3} [mg · dm^{-3}]	0.43 (0.25–0.65)	1.26 (0.40–2.60)	0.95 (0.15–2.21)	3.24 (2.69–3.97)	0.44 (0.22–0.65)	0.51 (0.36–0.79)	1.02 (0.16–2.41)	0.77 (0.64–0.99)
Fe^{2+} [mg · dm^{-3}]	0.02 (0.01–0.04)	0.02 (0.01–0.02)	0.02 (0.01–0.02)	0.03 (0.02–0.04)	0.03 (0.01–0.04)	0.01 (0.01–0.02)	0.04 (0.01–0.06)	0.02 (0.01–0.03)
Total Fe [mg · dm^{-3}]	0.09 (0.02–0.22)	0.02 (0.01–0.03)	0.04 (0.03–0.04)	0.05 (0.03–0.06)	0.03 (0.02–0.03)	0.03 (0.02–0.03)	0.06 (0.03–0.09)	0.04 (0.02–0.06)

Table 1 contd.

Parameters	Subsurface layer				Near-bottom layer			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Lake Sitno								
pH	7.75 (7.50–7.90)	8.00 (7.16–8.86)	7.97 (7.78–8.10)	8.08 (8.03–8.13)	7.73 (7.48–7.89)	7.94 (7.38–8.62)	8.00 (7.95–8.07)	7.97 (7.82–8.09)
Temperature [°C]	13.9 (12.7–14.6)	20.8 (17.6–25.5)	12.1 (8.4–18.1)	4.8 (2.8–6.4)	13.1 (11.9–13.8)	19.7 (17.2–23.1)	11.3 (8.6–16.5)	4.5 (2.4–6.4)
O_2 [mg · dm^{-3}]	12.9 (12.4–13.5)	11.0 (8.6–15.5)	7.6 (6.0–8.7)	10.4 (9.9–11.4)	11.9 (11.5–12.6)	10.0 (7.0–14.5)	7.6 (7.3–8.0)	9.7 (8.0–11.1)
NO_2-N [mg · dm^{-3}]	0.028 (0.024–0.035)	0.019 (0.014–0.024)	0.050 (0.032–0.063)	0.030 (0.022–0.035)	0.031 (0.025–0.039)	0.019 (0.009–0.035)	0.060 (0.032–0.079)	0.024 (0.011–0.044)
NO_3-N [mg · dm^{-3}]	9.6 (7.6–12.0)	8.1 (1.4–11.9)	6.8 (5.4–8.5)	8.1 (4.7–10.6)	9.6 (7.2–12.0)	8.1 (1.4–8.5)	6.4 (5.3–7.2)	8.7 (5.5–10.6)
PO_4^{-3} [mg · dm^{-3}]	0.64 (0.37–1.18)	1.25 (0.52–2.41)	0.40 (0.32–0.44)	0.53 (0.15–0.80)	0.63 (0.33–1.18)	0.88 (0.62–1.16)	0.34 (0.28–0.43)	0.99 (0.76–1.30)
Fe^{2+} [mg · dm^{-3}]	0.06 (0.04–0.08)	0.05 (0.02–0.08)	0.06 (0.03–0.09)	0.06 (0.03–0.09)	0.05 (0.04–0.05)	0.06 (0.04–0.08)	0.05 (0.02–0.08)	0.06 (0.02–0.08)
Total Fe [mg · dm^{-3}]	0.19 (0.06–0.40)	0.08 (0.06–0.09)	0.09 (0.05–0.12)	0.08 (0.04–0.13)	0.14 (0.06–0.25)	0.06 (0.04–0.09)	0.08 (0.06–0.10)	0.07 (0.04–0.10)

populations of vendace and whitefish in Lake Marta and lake trout in Lake Sitno [oral information from the Drawa National Park]. Additionally, reproduction of these fish species may have been reduced by progressing eutrophication [15].

Table 2

Average values of physiochemical parameters for lakes Marta and Sitno for the whole 2005–2007 period and for individual seasons (spring, summer, autumn, winter)

Parameters	Lake Marta	Lake Sitno	U*	Z	p-value	Z corrected
2005–2007 period						
pH	7.99	7.93	8169.5	2.88	0.004	2.88
Temperature [^{o}C]	13.9	12.5	8552.5	2.43	0.015	2.43
NO_2^--N [mg · dm^{-3}]	0.012	0.033	264.5	–7.54	0.000	–7.55
Fe^{2+} [mg · dm^{-3}]	0.02	0.06	454.5	–6.60	0.000	–6.68
Total Fe [mg · dm^{-3}]	0.05	0.11	415.0	–6.82	0.000	–6.86
Spring						
Temp.[°C]	14.3	13.5	435.5	2.90	0.004	2.91
O_2 [mg · dm^{-3}]	10.8	12.4	322.5	–3.81	0.000	–3.81
NO_2^--N [mg · dm^{-3}]	0.011	0.030	8.0	–4.13	0.000	–4.14
Fe^{2+} [mg · dm^{-3}]	0.03	0.06	12.5	–4.13	0.000	–4.23
Total Fe [mg · dm^{-3}]	0.06	0.17	49.5	–2.59	0.009	–2.61
Summer						
pH	8.00	7.97	1247.5	2.04	0.041	2.04
Temperature [^{o}C]	21.1	20.2	1190.0	2.37	0.018	2.37
NO_2^--N [mg · dm^{-3}]	0.010	0.019	61.5	–4.52	0.000	–4.54
Fe^{2+} [mg · dm^{-3}]	0.02	0.06	89.5	–3.92	0.000	–3.98
Total Fe [mg · dm^{-3}]	0.03	0.08	76.0	–4.21	0.000	–4.24
Autumn						
pH	8.22	8.00	744.0	2.88	0.004	2.88
Temperature [^{o}C]	15.0	11.7	872.0	2.09	0.036	2.10
NO_2^--N [mg · dm^{-3}]	0.017	0.055	10.5	–4.82	0.000	–4.84
Fe^{2+} [mg · dm^{-3}]	0.03	0.06	60.0	–3.31	0.001	–3.34
Total Fe [mg · dm^{-3}]	0.05	0.09	32.5	–4.15	0.000	–4.18
Winter						
pH	7.99	8.03	193.5	–3.25	0.001	–3.26
O_2 [mg · dm^{-3}]	9.1	10.1	268.5	–2.84	0.005	–2.84
NO_2^--N [mg · dm^{-3}]	0.012	0.027	12.0	–2.54	0.011	–2.56
PO_4^{-3} [mg · dm^{-3}]	2.00	0.76	8.0	2.87	0.004	2.87
Fe^{2+} [mg · dm^{-3}]	0.03	0.06	10.5	–2.66	0.008	–2.71
Total Fe [mg · dm^{-3}]	0.05	0.08	1.0	–3.46	0.001	–3.48

* Confidence semi-intervals calculated with the non-parametric Mann-Whitney U test: U – the Mann-Whitney U test; Z – standardized value of variable at normal distribution; Z corrected – correction due to tied ranks; p-value – significance level, $p < 0.05$.

Monitoring of nutrients revealed that in years 2005–2007, nitrate(V) content in subsurface water of Lake Marta was the lowest in summer (on average 6.7 mg · dm^{-3}), and the highest in spring and autumn (on average 8.6 mg · dm^{-3}) (Table 1). A regularity was observed that concentrations of mineral nitrogen compounds for subsurface water were higher than for near-bottom one (except for spring). Differently in Lake Sitno, nitrate(V) concentrations in each season were similar for both water layers. Near--bottom nitrate(V) concentrations for spring were higher than for the other seasons. For the 2005–2007 period, average nitrate(V) concentrations in subsurface and near-bottom waters were higher for Lake Sitno than for Lake Marta in spring, summer and winter seasons, however the differences have not been statistically confirmed (Table 2). At the same time, average nitrate(III) concentrations in Lake Marta ranged from 0.008 mg · dm^{-3} for summer to 0.017 mg · dm^{-3} for autumn, while in Lake Sitno nitrate(III) levels were higher and varied from 0.019 mg · dm^{-3} for summer to 0.060 mg · dm^{-3} for autumn (Table 1), and this difference between lakes was statistically significant. The relationships observed might have resulted from different sediment ability to retain chemicals, including nitrates(III) and (V). As nitrates(III) and (V) poorly adhere to organic particles in sediments, they may be easily released from the substratum. Nitrate content is also modified by denitrification intensity. The results obtained indicate that denitrification is more intensive at higher temperature, as evidenced by the fact that the lowest nitrate content occurred in summer in near-bottom water of both lakes [16]. A detailed study revealed that average level of nitrate removed from Lake Sitno by the Plociczna River was higher than average level delivered [12]. The source of the river, located at an agricultural area, may contribute to nutrient inflow. According to Lossow [17], agricultural fertilizers may be responsible for 10–20 % of nitrogen and 5 % of phosphorus delivered to freshwaters. Phosphate levels in Lake Marta tended to be the highest in winter in the subsurface layer and the lowest in spring in both layers (Table 1). Significantly higher winter concentrations of phosphates in Lake Marta may have resulted from occurrence of free phosphorus form, not built in animal or plant tissues. A considerable amount of nutrients is accumulated in the sediments and invertebrates [8]. Release of phosphorus from sediments may be intensified by mixing of near-bottom water with water from the above layers [4]. This took place in Lake Sitno where, in wintertime, near-bottom water contained more phosphorus than subsurface one (Table 1). Lake Sitno, therefore, being a shallow lake with high dynamics of water masses, is more susceptible to eutrophication. Similar regularities were reported by Kubiak [18]. A significant role in the processes of phosphorus sorption and binding to sediments is played by iron hydroxides and calcium carbonate, while sediment bacteria have a significant role in anaerobic release of iron-bound phosphorus [19]. Concentrations of Fe^{2+} in Lake Marta were nearly constant (from 0.01 to 0.06 mg · dm^{-3}) during the whole study period (Table 1). The highest concentration of total iron occurred in spring, while in the other periods its levels ranged from 0.01 to 0.09 mg · dm^{-3} (Table 1). Fe^{2+} concentrations in Lake Sitno were significantly higher than in Lake Marta (Table 2). In both lakes, total iron levels were the highest in spring.

The results of this study indicate that the examined lakes were characterized by high nutrient concentrations and temporary near-bottom hypoxia, while the dynamics of nutrient accumulation and bioaccumulation ensured maintenance of balance.

Conclusions

1. Marta and Sitno lakes were characterized by high nutrients concentrations and near-bottom hypoxia.

2. The observed dynamics of nutrient concentrations may reflect the compounds accumulation and bioaccumulation in sediments as well as their dynamic mobilization, confirmed by balanced concentrations in the whole water body.

Acknowledgements

The authors wish to thank the Management and Staff of the Drawa National Park (Poland) for help in collecting the material.

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SEZONOWE ZMIANY WARUNKÓW HYDROCHEMICZNYCH WYBRANYCH JEZIOR DRAWIEŃSKIEGO PARKU NARODOWEGO

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Abstrakt: Celem pracy było określenie sezonowych zmian warunków hydrochemicznych w warstwie powierzchniowej i przydennej jezior Marta i Sitno w DPN. Badania hydrochemiczne przeprowadzono w latach 2005–2007, podczas których mierzono pH, temperaturę, zawartość tlenu, azotanów(III i V), fosforanów, żelaza dwuwartościowe i ogólną zawartość żelaza.

Pomiary zawartości O_2 wykazały, że warstwy powierzchniowe i przydenne w litoralu obydwóch jezior były maksymalnie wysycone tlenem wiosną, a minimalnie jesienią. W całym okresie badań stwierdzono niedotlenienie warstw przydennych w obydwóch jeziorach. Kontrola warunków termicznych wykazała, że średnia temperatura wody w obu warstwach w jeziorze Marta jest znacznie wyższa aniżeli w jeziorze Sitno. Analiza stężeń azotanów(III i V) wykazała większą ich zawartość w obu warstwach w jeziorze Sitno, aniżeli w jeziorze Marta. Różnice te udowodniono statystycznie dla warstwy przydennej. Wykazana w obydwóch zbiornikach podwyższona zawartość pierwiastków biogennych klasyfikuje je jako eutroficzne. Mniejsze stężenie azotanów(III) w jeziorze Marta może świadczyć o zachodzącym w nim procesie denitryfikacji.

Słowa kluczowe: właściwości hydrochemiczne, jeziora, Drawieński Park Narodowy

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[2] Nowak S: Chemia nieorganiczna, WNT, Warszawa 1990.

[3] Bruns I., Sutter K., Neumann D. and Krauss G.-J.: *Glutathione accumulation – a specific response of mosses to heavy metal stress*, [in:] Sulfur Nutrition and Sulfur Assimilation in Higher Plants, P. Haupt (ed.), Bern, Switzerland 2000, 389–391.

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[3] Bruns I., Sutter K., Neumann D. and Krauss G.-J.: *Glutathione accumulation – a specific response of mosses to heavy metal stress*, [in:] Sulfur Nutrition and Sulfur Assimilation in Higher Plants, P. Haupt (ed.), Bern, Switzerland 2000, 389–391.

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