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FOLLOW-UP EFFECT OF HILLING ON GROWTH AND YIELDING OF MISCANTHUS (*Miscanthus x giganteus* Greef et Deu.)

NASTĘPCZY WPŁYW OBREDLANIA NA ROZWÓJ I PLONOWANIE MISKANTA OLBRZYMIEGO (*Miscanthus x giganteus* Greef et Deu.)

Abstract: In 2005–2007 in Pawlowice near Wroclaw, Poland, field experiments were conducted on the follow-up effect of hilling of Miscanthus in autumn after seeding. The split-plot experiment was set for the following three variable factors: I. Harvest date: a – autumn harvest after the vegetation period ends (11.07.2005, 14.12.2006; b – winter harvest before the vegetation starts (10.03.2006, 09.03.2007); II. Autumn treatment of rhizomes after seeding: a – with hilling; b – without hilling; III. N fertilization: 100, 150 and 200 kg N · ha^{-1}.

In the first years after the experiment had been set up, the morphological features, dry matter yield, water and ash contents were related to the age of the plantation.

Among the investigated factors, harvest dates had the most significant influence on yielding. Winter harvest resulted in a lower by 18.4 % dry matter yield and a decrease in water content in green matter by 23.8 % and in ash content by 43 %. However, it increased energy value in green matter by 52 %. In the second and the third year of the cultivation, out of 1 ha field of Miscanthus, it is possible to obtain biomass yield with a mean energy value of 294 GJ, which corresponds to 7.03 toe.

It is possible to obtain high dry matter yields of Miscanthus with autumn hilling and applying 150 kg N · ha^{-1}.

Keywords: Miscanthus, harvest date, hilling, N fertilization

Introduction

Energy development strategies of the developed countries focus more and more on obtaining renewable energy sources. In Poland, according to "The strategy for development of renewable energy" passed by the Polish Parliament, the renewable energy

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sources should make for 7.5 % of all the energy in 2010 and reach 14 % in 2020. In order to achieve these levels, it will be necessary to produce biomass on energy plantations located on arable lands additionally to firewood and agricultural by-products.

Potentially useful species for energy production in Polish environment include willow (*Salix* sp.), Miscanthus (*Miscanthus x giganteus* Greef et Deu), Virginia mallow (*Sida hermaphrodita* Rusby) and Jerusalem artichoke (*Helianthus tuberosus* L.).

Miscanthus x giganteus is a hybrid of a tetraploid *M. sinenss* and a diploid *M. sacchariflorus*. It was imported from Japan to Denmark in 1930 by Aksel Olsen and it gave start to cultivars grown at present in Europe. Since 1983 field experiments have been conducted with this species in the Northern Europe. The plant has been proved to be an effective energy crop and produce over 20 Mg d.m. per $ha^{-1} \cdot year^{-1}$ [1]. Miscanthus can be harvested twice: in autumn from October to November (and with favourable conditions in the first decade of December) when the vegetation period ends or in winter from February to March before the vegetation starts. Harvest dates affect the yield and the quality of the combusted biomass [1, 2].

Dry matter content in the harvested biomass in autumn is between 35–45 %, and in the winter harvest it is from 60 to 70 % [3]. The lower dry matter yield in winter is caused by some leaves falling down. The reduced yield is compensated by the increase in cellulose content which is low in leaves. The other reason for a lower yield of the aerial parts of the plants is the fact that in autumn the plants transport nutrients to the rhizomes which increase in mass and change in their chemical content. Since these are the rhizomes that determine winter survival, the plants gather in them osmotic active compounds which reduce the plants' susceptibility to low temperatures [4].

The yield of biomass in the autumn harvest is higher than in the spring harvest, the differences can be from 14 to 30 % [5]. The disadvantage of the autumn harvest is not only high moisture of the obtained biomass but also higher removal (accumulation and extraction) of the nutrients from the fields [6].

During the winter period the plants shed leaves, which makes potassium drain to soil [7]. As a consequence, sodium and potassium contents are reduced in Miscanthus straw and later in ashes [5]. Biomass of the highest quality for energy can be obtained in a winter–spring harvest. A later harvest results in biomass that is on average 30 % drier than in autumn. Winter harvest reduces also biomass chlorine content. The presence of chlorine in the biomass can cause emission of hydrogen chloride and dioxins in combustion [5, 8].

A drawback in growing Miscanthus is its little tolerance of low temperature in the first year after seeding. During the first winter after starting the plantation, rhizomes which are planted too shallow and those which are not fully formed are destroyed by frost and high moisture. Literature does not provide information on similar problems with winter survival of Miscanthus in the second and the following years of the cultivation [1]. After the second year of growth, Miscanthus survives well temperature drops below – 20 ^{o}C, even without a snow cover [4].

In the Danish and Irish experiments the majority of Miscanthus plants grown through micropropagation (*in vitro*) did not survive the first winter after seeding, while the plants obtained from rhizomes managed well through the winter period. Numerous

experiments indicate that the method of breeding of Miscanthus can affect its ability to survive in winter conditions [9].

Miscanthus needs relatively little fertilisation thanks to use and transport of nutrients in the plants from rhizomes and accumulation of large amounts of nutrients in rhizomes by the end of the vegetation period. It has been determined that a dose of 60 kg N · ha^{-1} is sufficient for an appropriate development of rhizomes. The leaves usually fall down and stay on the fields so nutrients absorption is relevant only for rhizomes. Miscanthus is capable to make a good use of nitrogen because it translocates it from rhizomes to shoots in spring and is again able to cumulate it in them at the end of the vegetation period [1, 10].

By the end of the vegetation period, 21–46 % of N, 36–50 % of P, 14–30 % of K and 27 % Mg accumulated in the aerial parts of the plants is translocated into the rhizomes [11].

The aim of the study was to determine the effect of hilling in *Miscanthus giganteus* in autumn after its seeding on growth, yielding and chemical content of the yield as well as on the energy value of this crop.

Materials and methods

In 2005–2007 in Pawlowice near Wroclaw, Poland, field experiments were conducted on the follow-up effect of autumn hilling of Miscanthus after seeding. The split-plot experiment was set for three following variable factors: I. Harvest dates: a – autumn harvest after the vegetation period ended – 11.07.2005 and 14.12.2006; II. Autumn treatment of rhizomes after seeding: a – with hilling, b – without hilling; III. N Fertilization: 100, 150 and 200 kg per ha^{-1}.

The experiments were set on a very light river alluvial soil with loose sand and sandy gravel. In 2004–2006 soil pH was acid to very acid with the following concentrations of microelements: P – very high, K – medium to high, Mg – very low.

Important features of Miscanthus growing in the year of seeding:

– harvest date: 07.05.2004,
– depth of rhizome seeding: 5–10 cm,
– row density: 70 cm,
– plant density in a row: 45 cm,
– number of rhizomes planted in a plot: 16 pieces,
– fertilization [kg · ha^{-1}]: N – 60,
– fertilization in all the years of the experiment [kg · ha^{-1}]: P_2O_5 – 60 (26 – P); K_2O – 100 (83 – K),
– size of a plot: 5.04 m^2,
– harvest date: 03.12.2004.

In 2004–2006 weed control was carried out – Roundup 360 SL at the dose of 4 dm^3 · ha^{-1} was applied focally. The plots were sporadically infested with White goosefoot (*Chenopodium album* L., Shepherd's purse (*Capsella bursa-pastoris* L.) and Common barnyard grass (*Echinochloa crus-galli* L.).

From the vegetation start in 2005 and 2006 observations and measurements were conducted on the growth and height of plants as well as diseases and insect infestations

were monitored. After the vegetation period had ended, the shoots were numbered on every plot, the results were calculated per 1 m^2. From 10 shoots from every plot the following features were measures: plants' height, flag-leaf length, and stem diameter (on the height of 10 cm above the soil surface).

Autumn and winter harvests were done manually with use of a chainsaw for hedges and a circular saw. After harvest, Miscanthus green matter yield was measured from every plot.

Chemical analyses were conducted in the laboratory of the Department of Crop Production at the Wroclaw University of Environmental and Life Sciences. The following parameters were determined:

– dry matter – through drying of minced plant material for 4 hours at 105 ^oC,
– crude ash – by combusting plant material in 600 ^oC in an electric oven,
– total nitrogen content with Kjeldahl's method,
– mineral compounds contents: K, Ca (flame photometry), P, Mg (coulometry).

Based on the results obtained from the chemical analyses, water percentage content in the plant material and dry matter yield were calculated. Additionally, the contents of crude ash and of the investigated macronutrients were determined. Energy value of biomass was measured in the Institute of Agricultural Engineering, Wroclaw University of Environmental and Life Sciences. The analysis was conducted with use of a semi-automatic calorimeter Precyzja-BIT KL-10 which is designed to measure heat in solid fuels combustion such as turf, lignite, coal, coal and lignite pellets, coke and non-explosive, combustive organic substances. The method of measure is compatible with the requirements of the official Polish Standard for measuring heat of combustion and calculating of calorific value of solid fuels from 1981 [12]. On the basis of the research the relationship between biomass energy value and moisture was determined (Figs. 1 and 2).

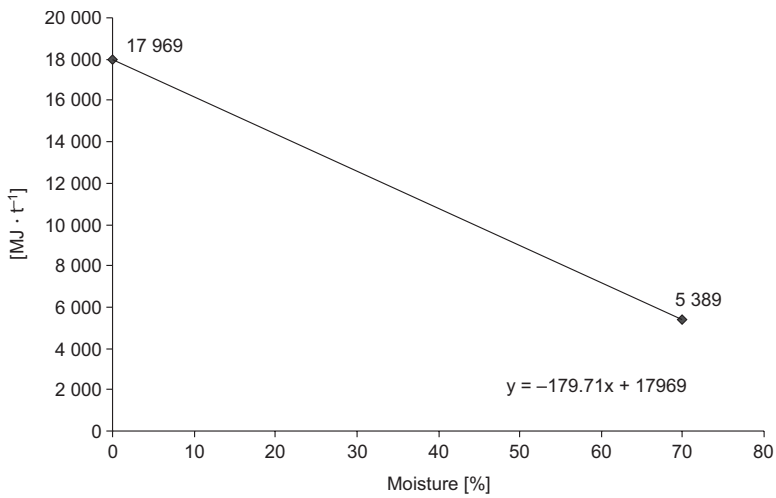

Fig. 1. The relationship between energy value and biomass moisture content (autumn harvest)

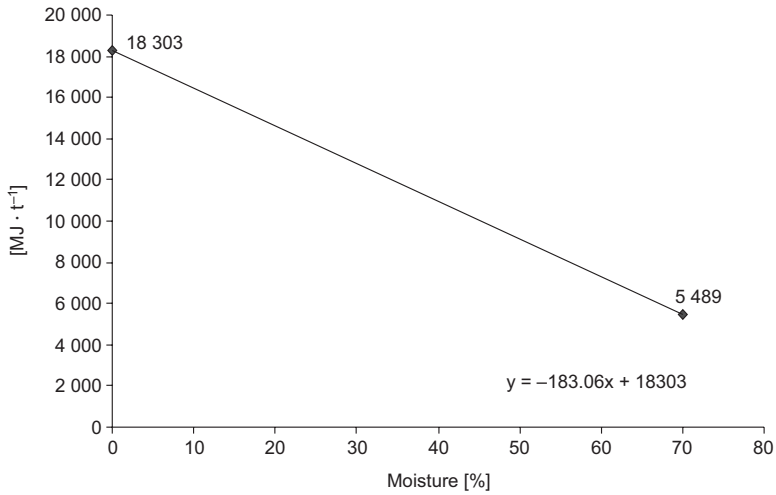

Fig. 2. The relationship between energy value and biomass moisture content (winter harvest)

In 2004–2006 the Department of Plant Protection, Wroclaw University of Environmental and Life Sciences, monitored diseases and insect infestation in Miscanthus. Low numbers of phytophagous organisms were observed in all the years of the experiment. A higher infestation was noted by aphids: the bird cherry-oat aphid *Rhopalosiphum padi* L. and rose-grain aphid *Metopolophium dirhodum* Walk, and thrips: *Frankliniella tenuicornis* Uzel., and *Haplothrips aculeatus* Fabricius.

In 2005–2006 on Miscanthus leaves was sparsely recorded one fungi species: *Alternaria alternata* (Fr.) Keissl.

Results and discussion

Before the autumn harvest 03.12.2004 it was calculated that for 1 m^2, Miscanthus produced 14 shoots with a mean length of 112 cm and the stem diameter of 6.7 mm, and a 66 cm long flag-leaf. Green matter yield was 3.8 $t \cdot ha^{-1}$ with 64 % water content, and dry matter yield was 1.37 $t \cdot ha^{-1}$.

In 2005 and 2006 the vegetation started on April 20, and ended on November 10, 20, respectively. In these two years of the experiment, the mean monthly temperatures from April to October, except for August, were higher than multiyear means. The sum of precipitations was similar to the mean values of a multiyear. It needs to be underlined that the pattern of precipitation was unfavourable (Table 1).

So far the research has indicated that Miscanthus starts growing in spring when the soil temperature reaches 10–12 ^oC. Air temperature of 5–10 ^oC is a threshold from which leaves start growing [1, 13].

Despite a low transpiration ration of 300 dm^3 of water per kg d.m., Miscanthus has high water requirements because of a high production of biomass [2]. According to Beale et al [14] Miscanthus plants use from 80 to 330 g of water per 1 g of dry matter.

Table 1

Mean monthly air temperature [°C] and precipitation sum [mm] in 2004–2007

Year \ Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Temperature												
2004	–2.9	5.9	4.8	9.8	13.2	16.7	18.6	19.6	14.4	10.5	4.9	2.0
2005	2.1	–1.5	1.7	9.8	14.3	16.9	19.7	17.7	15.2	9.9	3.3	0.8
2006	–6.0	–1.9	0.6	9.9	14.3	18.5	23.4	17.3	16.1	11.0	6.7	4.3
2007	4.9	2.7	6.5	10.9	16.2	19.2	19.2	18.9	12.9	8.3	2.8	1.0
Multiyear means for 1970–2000	–1.0	0.1	3.7	8.1	13.9	16.7	18.5	17.7	13.3	8.8	3.6	0.5
Precipitation												
2004	36.6	32.8	54.9	21.5	39.1	43.9	66.1	33.0	25.8	51.4	77.7	15.8
2005	41.7	39.1	9.3	25.5	121.0	36.3	109.3	51.0	20.2	5.4	26.3	95.9
2006	23.5	39.3	22.1	51.1	15.9	56.6	12.0	166.7	17.6	57.9	68.3	35.2
2007	52.0	59.0	48.8	2.7	50.3	69.2	92.4	52.8	46.1	21.7	53.9	21.0
Multiyear sums for 1970–2000	30.5	24.8	33.2	31.9	49.9	64.9	75.4	63.5	44.7	35.5	33.9	36.3

There was no correlation noted between the investigated factors and the growth of Miscanthus during the vegetation period. Therefore, mean measures from the particular years of the experiment were used to describe the plant growth as a function of time. From the vegetation start to mid-June, Miscanthus reached 80 % of its full height (Figs. 3 and 4). In a two-year experiment conducted in Greece, where the plants were watered every 6–7 days, Miscanthus shoots were growing on average 3 cm per 24 hours from May to the end of June, reaching the height of 170 cm. In the next months the shoots

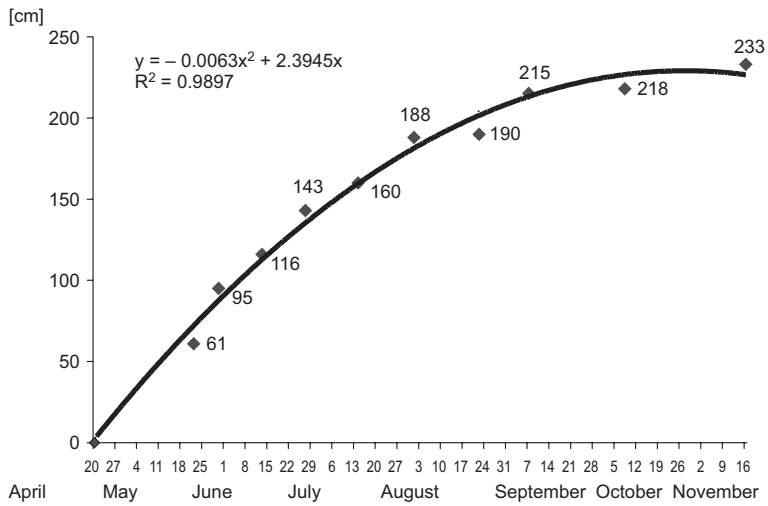

Fig. 3. Growth of Giant Miscanthus (*Miscanthus x giganteus* Greef et Deu.) in 2005

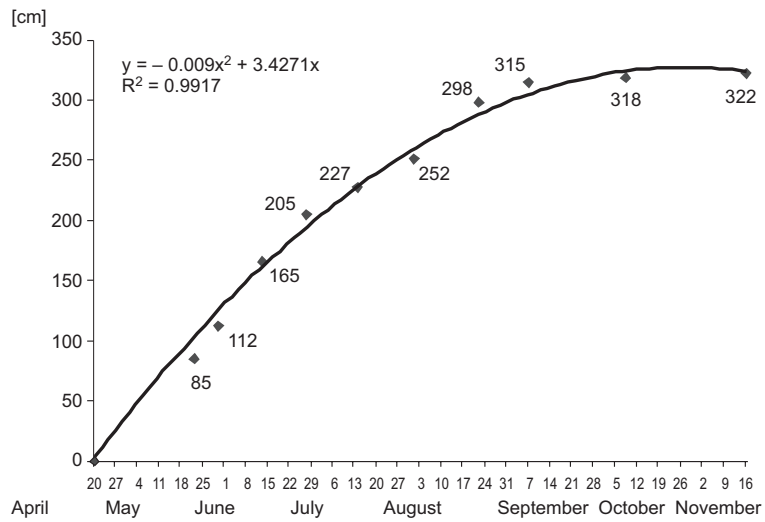

Fig. 4. Growth of Giant Miscanthus (*Miscanthus x giganteus* Greef et Deu.) in 2006

grew from 0.5 to 1 cm per 24 h until they reached their maximal height in August. The full length of shoots was 233 cm in the first year after seeding and 323 cm in the second year [15].

The number of shoots before harvest and the morphological features of the plants were affected mostly by N fertilization, and later to a lesser and lesser degree by hilling and harvest dates (Table 2). Autumn hilling, compared to the control, considerably increased the height of plants, the length of the flag with leaf and the stem diameter. Because of dynamic growth of Miscanthus in the second and the third year after seeding, significant differences were recorded in the number of shoots per 1 m^2, the height of plants and the length of the flag-leaf between the years of the experiment.

Table 2

Number of shoots before harvest and morphological features of Miscanthus plants (*Miscanthus x giganteus* Greef et Deu.) in 2005–2006 (means for factors 2005–2006)

Harvest date	Type of growing	N rate [kg · ha^{-1}]	Number of shoots per 1 m^2	Plant height [cm]	Flag-leaf length [cm]	Stem diameter [mm]
Autumn			39	280	46.0	9.4
Winter			40	276	46.1	9.2
LSD (α = 0.05)			NSD	NSD	NSD	0.15
	with hilling		39	288	48.2	9.5
	without hilling		40	268	43.8	9.2
LSD (α = 0.05)			NSD	6	1.0	0.25
		100	37	266	44.5	9.1
		150	41	278	46.3	9.2
		200	41	290	47.3	9.6
LSD (α = 0.05)			2	6	1.6	0.19
Years		2005	25	233	55.1	9.3
		2006	54	322	37.0	9.3
LSD (α = 0.05)			4	4	1.3	NSD

NSD – no significant difference.

In the environment of Greece, the number of shoots per 1 m^2 from May to June was: 80–85 in the first year and 90–100 in the second year. Later it gradually decreased down to 75 shoots [15]. Jezowski et al [16] stated that Miscanthus yielding depends mostly on the growth stage of the plants, their branching and the diameter of the clump.

Miscanthus green and dry matter yields as well as energy yield and water content depend mostly on the harvest date. In the autumn harvest, the dry matter yield was higher by 23 % than in the winter harvest, but it also contained 23.8 % more water and its energy value per 1 kg of green matter was 34 % lower (Table 3). Hilling increased, among other things, dry matter yield by 75 %. The highest dry matter yields were obtained with 150 kg N · ha^{-1} fertilization.

Table 3

Green and dry matter yields, water content in green matter and energy value in Miscanthus (*Miscanthus x giganteus* Greef et Deu.) in 2005–2006 (means for factors in 2005–2006)

Harvest date	Type of growing	N rate [kg · ha^{-1}]	Green matter				Dry matter		
			yield [t · ha^{-1}]	water content [%]	energy value		yield [t · ha^{-1}]	energy value	
					[GJ · t^{-1}]	[GJ · ha^{-1}]		[GJ · ha^{-1}]	[toe · ha^{-1}]
Autumn			36.9	52.3	8.58	322	17.9	322	7.68
Winter			20.6	28.5	13.09	267	14.6	267	6.38
LSD ($\alpha = 0.05$)			1.4	3.1	0.57	12	0.6	12	0.27
	with hilling		29.6	40.3	10.85	305	16.8	305	7.28
	without hilling		27.8	40.4	10.82	284	15.7	284	6.78
LSD ($\alpha = 0.05$)			1.2	NSD	NSD	6	0.3	6	0.15
		100	27.2	40.7	10.77	279	15.4	279	6.66
		150	29.8	41.0	10.72	305	16.8	305	7.28
		200	29.2	39.4	11.02	300	16.5	300	7.16
LSD ($\alpha = 0.05$)			1.3	NSD	NSD	14	0.8	14	0.33
Years		2005	20.7	42.6	10.43	199	11.0	199	4.75
		2006	36.8	38.1	11.24	390	21.5	390	9.31
LSD ($\alpha = 0.05$)			1.4	3.1	0.57	12	0.6	12	0.27

NSD – no significant difference; dry matter calorie content in 1 t of Miscanthus: autumn harvest: 17.97 GJ; winter harvest: 18.30 GJ.

Many researchers [1, 2] claim that the quality of Miscanthus biomass for combustion and the yield are determined by the harvest date. The autumn harvest yield is higher than from the spring harvest, and the losses can range from 14 to 30 % [5]. The disadvantage of the autumn harvest is not only high moisture of the obtained biomass but also higher removal (accumulation) of the nutrients from the fields [6].

According to several researchers [1, 11] N fertilization at the rate of 50–70 kg · ha^{-1} · year^{-1} is sufficient for Miscanthus's requirements when applied in spring at the beginning of shooting. Kaack and Schwarz [17] argue that high doses of N in growing *M. x giganteus* (over 75 kg of N · ha^{-1}) can result in an increased lodging, on fields not sheltered from wind.

The harvest dates and the age of the plantation affected chemical content of Miscanthus biomass. Harvested in winter, Miscanthus contained 40 % less ash and 25–48 % less of all the investigated macronutrients than the crops harvested in autumn. This was due to a translocation of nutrients from shoots to rhizomes and their draining with rain during the winter period.

Table 4

Chemical composition of Miscanthus biomass [%] (*Miscanthus x giganteus* Greef et Deu.) (means for factors in 2005–2006)

Harvest date	Type of treatment	N rate [kg · ha^{-1}]	Crude ash	N	P	K	Ca	Mg
Autumn			3.16	0.31	0.06	0.43	0.23	0.08
Winter			1.79	0.23	0.04	0.22	0.12	0.06
LSD (α = 0.05)			0.14	0.02	0.01	0.02	0.03	0.01
	with hilling		2.49	0.27	0.05	0.32	0.18	0.07
	without hilling		2.46	0.27	0.05	0.33	0.17	0.07
LSD (α = 0.05)			NSD	NSD	NSD	NSD	NSD	NSD
		100	2.55	0.27	0.05	0.33	0.17	0.07
		150	2.41	0.27	0.05	0.32	0.18	0.07
		200	2.46	0.27	0.05	0.32	0.18	0.07
LSD (α = 0.05)			NSD	NSD	NSD	NSD	NSD	NSD
Years		2005	3.04	0.28	0.07	0.48	0.26	0.07
		2006	1.91	0.26	0.03	0.17	0.09	0.07
LSD (α = 0.05)			0.14	NSD	0.01	0.02	0.03	NSD

NSD – no significant difference.

According to the studies conducted by Lewandowski et al [18] a delayed harvest decreased on average ash content in Miscanthus by 28 % in Portugal and the Great Britain, by 42 % in Germany, and by 50 % in Sweden and 54 % in Denmark.

All the agrotechnical factors and the age of the stand affected the accumulation of crude ash and the investigated macronutrients in Miscanthus biomass (Table 5). The winter harvest lowered crude ash content by 50 % and nutrient content by 40–58 % compared with the autumn harvest.

Table 5

Accumulation of crude ash and macrocomponents in Miscanthus biomass [kg · ha^{-1}] (*Miscanthus x giganteus* Greef et Deu.)

Harvest date	Type of growing	N rate [kg · ha^{-1}]	Crude ash	N	P	K	Ca	Mg
Autumn	with hilling	100	513	53.1	7.87	57.3	29.8	14.2
		150	566	57.6	9.86	62.7	35.1	16.7
		200	541	59.9	9.07	65.9	38.6	13.9
	without hilling	100	496	50.5	7.92	60.6	29.9	13.1
		150	458	53.7	8.83	64.2	37.1	13.4
		200	538	54.0	8.89	67.1	34.5	13.4
Winter	with hilling	100	248	30.7	4.70	29.3	13.2	9.3
		150	264	35.2	4.22	29.6	16.0	7.5
		200	240	32.2	3.92	27.1	14.5	10.1
	without hilling	100	238	31.6	5.06	28.3	14.7	9.0
		150	239	33.9	4.80	27.9	13.7	8.3
		200	242	32.8	4.49	28.3	14.6	8.2
LSD ($\alpha = 0.05$)			35	NSD	0.5	NSD	1.7	1.5

Table 5 contd.

Harvest date	Type of growing	N rate [kg · ha^{-1}]	Crude ash	N	P	K	Ca	Mg
Means for factors								
Autumn			519	54.8	8.74	63.0	34.2	14.1
Winter			245	32.7	4.53	28.4	14.5	8.7
LSD ($\alpha = 0.05$)			12	1.6	0.17	1.1	0.6	0.6
	with hilling		395	44.8	6.60	45.3	24.6	11.9
	without hilling		369	42.8	6.66	46.1	24.1	10.9
LSD ($\alpha = 0.05$)			6	0.8	NSD	0.7	0.4	NSD
		100	374	41.5	6.38	43.9	21.9	11.4
		150	382	45.1	6.93	46.1	25.5	11.5
		200	390	44.7	6.59	47.1	25.6	11.4
LSD ($\alpha = 0.05$)			NSD	2.1	0.25	1.6	0.9	0.8
Years		2005	340	30.8	7.58	53.6	29.5	7.8
		2006	423	56.8	5.69	37.7	19.1	15.1
LSD ($\alpha = 0.05$)			12	1.6	0.17	1.1	0.6	NSD

NSD – no significant difference.

In the experiments carried out by Clifton-Brown et al [19] on a 16-year old Miscanthus stand, it was recorded that the contamination of nutrients in the autumn harvest was as follows: 145 ± 9.4 kg N · ha^{-1}, 23 ± 1.1 kg P · ha^{-1} and 111 ± 9.9 kg K · ha^{-1}. When the harvest date was moved to March, the concentration of nutrients was lowered: 51 ± 6.1 kg N · ha^{-1}, 8.3 ± 0,7 kg P · ha^{-1} and 42 ± 7.9 kg K · ha^{-1}. The mean yields for 15 years were as follows: 13.4 ± 1.1 kg d.m. · ha^{-1} year^{-1} for autumn harvests and 9.0 ± 0.7 kg d.m. · ha^{-1} year^{-1} in winter harvests.

Conclusions

1. In the first years of the experiment on Miscanthus, morphological features, d.m. yield, water and ash contents depended on the age of the stand.

2. Harvest dates proved to have the most significant effect on Miscanthus yielding among all the investigated factors. Compared with the autumn harvest, the winter harvest lowered d.m. yield by 18.4 % and water content by 23.4 % in green matter. With winter harvest crude ash content also decreased by 43 %, while green biomass energy value increased by 52 %.

3. It is possible to obtain a mean biomass energy yield of 294 GJ (which corresponds to 7.03 toe*) from 1 ha of Miscanthus field in the second and the third year of the cultivation.

4. It is possible to obtain high d.m. yields in Miscanthus with autumn hilling and by applying 150 kg N · ha^{-1}.

5. Although the winter harvest results in lower biomass yields, it is more favourable in terms of quality of the plant material for energy production than the autumn harvest.

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* toe – ton of oil equivalent (fuel with caloric value of 41.87 GJ · t^{-1}).

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NASTĘPCZY WPŁYW OBREDLANIA NA ROZWÓJ I PLONOWANIE MISKANTA OLBRZYMIEGO (*Miscanthus x giganteus* Greef et Deu.)

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Abstrakt: W latach 2005–2007 w Pawłowicach koło Wrocławia prowadzono badania polowe nad następczym wpływem obredlania miskanta olbrzymiego jesienią, po posadzeniu. Doświadczenie założono w układzie „split–plot" na trzy czynniki zmienne, którymi w kolejności były: I. Terminy zbioru: a – jesienny po zahamowaniu wegetacji – 11.07.2005 r., 14.12.2006 r., b – zimowy przed ruszeniem wegetacji – 10.03.2006 r., 09.03.2007 r., II. Jesienna pielęgnacja kłączy po posadzeniu a – z obredlaniem, b – bez obredlania, III. Nawożenie w kg N · ha^{-1}: 100, 150 i 200.

W początkowych latach po założeniu doświadczenia z miskantem olbrzymim cechy morfologiczne, plon suchej masy, zawartość wody i popiołu zależały od wieku plantacji.

Spośród badanych czynników agrotechnicznych największy wpływ na poziom plonu i jego jakość miał termin zbioru. Zimowy zbiór, w porównaniu z jesiennym, spowodował zmniejszenie: plonu suchej masy o 18,4 %, zawartości wody w świeżej masie o 23,8 % i popiołu surowego 43 % oraz wzrost wartości

energetycznej świeżej masy o 52 %. Z 1 ha uprawy miskanta olbrzymiego w drugim i trzecim roku uprawy można uzyskać plon biomasy o średniej wartości energetycznej 294 GJ, co odpowiada 7,03 toe.

Duże plony suchej masy miskanta olbrzymiego można uzyskać przy jesiennym obredlaniu plantacji i zastosowaniu 150 kg N · ha^{-1}.

Słowa kluczowe: miskant olbrzymi, terminy zbioru, obredlanie, nawożenie azotem

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MISCANTHUS – UNUSUAL GRASS: BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERISTIC: A REVIEW

MISCANTHUS – TRAWA NIEZWYKŁA: CHARAKTERYSTYKA BIOCHEMICZNO-FIZJOLOGICZNA: PRZEGLĄD LITERATUROWY

Abstract: *Miscanthus* × *giganteus* (Giant Miscanthus) is one of the most promising plants cultivated for biomass production. In spite of its origin from south-east Asia and being warm adapted plant it grows well and produces high biomass in temperate latitudes. *Miscanthus* × *giganteus* is a C_4 plant and hence this study presents a brief description of C_4 photosynthesis and the enzymes involved in this process. On the basis of data from current literature, the biochemical bases of relatively high tolerance of miscanthus to cold temperatures (0–15 ^oC) were evaluated. Moreover, it was reviewed numerous ecophysiological features of *Miscanthus* × *giganteus* were reviewed (high productivity, low fertiliser and pesticides requirements, possibility to use in phytoremediation) which showed that it is a proecological and environmentally friendly crop. This causes that *Miscanthus* × *giganteus* might be recognize as a leading crop in non-food cultivations.

Keywords: miscanthus, C_4 photosynthesis, chilling tolerance, phytoremediation, allelopathy

Introduction

Plants from genus of *Miscanthus* originate from south-east Asia and are widely distributed in Asia and Pacific Islands. *Miscanthus* belongs to *Poaceae* family and tribe of Andropogoneae [1]. *Miscanthus* is rhizomatous perennial grass reaching height of 5 m and offers very high biomass yield. In 1935 it was introduced into Europe and initially was cultivated in parks and gardens as ornamental plants. Over the past 20 years, there is increasing interest in *Miscanthus* as a raw material for industry (paper production, insulation material) and renewable source of energy. Heating value of *Miscanthus* is about 17–19 MJ · kg^{-1} and is similar to energetic value of wood fuel and it accounts for about 60 % of that of hard coal [2, 3].

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Plants from *Miscanthus* genus are frequently so-called Chinese Grass or Elephant Grass. The most famous representatives of these grasses are: *Miscanthus sinensis, Miscanthus sacchariflorus* and *Miscanthus* × *giganteus* (Giant Miscanthus). *Miscanthus* × *giganteus* is a sterile triploid (2n = 3x = 57) arising through natural crossing of tetraploid *Miscanthus sacchariflorus* (2n = 4x = 76) and diploid (2n = 2x = 38) *Miscanthus sinensis* [4]. Generated hybrid is sterile, does not produce seeds and hence it must be propagated vegetatively by rhizome division or *in vitro* cultures. *Miscanthus* × *giganteus* displays a good balance of traits from each parent, combining rapid growth with a tolerance to low temperatures [5]. *Miscanthus* × *giganteus* (hereafter referred to as miscanthus) is a robust woody perennial which may be cultivated at one site for 15–20 years. It reaches full productivity over the first 3 years after planting. Miscanthus appears in numerous genotypes [3, 6].

Miscanthus is closely related to three important agricultural crops: sugarcane (*Saccharum officinarum*), sorghum (*Sorghum bicolor*) and maize (*Zea mays*). All these crop species are C_4 plants and exhibit high productivity particularly in warmer and well radiated regions.

Miscanthus – C_4 plant (biochemical characteristic)

C_4 plants have evolved mechanisms to improve photosynthetic efficiency and decrease water loss in hot, dry environments. In *Miscanthus*, as in other C_4 plants, CO_2 fixation is a two-step process. CO_2 is initially fixed in the cytosol of mesophyll cells surrounding the bundle sheath [7]. This reaction is catalyzed by *phosphoenolopyruvate carboxylase* (PEPC; EC 4.1.1.31). PEPC is homotetrameric enzyme that has high affinity to CO_2 and is activated by divalent cation [8]. PEPC can fixe CO_2 at very low its concentration and may use CO_2 produced in respiration. PEPC catalyses the β-carboxylation of phosphoenolopyruvate using HCO_3^- as substrate in a reaction that yields oxaloacetate (a four carbon dicarboxylic compound) and phosphate (Pi):

$$\textit{phosphoenolopyruvate}\ (\text{PEP}) + HCO_3^- \rightarrow \text{oxaloacetate (OAA)} + \text{Pi}.$$

Then oxaloacetate is converted to malate by NADPH-malate dehydrogenase:

$$\text{Oxaloacetate} + \text{NADPH} + H^+ \rightarrow \text{malate} + NADP^+.$$

Formed malate is transported by plazmodesmata to the bundle sheath cells, where it is decarboxylated in reaction catalysed by NADP-malic enzyme (NADP-ME):

$$\text{malate} + NADP^+ \rightarrow CO_2 + \text{pyruvate} + \text{NADPH} + H^+.$$

Released CO_2 is fixed by Rubisco (*ribulose-1,5-bisphosphate carboxylase/oxygenase*; EC 4.1.1.39) and converted to carbohydrate in the Calvin cycle. Pyruvate (C_3 acid) diffuses back to the mesophyll cells to regenerate phosphoenolopyruvate – primary acceptor of CO_2:

$$\text{pyruvate} + \text{ATP} + \text{Pi} \rightarrow \text{phosphoenolopyruvate} + \text{AMP} + \text{PPi}.$$

This reaction is very important because it leads to regeneration of primary CO_2 acceptor and allows to remaining its concentration at suitable level. Reaction occurs in mesophyll chloroplasts and is catalyzed by *pyruvate orthophosphate dikinase* (PPDK, EC 2.7.9.1). Enzyme action consumes two high-energetic bounds of ATP and requires orthophosphate. PPDK activity is regulated by light. Fully active PPDK is a tetramer that may dissociate at low temperature and it leads to a loss of its activity. Divalent cations (Mn^{2+}, Mg^{2+}, Ca^{2+}), phosphoenolopyruvate, polyols (glycerol, sorbitol) and proline protect PPDK against cold inactivation [9–11].

It is worth to note that among the C_4 plants there are three subtypes characterized according to the type of decarboxylating enzyme in the bundle sheath cells. *Miscanthus* belongs to described above the NADP-malic enzyme (NADP-ME) pathway. Plants using this pathway are characterized by very high *nitrogen use efficiency* (NUE).

The physiological significance of two-step CO_2 fixation process is increasing CO_2 concentration in cells of bundle sheath (it is estimated 10-fold over atmospheric concentration). This leads to elimination of oxygenase activity of Rubisco and carbon loss in photorespiration process. As a result, in favourable environments, C_4 plants are the most productive crops. They show high photosynthetic rates (both interception of solar radiation and CO_2 fixation) and higher water and nitrogen use efficiency. The field trials performed with *Miscanthus* × *giganteus* and *Triticosecale* (C_3 plant) showed that NUE, defined as the ratio of biomass yield to N supply (sum of soil NO_3^- and N fertilization) were 0.35 and 0.14 Mg dry biomass per kg N, respectively for miscanthus and triticale [12].

C_4 plants have high rates of photosynthesis and productivity but this is realized in favourable conditions – high radiation and warm, humid environments. In temperate zones, low temperature is a major constraint limiting rate of photosynthesis and consequently productivity of C_4 plants. *Miscanthus* × *giganteus* appears to be unusual among C_4 species and cultivated at 52° northern latitude produces 30 tonnes of dry matter per hectare per year, exceeding the most productive C_3 crops [13].

Cold tolerance of *Miscanthus* × *giganteus*

Low temperature is a major factor that influences plant metabolism and physiology and affects its productivity. The most of C_4 species influenced by chilling temperatures (0–15 °C) react by apparent reduction of CO_2 assimilation and consequently, utilization of absorbed solar energy is limited. This leads to photoinhibition and photodamage of photosynthetic apparatus. *Miscanthus* × *giganteus* is relatively tolerant to chilling temperatures (avoids these damages) and similarly to maize may be cultivated in temperate climates. Nevertheless, in comparison with maize, miscanthus considerably better tolerates suboptimal conditions, particularly too low temperatures in spring and autumn [14]. Maize growing at 14 °C shows 90 % reduction in CO_2 uptake comparing with plants grown at 25 °C, whereas in *Miscanthus* × *giganteus* this negative response does not happen [15, 16]. Some researchers have postulated that Rubisco is the most likely candidate for limiting C_4 photosynthesis at chilling temperatures [17]. However experiments conducted by Wang et al [18] on Rubisco from *Miscanthus* × *giganteus*

grown at 14 and 25 ^{o}C and *Zea mays* grown at 25 ^{o}C showed that there were no significant differences in enzyme catalytic proprieties. Rubisco did not differ significantly, either between the two species or between growth temperature. These results suggest that higher cold tolerance of miscanthus than maize does not result from Rubisco activity [18]. Wang et al [11] concluded that pyruvate orthophosphate dikinase (PPDK) is responsible for greater photosynthetic capacity in miscanthus than other C_4 species cultivated under suboptimal temperature. In experiments of Wang et al [11], lowering of growth temperature from 25 to 14 ^{o}C caused different reaction in miscanthus and maize. On the first day at low temperature, PPDK protein declined slightly in miscanthus but then accumulated above the initial level and was nearly doubled after 7 days, whereas Rubisco level did not change significantly. In contrast to miscanthus, PPDK in maize leaves declined throughout in chilling period and Rubisco level was also significantly reduced. Naidu et al [19] obtained similar results. In miscanthus low temperature caused accumulation of PPDK protein while level of Rubisco remained unaffected. Whereas in maize leaves there was recorded decrease in quantity and activity of both crucial enzymes – PPDK and Rubisco.

Concluding, it can be stated that increases in either protein content and PPDK activity in leaves of C_4 plants growing in chilling conditions may be one of the mechanisms increasing their tolerance to low temperatures.

As mentioned above, low temperatures can disrupt balance between absorbed excitation energy of light and its utilization by photosynthetic apparatus [20]. Low temperatures decrease rates of CO_2 uptake and assimilation, and in turn, plant demand for ATP and NADPH is markedly reduced. Consequently, it leads to secondary effects associated with formation and accumulation of *reactive oxygen species* (ROS). The most important ROS are: superoxide ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). These molecules are potentially harmful and may attack cellular components (macromolecules and membranes) and cause serious damage of photosynthetic apparatus (photoinhibition), ultimately leading to severe cellular damage. Plants have the capacity to eliminate ROS and antioxidant molecules such as ascorbic acid, carotenoids, glutathione play important role in this protective system. Carotenoids, lipid-soluble pigments presented in chloroplasts, have the ability to dissipation of surplus excitation energy and act as quencher of its excess and hence they are the main compounds involved in preventing photoinhibition. A particular class of carotenoids – the xananthopylls – play a key role in protection against oxidative damage. Zeaxanthin, the de-epoxidised form, better dissipates excitation energy and is derived from the epoxidised pigments violaxantin. The rapid changes between the two forms, brought about by special enzymes, constitute the "xantophyll cycle". It is worth to note that in chilling conditions miscanthus avoids photooxidative damages by maintaining a high rate of CO_2 uptake and assimilation, and in turn increased utilization of absorbed light energy [13]. On the other hand, in this plant very efficiently operates described above mechanism of non-photochemical quenching of excitation energy. This correlates with large increase in xanthophylls content and its de-epoxidation [16]. In comparison with the other C_4 plants, *Miscanthus* × *giganteus* has a lower temperature optimum for light-saturated photosynthesis [15].

It is worth noting that *Miscanthus* × *giganteus* shows high cold susceptibility, especially to freezing temperature, in the first year after planting. Periods of frost, typical during early spring and winter, may result in poor plants' survival and are potential obstacles to the establishment of miscanthus in northern regions of Europe [21–23]. Plazek et al [24] suggested that frost susceptibility of miscanthus is mainly caused by sensitivity of shoot apical meristems to frost. Farrell at al [23] have investigated genotypic variation in the *base temperature* (T_b) for shoot emergence and in the lethal temperature for shoots in four *Miscanthus* genotypes. In all genotypes, lowering temperature increased the time of shoot emergence. T_b for *Miscanthus* × *giganteus* accounted 8.5 ^oC and was slight higher compared with *Miscanthus sinensis*. All examined genotypes exhibited considerable leaf damage following exposure to –8 ^oC and they might be classed as freezing sensitive [23]. However, taking into account the information that tropical grasses fall within the temperature range –1.8 ^oC to –4.3 ^oC [25], it might be concluded that plants from *Miscanthus* genus are relatively tolerant to non severe frost. In the case of *Miscanthus* × *giganteus*, the lethal temperature at which 50 % of the shoots were killed was estimated as –8 ^oC [23].

Other promising features of *Miscanthus* × *giganteus*

Miscanthus × *giganteus* has several positive ecophysiological traits causing that is considered as environmentally benignant plant:

– **High productivity.** At temperate climate conditions miscanthus yield reaches 20–30 tonnes dry matter per hectar per year (since the second or third year after planting when the crop is well established) and it may be higher – 40 tonnes dry matter $year^{-1}$ ha^{-1} – in southern Europe (Mediterranean regions) with irrigation [12, 26, 27].

The first side-by-side large-scale field trials conducted on *Zea mays* and *Miscanthus* × *giganteus* in the U.S. Corn Belt (Illinois) showed that miscanthus was 59 % more productive than modern lines of grain maize bred for high productivity [28]. Nevertheless, it is interesting that examinations concerning some important parameters of photosynthesis (rate of both phosphoenolopyruvate carboxylation and phospho-enolopyruvate regeneration, quantum efficiency of CO_2 assimilation) demonstrated that they were higher in maize than in miscanthus. However, these biochemical and photochemical traits of maize were not enough to match up to miscanthus in productivity. Dohleman and Long [28] state that higher productivity of miscanthus in comparison with maize is related to larger leaf area and its longer growing season. Consequently, miscanthus intercepts more PAR (*photosynthetically active radiation*) and longer continues photosynthesis. Length of growing season (average for two years) for maize was 125 days whereas for miscanthus it was 199 days (60 % longer than for maize).

Dohleman et al [29] conducted field trials to find the reasons for hugely higher biomass production of *Miscanthus* × *giganteus* in comparison with *Panicum virgatum* (switchgrass; such as miscanthus is C_4 perennial grass considered as bioenergy crop). They concluded that there are numerous factors determined greater productivity of miscanthus: almost 40 % higher photosynthetic rates, significantly higher both nitrogen

and water use efficiencies, lower respiration, larger leaf canopy and its more vertical standing (more radiation penetrates into the deeper canopy).

– **Low fertiliser requirements.** The most field trials conducted at different sites showed that mineral fertilisation (particularly nitrogen supply) does not improve significantly yield of miscanthus especially in the case of full established plantations [26, 30–34]. In initial seasons of planting fertilisation may lead to an increase in above-ground biomass accumulation [35, 36]. Field experiments in southern regions of Europe demonstrated that nitrogen fertilisation significantly increase dry mass of aboveground biomass when water was not limiting (trials with irrigation) [36, 37].

Experiments conducted by Kalembasa et al [35] on five clones of *Miscanthus*, after two years of cultivation, showed that $N_{60}P_{50}K_{100}$ fertilisation cause decrease in dry mass of underground parts (rhizomes + roots). Amougou et al [33] stated that underground biomass was not significantly affected by nitrogen fertilization (120 kg N ha^{-1} $year^{-1}$) but it tended to decrease under N fertilization, particularly on 2-year-old miscanthus stand.

It is noteworthy that long-term trials (14 years) conducted in different locations in Europe also demonstrate that nitrogen fertilisation does not improve growth and biomass productivity of *Miscanthus* × *giganteus* [32]. This unpredictable response of miscanthus to nitrogen application results from very efficient minerals' movement within the plant during the growing season. Rhizomes act as a storage organ and in autumn nitrogen and other nutrients are translocated from aboveground parts (stem + leaves) to rhizome. In subsequent spring season, nutrients stored in rhizome are remobilized and transported into new growing shoots. This causes that miscanthus is partly independent of the actual nutrient supply from soil [26, 38]. Kalembasa et al [39] investigated 5 clones of *Miscanthus* in second year of cultivation, at five different term of growing season (from June to October) and they demonstrated that total nitrogen content was the highest in June and lowered significantly in subsequent months. At the end of growing season (in October) N concentration in dry matter lowered by 5.5-fold on average for all clones comparing with its concentration in June.

Effective minerals' translocation is very profitable phenomenon also in aspect of biomass utilization and its combustion quality. Low concentration of nutrients in harvestable biomass results in reduction of undesirable compounds released during combustion such as SO_2, NO_x, HCl, dioxin. Delayed harvest date (over winter) reduces moisture, ash and nutrient contents in yield and hence is an effective method to improve the biomass quality for combustion [2, 22, 40].

– **Poor needs for crop protection.** In well established plantations (from year two or three onwards), miscanthus very effectively competes with weeds. Mechanical or chemical weed control is necessary during crop establishment [26, 32, 34]. In subsequent years, fast growing plants and leaf litter layer on the soil create conditions that significantly limit growth of other plants and hence application of herbicides is not necessary every year. It is likely that *Miscanthus* × *giganteus* such as other representatives of *Poaceae* family shows high allelopathic activity [41]. It was documented that *Miscanthus transmorrisonensis* and *Miscanthus floridulus* (species growing in Taiwan) produce phenolic compounds that inhibit growth of other plants [1, 41, 42]. Miscanthus

is characterized by relatively high resistence to pests and diseases and chemical products against them are not necessary [26, 32, 34].

– **Potential possibility to using in phytoremediation.** *Miscanthus* ssp. may grow on acidic soils where increases bioavailability of heavy metals and in turn raises their phytotoxicity [43, 44]. Under low pH conditions aluminium (Al), the third most abundant element in earth's crust, is a major soil mineral constituent that may restrict plant growth. It has been reported that Al promotes growth of *Miscanthus sinesis* and is considered as beneficial element for this plant [45]. Besides, it was shown that *Miscanthus sinesis* is also tolerant to other heavy metals: chromiun and zinc [44]. Aluminium presented in plant tissues may enhance herbivore defence and promote phosphorus uptake [45]. Arduini et al [46] have shown that *Miscanthus* × *giganteus* is not particularly tolerant to cadmium. Their experiments conducted in a controlled environment demonstrated that biomass production is declined by 50 % with cadmium concentrations ranging from 0.75 to 2.25 mg · dm^{-3} in nutrient solution and Cd concentration in roots was more than 10-fold higher than that of the shoot. However, these authors suggest that even Cd-sensitive species like miscanthus could effectively accumulate cadmium and widen the range of species using in phytoremediation.

Plantations of *Miscanthus* ssp. may prevent and reduce soil erosion as well as stabilize and extract contaminations from soil [47, 48].

Conclusions

Miscanthus × *giganteus* in spite being C_4 plant adapted to warmer climate, is relatively tolerant to chilling temperatures and in temperate zones its growth is fast and biomass production very high. *Miscanthus* × *giganteus* displays a good combination between high productivity and efficiencies in using of water, nitrogen and solar radiation. Besides, it shows numerous ecophysiological traits causing that it might be recognize as leading crop in non-food cultivations.

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MISCANTHUS – TRAWA NIEZWYKŁA: CHARAKTERYSTYKA BIOCHEMICZNO-FIZJOLOGICZNA: PRZEGLĄD LITERATUROWY

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Abstrakt: *Miscanthus × giganteus* (Miskant olbrzymi) jest jedną z bardziej obiecujących tzw. roślin alternatywnych uprawianych z przeznaczeniem na cele energetyczne. Pomimo że pochodzi z południowo-

-wschodniej Azji i jest rośliną ciepłolubną, to bardzo dobrze rośnie i charakteryzuje się wysoką produktywnością w strefie umiarkowanych szerokości geograficznych. *Miscanthus* × *giganteus* jest rośliną typu C_4, dlatego w pracy przedstawiono krótki opis procesu fotosyntetycznego wiązania CO_2 w tzw. szlaku C_4 oraz uczestniczących w nim enzymów. Korzystając z najnowszych danych literaturowych, przeanalizowano podstawy biochemiczne stosunkowo dużej odporności tej rośliny na niskie temperatury (0–15 ^oC). Opisano również szereg ekofizjologicznych właściwości *Miscanthus* × *giganteus* (wysoka produktywność, niewielkie wymagania nawozowe, brak konieczności stosowania pestycydów, możliwość wykorzystania w procesach fitoremediacji), które sprawiają, że jest on określany jako roślina ekologiczna i szczególnie przyjazna środowisku. Opisane cechy rośliny sprawiają, że można ją uznać za lidera wśród roślin uprawianych na cele nieżywnościowe.

Słowa kluczowe: miskantus, tolerancja na chłód, C_4 fotosynteza, fitoremediacja, allelopatia

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INITIAL RESEARCH ON THE EFFECT OF THE NANOGRO PLANT GROWTH STIMULATOR ON *Fusarium culmorum* (W.G. Smith) Sacc.

WSTĘPNE BADANIA NAD WPŁYWEM STYMULATORA WZROSTU ROŚLIN NANOGRO NA *Fusarium culmorum* (W.G. Smith) Sacc.

Abstract: An *in vitro* experiment determined the effect of the Nanogro plant growth and development stimulator, newly introduced in Poland, on *Fusarium culmorum* (W.G. Smith) Sacc. phytopathogenic fungus. A modification of linear growth and sporulation of *F. culmorum* mycelium caused by Nanogro was observed after the contact with the fungus spores, vegetative mycelium and the added medium. In the experiments where limited linear growth of Nanogro was observed as a result, stimulation of mycelium sporulation occurred the most frequently. The application of Nanogro in practice will not contribute to limiting the harmfulness of this pathogen.

Keywords: *F. culmorum*, Nanogro, linear growth, sporulation

Research on agro-homeopathy has been conducted worldwide and preparations with ultra low content of elements or chemical compounds positively affecting growth and development of plants have been introduced to the agro-market. Scientific research on agro-homeopathy revealed a positive effect of ultra-low concentrations of the substances selected for the experiments on the growth of various plants, particularly during germination [1–7]. Like medical homeopathy, also agro-homeopathy has both supporters and opponents [8].

Nanogro, a plant growth and development stimulator, was first marketed in Poland in 2007 as one of the few agro-homeopathic products. The preparation consists of oligosaccharide granules (pellets) saturated with metal sulphates (Fe, Co, Al, Mg, Mn, Ni and Ag) in nanomolar concentrations. Nanogro is recommended for treatment and

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watering agronomic and horticultural plants, which according to its producer causes an increase in yields, more exuberant plant development, shortens vegetation and improves plant viability and vigour.

Fungi of the *Fusarium* genus are dangerous cosmopolitan pathogens of crops. Diseases caused by these fungi lead not only to a decline in yield and its parameters but also worsens its quality due to the presence of micotoxins dangerous for humans and animals. The species dominant in Europe is *Fusarium culmorum* (W.G. Smith) Sacc. [9–12].

The research was conducted to assess the effect of Nanogro on the vegetative mycelium and spores of *Fusarium culmorum*.

Material and methods

F. culmorum strain purchased from the collection of the Plant Protection Institute (the Plant Pathogen Bank), isolated from cabbage was used for the analyses. The experiment was conducted *in vitro* to test the effect of Nanogro on the concentrated spore suspension and vegetative mycelium of *F. culmorum*.

Nanogro in the concentrations recommended by the producer (10 granules per 10 dm^3 of water) and three times increased (30 granules per 10 dm^3 of water) was added to the fungus spore suspension prepared in sterile distilled water. The control was the suspension with the same spore concentration without the Nanogro supplement. The spore suspension was shaken in 300 cm^3 flasks for 81 hours and after 1, 2, 3, 6, 9 and 81 hours some amount of suspension, adequate for further culturing was collected. *F. culmorum* spore suspension was inoculated on Petri dishes with solid PDA medium in 7 replications. The culture was maintained at the temperature of 21 ^oC.

Linear growth of the cultured mycelium was measured. Once the culturing was completed, spore suspensions were prepared of the mycelia discs with a 50 mm diameter and shaken in 100 cm^3 of distilled water. After filtration the spores number was measured in the obtained suspensions using the spectrophotometric method.

The subsequent *in vitro* experiment aimed at an assessment of the effect of Nanogro on *F. culmorum* vegetative mycelium. Mycelial discs with a 5 mm diameter from the Department's own collection were inoculated on Petri dishes with solid PDA medium. Nanogro solutions were prepared in sterile distilled water (10, 20 and 30 granules per 10 dm^3 of water). Subsequently, 30 mm^3 (μl) of the solution was dripped with a micropipette onto the discs inoculated on the Petri dishes. 7 replications were made for each Nanogro concentration, whereas the fungus culture from discs dripped with sterile distilled water constituted the control. The culture was maintained and mycelial linear growth was measured until sporulation was obtained, which was assessed as presented above.

The effect of Nanogro supplied to PDA medium in the concentration of 10 granules per 10 dm^3 was also tested. As in the other experiments, assessed were mycelia linear growth and its sporulation after culturing. The control was the culture maintained on the standard PDA medium. The obtained results were verified statistically.

Results and discussion

Figures 1 and 2 present the results obtained in the experiment assessing the effect of Nanogro on *F. culmorum* spores. After 1, 2, 3, 6 and 9-hour spore contact with the preparation in the concentration recommended by the producer (10 granules per 10 dm^3)

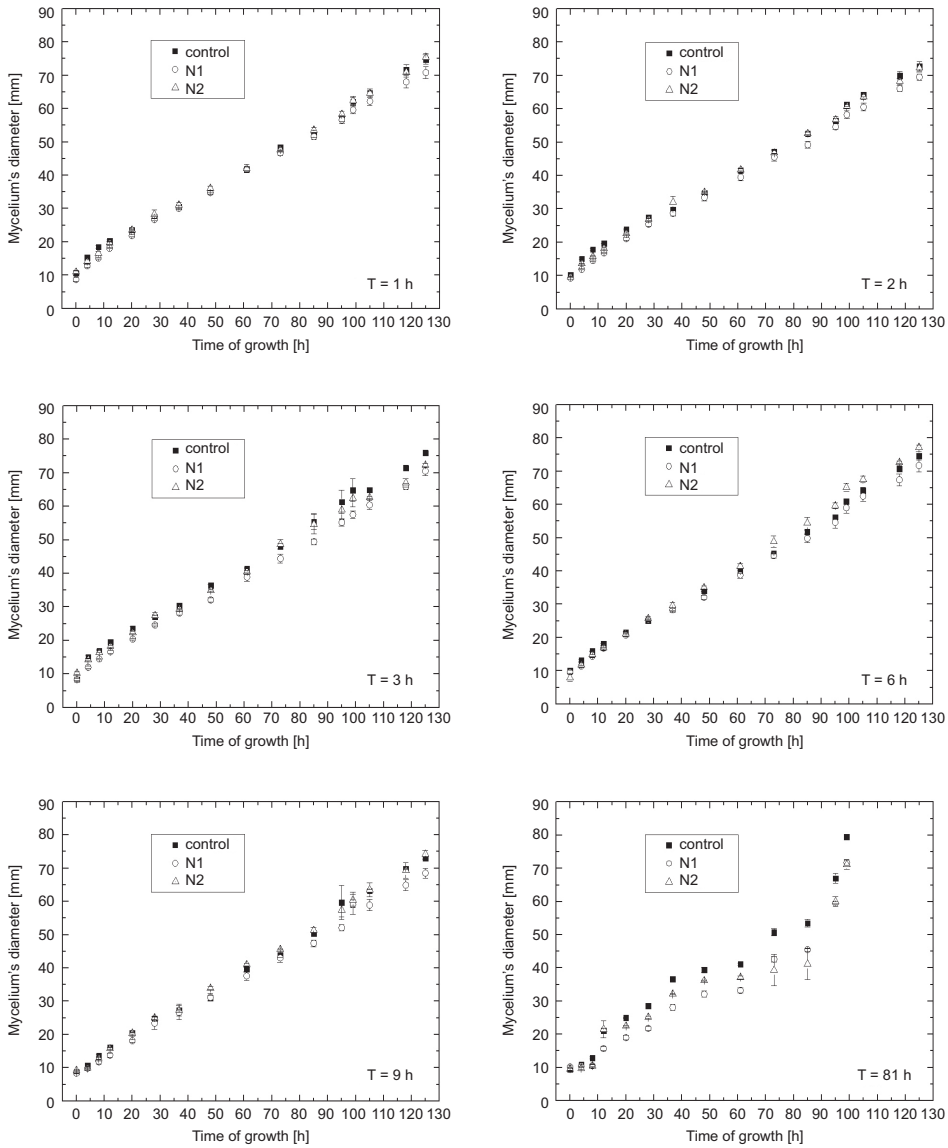

Fig. 1. The effect of Nanogro on *F. culmorum* spores expressed by linear growth in consecutive culturing after contact (T) with preparation in the concentrations of 10 granules per 10 dm^3 (N1) and 30 granules per 10 dm^3 (N2)

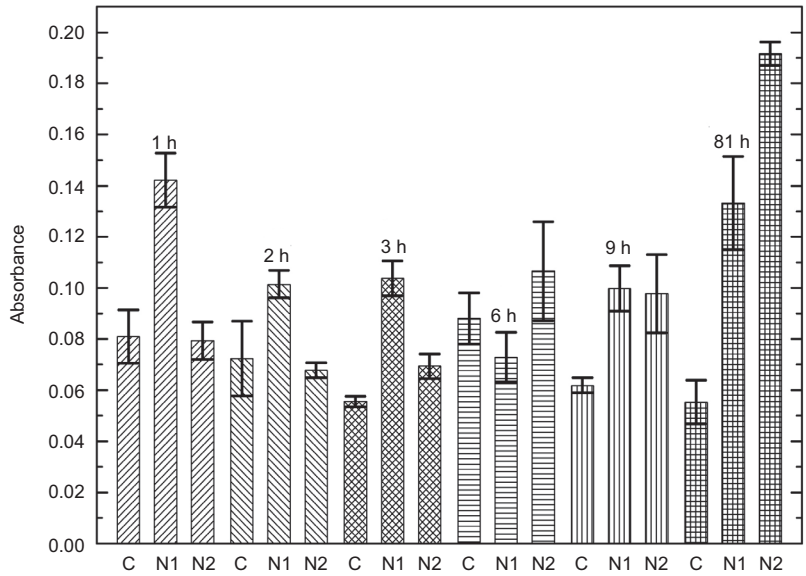

Fig. 2. The effect of Nanogro on *F. culmorum* spores expressed by mycelial sporulation obtained in consecutive culturing after contact with the preparation (1, 2, 3, 6, 9 and 81 h) in concentration of 10 granules per 10 dm^3 (N1) and 30 granules per 10 dm^3 (N2), c – control

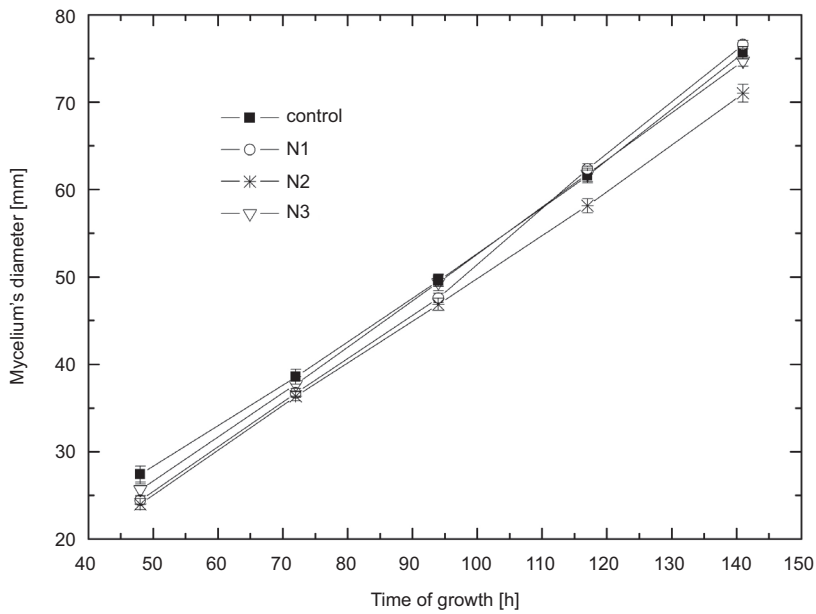

Fig. 3. The effect of Nanogro on *F. culmorum* vegetative mycelium growth, applied Nanogro concentrations: 10 granules per 10 dm^3 (N1), 20 granules per 10 dm^3 (N2) and 30 granules per 10 dm^3 (N3)

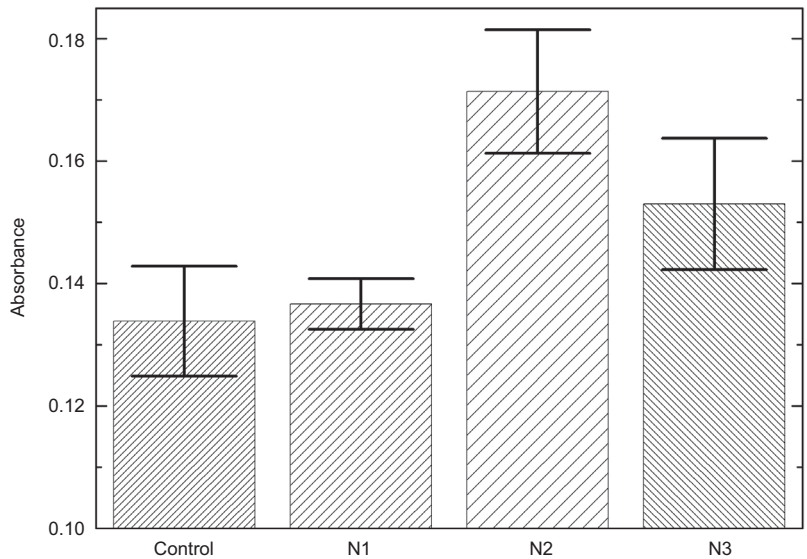

Fig. 4. The effect of Nanogro applied on vegetative mycelium on sporulation obtained in consecutive culturing. Applied Nanogro concentrations: 10 granules per 10 dm^3 (N1), 20 granules per 10 dm^3 (N2) and 30 granules per 10 dm^3 (N3)

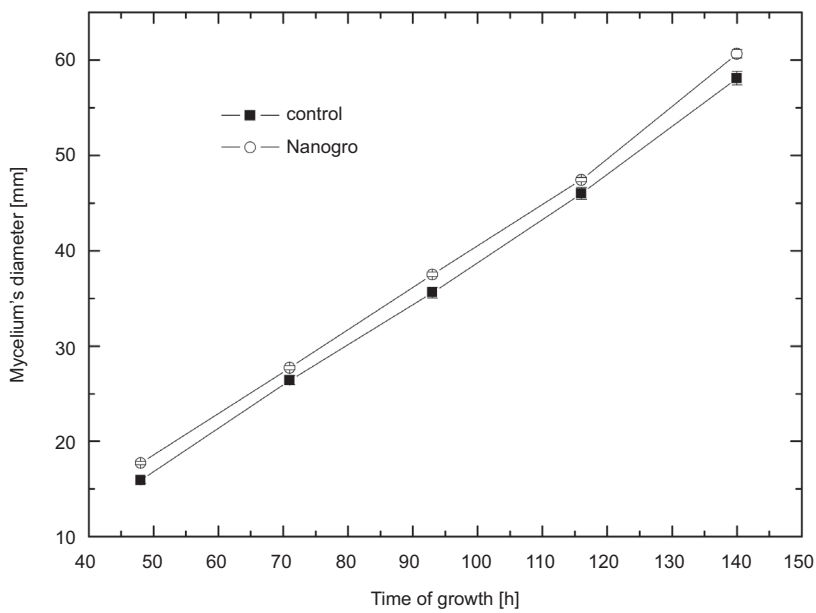

Fig. 5. The effect of Nanogro supplied to the medium in concentration of 10 granules per 10 dm^3 on *F. culmorum* linear growth

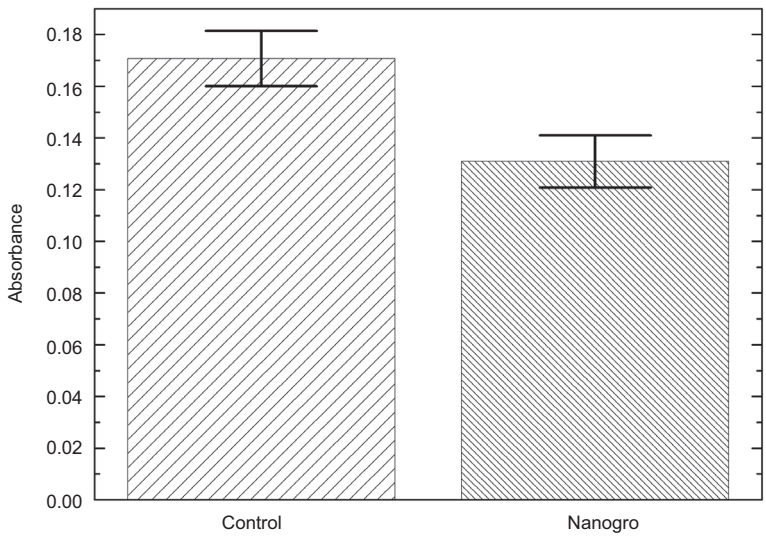

Fig. 6. The effect of Nanogro supplied to the medium in the concentration of 10 granules per 10 dm^3 on *F. culmorum* sporulation

a slight reduction of linear growth of mycelium cultured from spores was revealed in comparison with the control (Fig. 1), which was not registered for the higher concentration (30 granules per 10 dm^3). The longest (81 h) spore contact with the preparation in both applied concentrations significantly limited the fungus growth in the consequent culturing. The analyzed mycelia sporulation after culturing (Fig. 2) evidences that despite limiting mycelia linear growth, Nanogro affects the strong stimulation of *F. culmorum* sporulation.

The results obtained from the experiment on the effect of Nanogro on *F. culmorum* vegetative mycelium show that only in the concentration of 20 granules per 10 dm^3 of water the preparation significantly limits the linear growth of the pathogen mycelium in comparison with the control (Fig. 3). Like in the experiments on spores, Nanogro in the concentration limiting the linear growth caused a markedly greater mycelium sporulation (Fig. 4).

On the other hand, Nanogro supplied to the medium slightly stimulated linear growth of *F. culmorum* (Fig. 5) in comparison with the control and significantly limited mycelium sporulation obtained in culturing on this medium (Fig. 6).

The obtained results demonstrated that Nanogro modifies linear growth and sporulation of *F. culmorum* – a dangerous crop pathogen. However, no fungistatic effect of Nanogro was registered, whereas practical application of this preparation will contribute to limiting this pathogen harmfulness.

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WSTĘPNE BADANIA NAD WPŁYWEM STYMULATORA WZROSTU ROŚLIN NANOGRO NA *Fusarium culmorum* (W.G. Smith) Sacc.

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Abstract: W doświadczeniu *in vitro* określono wpływ nowego w Polsce stymulatora wzrostu i rozwoju roślin Nanogro na grzyb fitopatogenny *Fusarium culmorum* (W.G. Smith) Sacc. Stwierdzono modyfikację wzrostu liniowego i zarodnikowania grzybni *F. culmorum* wywoływane przez Nanogro po kontakcie z zarodnikami grzyba, grzybnią wegetatywną, jak i dodanego do podłoża hodowlanego. W doświadczeniach, gdzie pod wpływem Nanogro obserwowano ograniczenie wzrostu liniowego, występowała najczęściej stymulacja sporulacji grzybni. Stosowanie Nanogro w praktyce nie przyczyni się do ograniczenia szkodliwości tego patogenu.

Słowa kluczowe: *F. culmorum*, Nanogro, wzrost liniowy, zarodnikowanie

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EFFECT OF CATCHMENT AREA USE ON LEAD AND ZINC ACCUMULATION IN THE BOTTOM DEPOSITS OF LAKES ARDUNG AND BUKWALD

WPŁYW UŻYTKOWANIA ZLEWNI NA AKUMULACJĘ OŁOWIU I CYNKU W OSADACH DENNYCH NA PRZYKŁADZIE JEZIOR ARDUNG I BUKWAŁD

Abstract: The study concerns with the effect of catchment area use on lead and zinc accumulation in the bottom deposits of lakes. It was carried out in two water bodies in the Olsztyn Lakeland. The catchment areas of the investigated lakes are used for various purposes, ranging from forests to agricultural production. Lake Ardung (N 53°45′, E 20°55′) is situated in the eastern part of the Masurian Lakeland, approximately 25 km east of Olsztyn. The lake has an area of 26.2 ha and a maximum depth of 3.6 m. The lake's catchment area of 1539 ha is covered by farmland in 2 %, grassland in 2 %, fallow land overgrown with shrubs in 11.4 % and forests in 84.6 %. Lake Bukwald (N 53°58′, E 20°16′) is located in the vicinity of the village of Bukwald, municipality of Dywity, around 20 km north of Olsztyn. The lake's catchment area of 1156.8 ha comprises arable land in 60 %, forests and afforested areas in 31 % and wasteland in the remaining part.
The studied water bodies were characterized by low concentrations of the analyzed elements. The average lead and zinc levels reached 28.3 mg/kg d.m. and 32.3 mg/kg d.m., respectively in Lake Ardung, and 33.3 mg/kg d.m. and 91.9 mg/kg d.m., respectively in Lake Bukwald. The total zinc and lead accumulation in the bottom deposits of the investigated water bodies, in terms of the surface area of the lakes and their catchments, was significantly higher in Lake Bukwald than in Lake Ardung.

Keywords: lakes, bottom deposits, catchment area, trace elements, lead, zinc

Substance runoff from the catchment area into surface waters is determined by various factors, in particular land relief, soil cohesion and fertility, the type of land use, water relations and climate conditions, mostly the volume and distribution of precipita-

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tion [1]. Vast quantities of substances are also supplied by atmospheric precipitation which contributes to the leaching of chemical components from the soil [2, 3].

Bottom deposits are a combination of crystalline and amorphous minerals with a different grain size, a various content of organic matter and mineral or organic colloidal substances [4, 5]. Bottom deposits are formed by the sedimentation of allochtonic material created outside the sedimentation area as well as autochthonic material formed in the place of sedimentation [6]. The bottom deposits of aquatic ecosystems vary immensely from coarse-grained, nearly mineral deposits to fine--grained, mostly organic deposits in the deep strata of lakes. Deposits comprise mineral substances (silica, silicate, aluminosilicate, carbonate) as well as organic substances of various origin (catchment area, littoral and pelagial zones) and various degree of decomposition. Deposits found at deeper strata are generally characterized by greater thickness, fine-grained structure and a higher organic matter content [7].

The trace element content of bottom deposits is conditioned by numerous natural and anthropogenic factors. It is largely determined by the geological structure of the catchment area, its geomorphologic characteristics and climate conditions which are responsible for rock weathering, the mobilization, migration and accumulation of elements in the environment. In undeveloped areas, high concentrations of potentially harmful trace elements in bottom deposits are attributed mainly to different types of human activity in the catchment area, mostly agricultural production [8–10].

Materials and methods

In the study the effect of catchment area use on lead and zinc accumulation in the bottom deposits of lakes was carried out in two water bodies in the Olsztyn Lakeland. The catchment areas of the investigated lakes are used for various purposes, ranging from forests to agricultural production.

Lake Ardung (N 53°45′, E 20°55′) is situated in the eastern part of the Masurian Lakeland, approximately 25 km east of Olsztyn. The lake has an area of 26.2 ha and a maximum depth of 3.6 m. The lake's catchment area of 1539 ha is covered by farmland in 2 %, grassland in 2 %, fallow land overgrown with shrubs in 11.4 % and forests in 84.6 %. Lake Ardung is located in the catchment area of the Lyna River in the watershed of Lyna (tributary of the Pregoła River) and Omulew (tributary of the Narew River in the Vistula Basin) river systems. This area is characterized by a large number of small lakes, ponds and swamps. It is weakly populated, and the predominant types of human activity include farming, forestry and tourism. The parent rock of catchment area soils comprises sandy glaciofluvial deposits that fill the channel surrounded by more cohesive clay formations [11]. The catchment area features mostly podzolic soils in natural pine forests. Depression areas comprise mostly hydrogenic soils developed in the course of several drainage schemes. The lake's present catchment area was formed during land improvement projects carried out in the mid 19th and the early 20th century. As part of those schemes, the lake's water horizon was lowered to expose shallow waters. Today, Lake Ardung is a remnant of a multi-sectional lake with the original area of around 250 ha surrounding the town of Nerwik. The lake was periodically separated

to create 224 ha of farmland. The original lake basin which today forms Lake Ardung spanned an area of 62 ha.

Lake Bukwald (N 53°58′, E 20°16′) is located in the vicinity of the village of Bukwald, municipality of Dywity, around 20 km north of Olsztyn. Lake Bukwald is a flow through water body fed by four streams in its north-western and the south-western parts. Water is evacuated from the lake via a single watercourse in the village of Bukwald. The lake has an area of 36.2 ha and a maximum depth of 12.4 m. It is supplied from a hilly area marked by significant altitude variations – the difference between the highest and the lowest point in the catchment area is 23.5 m. Lake Bukwald's catchment area of 1156.8 ha comprises arable land in 60 %, forests and afforested areas in 31 % and wasteland in the remaining part. There is a predominance of light to medium-heavy loams in the north, and light and heavy loamy sands turning into light loams and slightly loamy sands in the south. Catchment area soils fall into quality classes IVa and IVb and, locally, IIIa and IIIb.

Surface samples from the bottom deposits of the studied lakes were collected for physical and chemical analyses in June 2008. Sampling sites were distributed along five perpendicular transects, three sites per transect (Fig. 1). The location of sampling sites was chosen based on barimetric charts to produce the most comprehensive overview of the morphological and chemical properties of deposits, subject to the lake-bottom topography, the shape of the lake basin, flow rate, tributaries, etc.

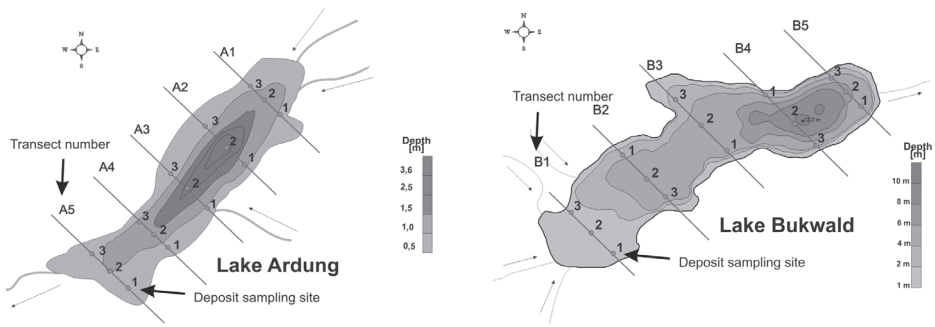

Fig. 1. Location of deposit sampling sites in Lake Ardung and Lake Bukwald

Deposit samples were collected using the Ekman grab (for collecting samples with a surface area larger than 250 cm^2). Three samples were collected from each site, and they were averaged into one sample. The deposits and sedimentation water sampled from each site were placed in air-tight glass containers. The physical properties of samples were analyzed by the Troels-Smith method immediately after collection [12].

At the laboratory of the Department of Land Improvement and Environmental Management at the University of Warmia and Mazury in Olsztyn, the following physical and chemical parameters were determined in fresh deposit samples directly after collection:

– solid residue – by the gravimetric method at 105 °C [g/kg d.m.],

– ignition losses – as an indicator of organic substance content, calculated as the difference between solid residue and residue after ignition [g/kg d.m.],

Pb and Zn levels were determined by *atomic absorption spectroscopy* (AAS) in deposit samples at the laboratory of the Department of Technology in Environmental Engineering and Protection at the Bialystok University of Technology.

Results and discussion

The bottom deposits of the analyzed water bodies were characterized by significant morphological diversity. Deposits from Lake Ardung (Nig. 4) were marked by a much greater degree of darkness than samples from Lake Bukwald (Nig. 2). Structural layering was not determined in samples from both lakes (Strf. 0), and the analyzed deposits were completely homogenous. Owing to continuous contact with water, the surface layers of bottom deposits were strongly hydrated (Sicc. at level 2–3), and they contained highly decomposed plant matter (Humo at level 3–4, H7–H10 on the von Post scale of humification). The investigated deposits had homogenous, greasy structure. Littoral zone samples contained fragments of hydrophyte roots with partially decomposed plant matter and small quantities of mollusk shells, while profundal zone samples were more homogenous (with an absence of plant remains or macrophyte roots). The analyzed deposits had the characteristic features of sapropel formations [12].

Significant differences were observed in the water content of deposit samples. The average dry matter content was 16.3 % in samples from Lake Ardung and 20.9 % in deposits from Lake Bukwald (Table 1). More profound differences in the dry matter content of deposits were found between sampling sites in Lake Ardung. The highest moisture content of 88.7 % was observed in samples from the central part of transect A3, and the lowest moisture content of 65.4 % was reported in transect A1, ie on the side of the inflow to the lake near the river head-streams. In Lake Bukwald, the highest water content of bottom deposits was determined in samples from the central part of transect B2 (86.8 %), and the lowest – on the northern side of transect B4 (66.3 %).

Table 1

Physical and chemical properties of bottom deposits in lakes

Parameter	Unit	Lake Ardung	Lake Bukwald
		mean (min – max)	mean (min – max)
Dry matter	[%]	16.3 (11.3–34.6)	20.9 (13.2–33.7)
Organic content	[% d.m.]	18.9 (14.9–26.0)	14.0 (8.1–19.9)
Pb	[mg/kg d.m.]	28.3 (20.0–35.0)	33.3 (24.0–56.0)
Zn	[mg/kg d.m.]	32.3 (19.7–68.8)	91.1 (45.9–112.9)

The organic content of deposits is formed by remnants of aquatic organisms that fall to the bottom of the lake, organic suspension, the precipitation and coagulation of organic substances dissolved in water. The organic matter content of deposit surface layers is determined mostly by the productive output of the trophogenic zone, the quantity of allochtonic matter and sedimentation time [13–17].

The average organic matter content of sediment samples from the analyzed lakes was 14.0 % in Lake Bukwald and 18.9 % in Lake Ardung. The reported values are characteristic of post-glacial lakes whose organic matter content ranges from 10 % to 70 % [16, 18]. The results of the study point to variations not only between the water bodies, but also in the spatial distribution of organic matter in the examined lakes. In Lake Ardung, the lowest organic matter concentrations (14.9 %) were noted in deposits from the southern part of the water body on the side of the outflow in transect A5 and in deposits from the side of the inflow to the lake (15.7 %), while the highest organic content at 26 % was reported in the central part of transect A2 (deepest waters). Similar results were noted in Lake Bukwald where the lowest organic matter concentrations were found in samples collected on the side of the outflow (8.1 %) and near the inflow (13.8 %), and the highest organic matter content was determined in deposits from the profundal zone of the lake (19.9 %). Organic matter produced by the precipitation and coagulation of organic substances dissolved in water has a capillary structure, and its specific gravity is similar to the specific gravity of water [12, 14, 15]. The above facilitates the lifting and the transport of substance particles, and it explains the limited organic matter content of samples collected on the side of the outflows and inflows because the deposits accumulating in those areas are depleted by moving water which washes out lighter particles. Runoff and feeder sections of the lake are shallower and more abundant in oxygen which contributes to the mineralization of deposits. The highest organic matter content was noted in the deepest zones of the lake owing to the gravitational force which pushes deposits deeper down the lake basin.

The studied deposits were characterized by an average lead content of 28.3 mg/kg d.m. in Lake Ardung and 33.3 mg/kg d.m. in Lake Bukwald. The geochemical background of lead in the bottom deposits of lakes in north-eastern Poland is 11 mg Pb/kg d.m., and the lead content of unpolluted deposits should not exceed 20 mg/kg d.m. [8]. In Lake Bukwald, the highest Pb levels were noted on the southern side of transect B4 (56.0 mg Pb/kg d.m.), while the lowest lead concentrations were found in deposits on the southern side of transect B1 (24.0 mg Pb/kg d.m.). In the mid-forest Lake Ardung, Pb levels were lower, in the range of 20.0 mg/kg d.m. to 35.0 mg/kg d.m. (Table 1).

The average zinc content of the analyzed deposits was low, ranging from 32.3 mg/kg d.m. in Lake Ardung to 91.1 mg/kg d.m. in Lake Bukwald. The geochemical background of zinc in soils usually does not exceed 50.0 mg/kg dm, while the average values noted in lakes are generally under 100 mg/kg d.m. [19]. The average Zn content of the investigated deposits approximated geochemical background levels. Zinc concentrations in the bottom deposits from Lake Ardung were within the 19.7–68.8 mg/kg d.m. range. The Zn content of samples from Lake Bukwald, whose catchment area is occupied by a housing estate and farmland, was determined at 45.9–112.9 mg/kg

d.m. (Table 1). According to the system for classifying the purity of bottom deposits proposed by Bojakowska and Sokolowska [20], the Zn and Pb concentrations noted in this study meet first class purity standards.

Agricultural production in the catchment area of Lake Bukwald, including the use of pesticides, contributes to high Pb values in deposit samples from the lake. Lead concentrations were further increased by the long-term use of tetraethyl lead as an antiknock agent in the fuel powering farming machines as well as atmospheric deposition. According to Nicholson [21], atmospheric deposition is the main source of lead in agricultural areas, and it accounts for as much as 77 % of its total supply.

A comparison of lead and zinc concentrations in deposit samples shows that they were significantly lower in the littoral zones than in the profundal zones of the analyzed lakes. The low Pb and Zn content of bottom deposits in littoral zones could be attributed to bioaccumulation. Deposits in deeper parts of the lake are characterized by high concentrations of organic substances and fine-grained mineral fractions that bind metals [22].

The results of the analysis indicate that the highest concentrations of trace elements in Lake Ardung were found on the side of the outflow and in the profundal zone of the lake, while the lowest Pb and Zn levels were observed on the side of the inflow near the river head-streams. In Lake Bukwald, lower lead and zinc levels were also noted in deposit samples collected on the side of the inflow, while the highest Pb and Zn concentrations were reported in transects on the side of the outflow.

An analysis of total lead and zinc load in the bottom deposits of the examined water bodies points to significantly higher accumulation levels in Lake Bukwald at 1372.6 kg Zn and 502.3 kg Pb. In Lake Ardung, zinc accumulation was 80 % lower (279.5 kg Zn) and Pb accumulation was approximately 50 % lower (247.2 kg Pb) than in Lake Bukwald (Table 2).

Table 2

Accumulation of trace elements in the 0–20 cm layer of bottom deposits in the studied lakes

Indicator	Lake			
	Ardung		Bukwald	
	Pb	Zn	Pb	Zn
kg/lake	247.2	279.5	502.3	1 372.6
kg /ha lake	9.43	10.7	13.88	37.9
kg/ha catchment area	0.14	0.16	0.43	1.2

An analysis of Zn and Pb accumulation in terms of the catchment area unit points to higher deposition in Lake Bukwald at 1.19 kg Zn/ha and 0.43 kg Pg/ha. In the mid-forest Lake Ardung, accumulation values reached 0.16 kg Zn/ha and 0.14 kg Pb/ha. In terms of the surface area of the lakes, the bottom deposits of Lake Bukwald also accumulated more Zn and Pb (13.88 kg Pb/ha, 37.9 kg Zn/ha) than the deposits of Lake Ardung (9.43 kg Pb/ha, 10.7 kg Zn/ha) (Table 2).

A comparison of lead accumulation values in the bottom deposits of the examined lakes indicates that lead concentrations are largely determined by the type of catchment area use. A higher level of trace elements accumulation was noted in the bottom deposits of Lake Bukwald, whose catchment area is used for agricultural production, than in the deposits of the mid-forest Lake Ardung.

Conclusions

1. Variations in the spatial distribution of lead and zinc were determined in the bottom deposits of the investigated lakes. Higher levels of the analyzed trace elements were noted in deposit samples collected from shallower lake sections on the side of the outflow.

2. The level of Zn and Pb accumulation in the bottom deposits of the examined water bodies was determined mostly by the type of catchment area use and the applied fertilization rates. In the bottom deposits of Lake Bukwald, whose catchment area is used for farming production and human settlement, the total accumulation of the analyzed elements was much higher (1372.6 kg Zn and 502.3 kg Pb) than in the bottom deposits of the mid-forest Lake Ardung (279.5 kg Zn and 247.2 kg Pb).

3. The total lead and zinc accumulation in bottom deposits in terms of the surface area of the lakes was significantly higher in Lake Bukwald (1372.6 kg Zn and 502.3 kg Pb) than in Lake Ardung (279.5 kg Zn and 247.2 kg Pb). The above differences resulted mainly from the type of activity performed in the catchment areas of the investigated water bodies.

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WPŁYW UŻYTKOWANIA ZLEWNI NA AKUMULACJĘ OŁOWIU I CYNKU W OSADACH DENNYCH NA PRZYKŁADZIE JEZIOR ARDUNG I BUKWAŁD

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Abstrakt: Do badań mających na celu określenie wpływu użytkowania zlewni jezior na akumulację ołowiu i cynku w ich osadach dennych wytypowano dwa zbiorniki położone na obszarze Pojezierza Olsztyńskiego. Zlewnie badanych jezior obejmują obszary o zróżnicowanym zagospodarowaniu – od obszarów leśnych po użytki rolne.

Jezioro Ardung (N 53°45′, E 20°55′) położone jest we wschodniej części Pojezierza Mazurskiego ok. 25 km na wschód od Olsztyna. Powierzchnia jeziora wynosi 26,2 ha, natomiast jego maksymalna głębokość 3,6 m. Na obszarze zlewni jeziora o powierzchni 1539 ha grunty orne stanowią 2 %, łąki i pastwiska 2 %, odłogi w znacznym stopniu zakrzewione 11,4 % i 84,6 % lasy. Jezioro Bukwałd (N 53°58′, E 20°16′) położone jest w okolicach wsi Bukwałd w gminie Dywity około 20 km na północ od Olsztyna. Całkowita zlewni jeziora Bukwałd wynosi 1156,8 ha, z czego 60 % stanowią grunty orne, 31 % to lasy i tereny zalesione, pozostałą część stanową nieużytki.

Badane osady charakteryzowały się niskim stężeniem badanych pierwiastków i w jeziorze Ardung średnie stężenie Pb wynosiło 28,3 mg Pb/kg s.m., natomiast Zn – 32,3 mg Zn/kg s.m., zaś w jeziorze Bukwałd było to 33,3 mg Pb/kg s.m. oraz 91,1 mg Zn/kg s.m. Także całkowita akumulacja cynku i ołowiu w osadach badanych zbiorników zarówno w odniesieniu do powierzchni lustra wody, jak i zlewni była zdecydowanie większa w osadach jeziora Bukwałd niż śródleśnego jeziora Ardung.

Słowa kluczowe: jeziora, osady denne, zlewnia, pierwiastki śladowe, ołów, cynk

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ALUMINIUM CONCENTRATION IN PLANTS DEPENDING ON SOIL CONTAMINATION WITH CADMIUM

ZAWARTOŚĆ GLINU W ROŚLINACH W ZALEŻNOŚCI OD ZANIECZYSZCZENIA GLEBY KADMEM

Abstract: The aim of the study has been to determine the effect of soil contamination with cadmium (10, 20, 30 and 40 mg Cd · ha^{-1} soil) on the concentration of aluminium in aboveground parts and roots of oats, maize, yellow lupine and radish. In order to neutralise cadmium, the following neutralising agents were introduced to soil: compost, brown coal, lime and bentonite. Apart from the plant species and type of organs, other factors which largely affected the concentration of aluminium were a rate of cadmium and type of a neutralising substance. Roots contained much more aluminium than aboveground parts of plants. The highest levels of aluminium were found in roots of yellow lupine and maize whereas the smallest concentrations of this metal were determined in grain and roots of oats. Soil contamination with cadmium caused bigger changes in the concentration of aluminium in aboveground parts of plants than in their roots, especially in the case of maize and yellow lupine. Cadmium applied at 20 mg (maize and yellow lupine roots) or 40 mg Cd · ha^{-1} soil (aboveground parts of maize and yellow lupine) caused increased levels of cadmium in plant tissues. Any further increase in the rates of the pollutant caused depression in the content of aluminium in roots of the above crops. In the case of aboveground parts and roots of radish and grain of oats, less aluminium was observed in all cadmium contaminated objects. The range of effects produced by the test neutralising substances on the concentration of aluminium was varied. The neutralising agents tended to depress the content of aluminium in plant tissues. Brown coal, bentonite and lime caused larger changes in the content of aluminium than compost. The concentration of aluminium was correlated with yields of the crops. For most of the plants, these correlations were negative in the case of aboveground parts (except radish) and positive in roots (except yellow lupine). The concentration of aluminium in particular plant organs was correlated with a number of macro- and microelements, with the correlations being usually positive for manganese, iron, cobalt, lithium, copper and zinc but negative for sulphur and boron.

Keywords: cadmium contamination, compost, brown coal, lime, bentonite, plants, aluminium content

Contamination of natural environment with heavy metals has many negative consequences to the nature. Among particularly harmful effects are modifications of the soil environment, especially changes in physicochemical, microbiological and bio-

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chemical properties of soils [1–3]. Such alterations disrupt the uptake of many nutrients by plants, which in turn deteriorates quality of crops [4]. Crops which contain excessive concentrations of heavy metals cannot be used as animal fodder or human foodstuff. Cadmium is one of the most dangerous heavy metals – found in soil, it not only increases its levels in the plants but also changes concentrations of other elements in plant tissues. The latter effect is caused by antagonistic and syngeristic interactions [5, 6]. The response of plants to an identical level of soil contamination with cadmium can be highly varied – from very small changes, in some species, that are invisible to the naked eye, to failed emergence or necrosis of seedlings of other plant species [1]. The risk of cadmium and other heavy metals passing to successive food chain links must be minimised by undertaking protective measures, which reduce the influence of this metal on plants. Liming is the most popular treatment for this purpose, but other methods can also be helpful.

Considering the above, a study has been conducted in order to assess the effect of soil contamination with cadmium on concentration of aluminium on aboveground parts and roots of oats, maize, yellow lupine and radish. Cadmium introduced to soil was neutralising with compost, brown coal, lime and bentonite.

Material and methods

A greenhouse experiment was conducted at the University of Warmia and Mazury in Olsztyn. Acidic soils of the granulometric composition of light loamy sand were used for the trials. A more detailed description of the soils can be found in our earlier publication [7]. Polyethylene pots were filled with 9 or 10 kg of soil. The soil was contaminated with cadmium at the following rates: 10, 20, 3 and 40 mg Cd · kg^{-1} soil. The effect produced by cadmium was tested on four plants: oats, maize, yellow lupine and radish. In order to reduce the influence of cadmium on plants, the following were applied in the trials with oats: compost, brown coal and lime. In the trials involving the other crops, bentonite was used in addition to the above neutralising agents. Compost and brown coal were introduced to soil at a rate of 4 % while bentonite was added at 2 % relative to the whole mass of soil in a treatment. Lime was added in an amount corresponding to 1 unit of hydrolytic acidity of soil. Simultaneously, the soils were fertilized with NPK, in the amounts adjusted to the crop species. The elements were introduced to soil in the following compounds and mixtures: cadmium as $CdCl_2$, nitrogen as $CO(NH_2)_2$, phosphorus as $Ca(H_2PO_4)_2 + H_3PO_4 + CaSO_4$ and potassium as KCl. Cadmium, the neutralising agents and the mineral fertilizers were carefully mixed with the soil when the trials were being established. The experiment was run with 4 replications. During the growing season, the soil moisture level was maintained at 60 % capillary water capacity. The test plants were harvested in the stage of full maturity (oats and radish), the inflorescence stage (yellow lupine) and the cob formation stage (maize).

During the harvest, samples of aboveground and underground parts of plants were collected. Plant roots were rinsed with water to remove soil. Samples of the plant material were fragmented, dried and ground. The concentration of aluminium in the

plant material was determined by *atomic absorption spectrophotometry* (AAS) using a Unicam 939 Solar spectrophotometer. The statistical calculations were done using the software package Statistica [8].

Results

Apart from the plant species and plant organ, other factors which strongly affected the concentration of aluminium in plant tissues were the rate of cadmium and the type of a neutralising agent applied. The roots contained much more aluminium than the aboveground parts of plants. Most aluminium was determined in roots of yellow lupine and maize; the smallest levels of Al were found in grains and straw of oats.

The soil pollution with cadmium caused more profound changes in the content of aluminium in aboveground rather than underground parts of plants, especially in maize and yellow lupine (Tables 1–2).

Table 1

Effect of cadmium on aluminium (Al) content in oats and maize [mg per kg of d.m.]

Cd contamination [mg · kg^{-1} of soil]	Oat			Maize	
	grain	straw	roots	aboveground parts	roots
0	32.1	63.3	1385.4	59.9	2821.8
10	25.6	54.8	1474.8	94.5	3100.3
20	23.7	60.9	1330.6	90.4	3488.9
30	28.4	64.7	1506.3	65.6	3280.2
40	26.2	65.5	1569.2	239.7	2962.2
Average	27.2	61.8	1453.3	110.0	3130.7
r	–0.440**	0.527**	0.662**	0.707**	0.278**

r – correlation coefficient; * significant at p = 0.05; ** significant at p = 0.01.

Table 2

Effect of cadmium on aluminium (Al) content in yellow lupine and radish [mg per kg of d.m.]

Cd contamination [mg · kg^{-1} of soil]	Yellow lupine		Radish	
	aboveground parts	roots	aboveground parts	roots
0	205.1	4945.2	295.4	548.3
10	327.6	5404.4	284.6	509.5
20	647.8	5972.2	283.9	424.8
30	939.7	4995.4	273.6	370.4
40	1265.3	5130.7	244.5	418.2
Average	677.1	5289.6	276.4	454.2
r	0.991**	–0.014	–0.917**	–0.869**

r – correlation coefficient; * significant at p = 0.05; ** significant at p = 0.01.

The cadmium rates of 20 mg (maize and yellow lupine roots) and 40 mg Cd · ha^{-1} (aboveground parts of maize and yellow lupine) increased the levels of aluminium in plant tissues. Under these rates of the soil pollutant, the content of aluminium rose by 24 % (r = 0.278) in maize roots, 21 % in yellow lupine roots, 300 % (r = 0.707) in aboveground parts of maize and up to 517 % (r = 0.991) in aboveground parts of yellow lupine. Any further increase in the rates of cadmium introduced to soil depressed the concentration of aluminium in roots of these two crops. Regarding the aboveground parts and roots of radish as well the oats grain, all cadmium-polluted objects revealed depressed aluminium concentrations in plant tissues. Under the highest cadmium rate, the difference varied from 17 % (r = –0.917) in aboveground parts to 24 % (r = –0.869) in roots of radish. The smallest fluctuations in the content of cadmium were demonstrated in oats straw and roots.

The range of effects produced by the neutralising agents on the concentration of aluminium was varied (Table 3).

Table 3

Effect of neutralization substances on aluminium (Al) content in plants [in mg per kg of d.m.]

Cd contamination [mg · kg^{-1} of soil]	Without additions	Compost	Brown coal	Lime	Bentonite	Average
Oats						
Grain	31.1	24.5	26.1	27.1	—	27.2
Straw	69.3	61.3	59.5	57.3	—	61.8
Roots	1622.0	1466.0	1333.7	1391.2	—	1453.3
Maize						
Aboveground parts	55.9	38.4	57.8	228.4	169.6	110.0
Roots	3275.6	2870.5	2898.9	2921.2	3687.4	3130.7
Yellow lupine						
Aboveground parts	1226.4	802.8	394.4	485.3	476.7	677.1
Roots	5266.5	4638.2	4278.9	5279.0	6827.0	5289.6
Radish						
Aboveground parts	410.4	288.5	204.5	288.4	273.4	276.4
Roots	631.9	411.2	376.6	433.7	554.3	454.2

The neutralising agents tended to depress the concentration of aluminium in plants. Brown coal, bentonite and lime caused bigger changes in the content of aluminium in plants than did compost. Brown coal reduced the aluminium level by 68 % in yellow lupine aboveground parts, 50 % in radish aboveground parts, 40 % in radish roots, 19 % in yellow lupine roots, and in oats roots, grain and straw – by 18, 16 and 14 %, respectively. Bentonite depressed the concentration of aluminium by 61 % in aboveground parts of yellow lupine, 33 % in radish aboveground parts and 12 % in the roots of the former crop. The relationships determined for yellow lupine roots and, especially, for aboveground parts of maize were reverse. Also liming caused a contrary effect on aluminium in aboveground parts of maize. Lime and bentonite produced, respectively, a

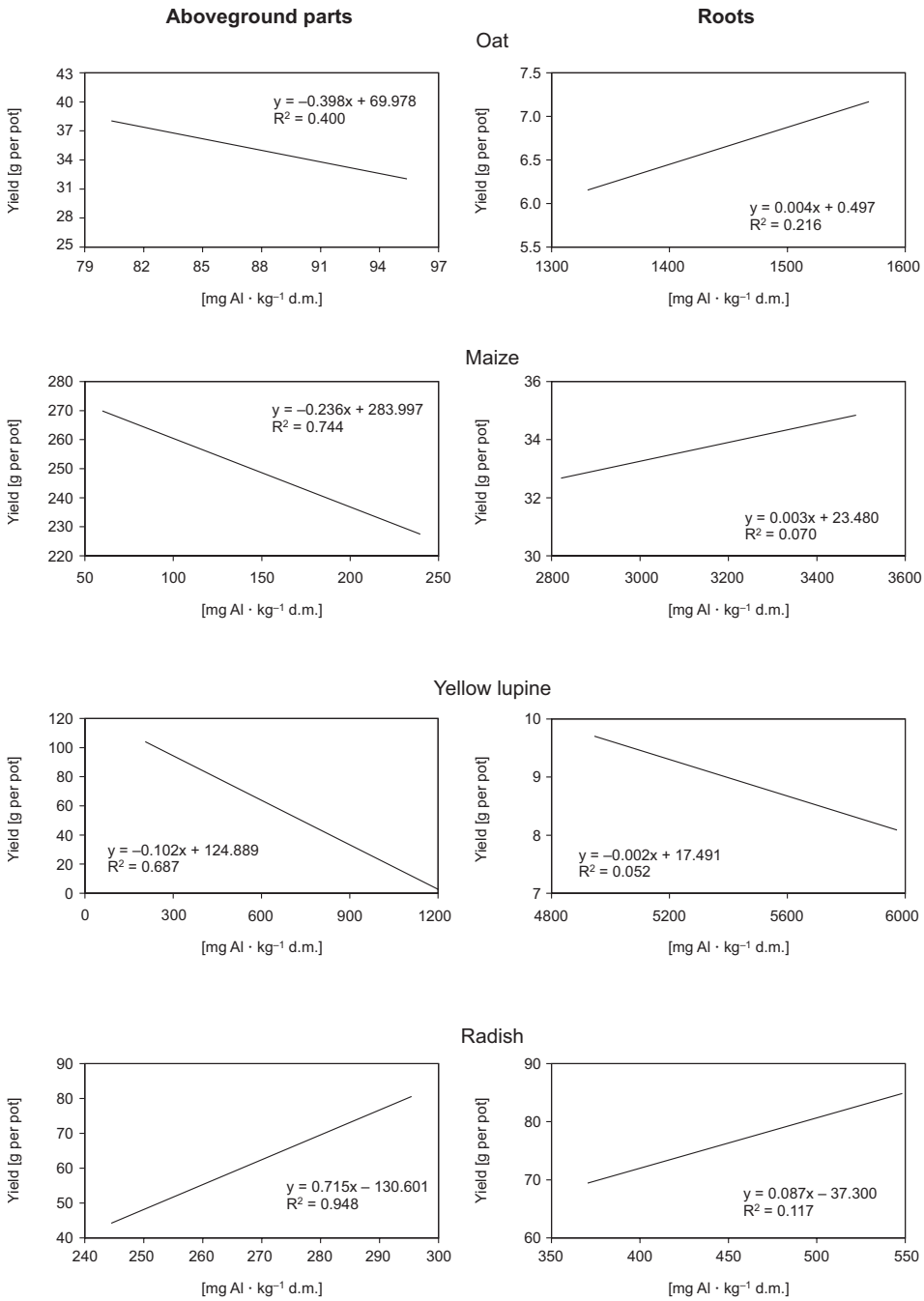

Fig. 1. Relationship between the content of aluminium (Al) and yield of plants

Table 4

Relationships (r) between the aluminium (Al) content versus the yield and the content of macro- and microelements in plants

Al content in plants	Yield	Content in plants																	
		N	P	K	Mg	Ca	Na	S	B	Mo	Mn	Fe	Co	Li	Cd	Pb	Cu	Ni	Zn
Oat grain	–0.42**	0.21	–0.06	0.72**	–0.27	0.13	0.22	–0.48**	–0.24	0.20	0.60**	0.34*	0.47**	0.57**	0.13	0.50**	0.12	–0.06	0.08
Oat straw	–0.28	–0.16	0.20	0.26	–0.07	–0.18	0.30	–0.24	–0.25	0.05	0.51**	0.65**	0.61**	0.30	0.53**	0.20	0.40**	0.53**	0.36*
Oat roots	0.46**	–0.17	0.06	–0.23	–0.40**	–0.22	–0.37*	–0.48**	–0.62**	–0.23	0.64**	0.84**	0.70**	0.96**	0.11	0.46**	0.51**	0.27	0.55**
Maize above-ground parts	–0.45**	–0.13	–0.36**	0.09	–0.22	–0.14	0.14	–0.06	–0.30*	–0.32	0.64**	0.00	0.10	0.13	–0.12	–0.29*	0.01	–0.05	–0.45**
Maize roots	0.05	–0.14	–0.25	–0.15	–0.15	–0.25	0.03	–0.18	0.21	0.87**	0.83**	0.82**	0.89**	–0.10	0.58**	0.21	0.30*	0.14	0.05
Yellow lupine aboveground parts	–0.59**	0.09	0.58**	–0.11	0.24	0.43**	–0.13	0.53**	–0.08	0.11	0.17	0.97**	0.93**	0.85**	0.89**	0.80**	0.18	0.96**	0.55**
Yellow lupine roots	0.36**	–0.95**	–0.84**	–0.90**	–0.50**	0.01	0.44**	–0.61**	–0.67**	–0.00	0.91**	0.97**	0.94**	0.99**	–0.47**	0.26	0.54**	–0.27	–0.74**
Radish above-ground parts	0.12	–0.13	–0.17	0.08	–0.58**	0.09	0.08	–0.43**	–0.31*	–0.21	0.18	0.89**	0.44**	0.47**	0.40**	0.04	0.22	–0.04	0.33*
Radish roots	0.44**	–0.47**	0.22	–0.61**	–0.38**	0.50**	0.62**	–0.17	–0.57**	–0.29*	0.04	0.74**	0.18	0.74**	–0.39**	–0.06	0.06	0.26	–0.21

* significant at p = 0.05; ** significant at p = 0.01.

four-fold and three-fold increase in aluminium found in aboveground parts of maize. Liming was also responsible for a 60 % decline in the aluminium concentration in yellow lupine aboveground parts, 30–31 % decrease in aboveground and underground parts of radish and 13–17 % drops in all parts of oats. The influence of compost on the concentration of aluminium in all the crops, and particularly in aboveground parts of yellow lupine and in aboveground and underground parts of radish, was significantly negative.

The regression curves and equations derived from the data suggest that the concentration of aluminium in plant tissues was correlated with the plant yields. Most of the test plants showed negative correlations between these two factors for the aboveground parts (except radish) and positive correlations for the root tissues (except yellow lupine) (Fig. 1). As regards the aboveground parts of radish and roots of yellow lupine, contrary correlations were observed. The concentration of aluminium in particular plant organs was correlated with many macro- and micronutrients, with the correlations being stronger in roots than in aboveground parts. Besides, the correlations involving mircoelements were stronger than those with macroelements (Table 4). Significant correlations were the least frequent in the case of maize. The content of aluminium was most often positively correlated with the content of manganese, iron, cobalt, lithium, cadmium, copper and zinc. Most negative correlations were determined between aluminium and sulphur or boron.

Discussion

The literature has very few references on the above issues. Guo Tian Rong et al [9] reported higher levels of aluminium in barley caused by cadmium. By introducing various substances to soil, concentrations of aluminium in plants can be modified. Alfaro et al [10] demonstrated that liming can depress the content of aluminium in grasses and legumes with a wide range of differences (from 35 to 61 %). Mongia et al [11] proved analogous relationships in rice grain and straw. Wallace [12] studied such interactions in maize; Patriam-Rai et al [13] investigated beans, Hahn and Marschner [14] analysed aluminium content in spruce roots and, finally, Kiepul [15] studied a variety of plant species in this respect.

Conclusions

1. Concentration of aluminium in plants depends not only on the plant species, type of plant organs but, to a large extent, on cadmium soil pollution (rates of the pollutants) and type of a neutralising agent introduced to the soil. Roots of the test plants contained much more aluminium than their aboveground parts did. The highest levels of aluminium were determined in yellow lupine and maize roots whereas the smallest amounts of this metal were discovered in oats grain and straw.

2. Contamination of soil with cadmium caused bigger changes in the concentration of aluminium in aboveground parts than in roots of the test plants, especially in maize and yellow lupine. Cadmium applied at 20 mg (maize and yellow lupine roots) or 40 mg

Cd · ha^{-1} (aboveground parts of maize and yellow lupine) caused increased concentrations of aluminium. Any further increase in the cadmium rates resulted in depressed amounts of aluminium in roots of these two crops. Concerning the aboveground parts and roots of radish as well as oats grain, the content of aluminium was found to have been depressed as a consequence of cadmium pollution of the soil.

3. The range of effects produced by the test neutralising agents on the concentrations of aluminium was varied. The neutralising agents tended to depress the content of aluminium in plants. Brown coal, bentonite and lime caused larger changes in the levels of aluminium in plant tissues than compost.

4. The content of aluminium was correlated with yields of the crops. In most of the test plants, we noticed negative correlations for the aboveground parts (except radish) and positive ones for roots (except yellow lupine). The concentration of aluminium in particular plant organs was correlated with many micro- and macroelements. These correlations were predominantly positive in the case of manganese, iron, cobalt, lithium, copper and zinc, but negative for the concentrations of sulphur and boron.

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ZAWARTOŚĆ GLINU W ROŚLINACH W ZALEŻNOŚCI OD ZANIECZYSZCZENIA GLEBY KADMEM

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Abstrakt: Celem badań było określenie wpływu zanieczyszczenia gleby kadmem (10, 20, 30 i 40 mg Cd · kg^{-1} gleby) na zawartość glinu w częściach nadziemnych i korzeniach owsa, kukurydzy, łubinu żółtego i rzodkiewki. Do neutralizacji kadmu wprowadzonego do gleby zastosowano: kompost, węgiel brunatny, wapno i bentonit. Na zawartość glinu w roślinach, oprócz gatunku i organu roślin, duży wpływ miała dawka kadmu, jak również rodzaj zaaplikowanej substancji neutralizującej. Korzenie zawierały zdecydowanie więcej glinu niż części nadziemne roślin. Najwięcej glinu stwierdzono w korzeniach łubinu żółtego i kukurydzy, a najmniej w ziarnie i słomie owsa. Zanieczyszczenie gleby kadmem spowodowało większe zmiany w zawartości glinu w częściach nadziemnych niż w korzeniach roślin, szczególnie w przypadku kukurydzy i łubinu żółtego. Dawki kadmu w wysokości 20 mg (korzenie kukurydzy i łubinu żółtego) lub 40 mg Cd · kg^{-1}

gleby (części nadziemne kukurydzy i łubinu żółtego) powodowały zwiększenie zawartości glinu. Dalszy wzrost dawek kadmu wywoływał zmniejszenie zawartości glinu w korzeniach tych roślin. W przypadku części nadziemnych i korzeni rzodkiewki oraz ziarna owsa odnotowano zmniejszenie zawartości glinu we wszystkich obiektach zanieczyszczonych kadmem. Zakres oddziaływania testowanych substancji neutralizujących na zawartość glinu był zróżnicowany. Dodatki neutralizujące na ogół obniżały zawartość glinu w roślinach. Węgiel brunatny, bentonit i wapno wywoływały większe zmiany w zawartości glinu w roślinach niż kompost. Zawartość glinu wykazywała skorelowanie z plonem roślin. U większości roślin wystąpiły relacje ujemne w częściach nadziemnych (oprócz rzodkiewki), a dodatnie w korzeniach (z wyjątkiem łubinu żółtego). Zawartość glinu w poszczególnych organach roślin była skorelowana z licznymi makro- i mikroelementami, w tym przeważnie dodatnio z zawartością manganu, żelaza, kobaltu, litu, kadmu, miedzi i cynku, a ujemnie z zawartością siarki i boru.

Słowa kluczowe: zanieczyszczenie kadmem, kompost, węgiel brunatny, wapno, bentonit, rośliny, zawartość glinu

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EFFECT OF SOIL CONTAMINATION WITH A MIXTURE OF HEAVY METALS ON BROAD BEAN (*Vicia faba* L.) SEED QUALITY

ODDZIAŁYWANIE SKAŻENIA GLEBY MIESZANINĄ METALI CIĘŻKICH NA JAKOŚĆ NASION BOBU (*Vicia faba* L.)

Abstract: The work aimed at an assessment of soil contamination with mixtures of heavy metals with zinc and nickel on two levels of pollution on the broad bean seed yield, degrees of injuries caused by broad bean beetle, and the germination energy and ability. The assessment of germination energy and ability of broad bean seeds was tested in laboratory, according to generally used standards. Soil contamination with mixtures of zinc and nickel with cadmium, copper and lead on III level of pollution acc. to the IUNG classification led to a significant decline in broad bean seed yield or its total loss, but the decrease in yield was lower than when the soil was contaminated by zinc or nickel used separately. Soil contamination with mixtures of zinc with nickel, zinc with copper and zinc with lead and nickel with copper on II level of pollution in the IUNG classification also caused a notable decrease in broad bean seed yield. Soil pollution with the tested mixtures of heavy metals did not affect the degree of seed injuries due to *Bruchus rufimanus* or their germination energy. Soil contamination with a mixture of zinc and nickel in II class of pollution in the IUNG classification leads to a worsening of the seed quality (the percentage of dead seeds increased and the condition of obtained seedlings worsened). The above-mentioned features were also negatively affected by the soil pollution with a mixture of nickel and cadmium on III level of pollution in the IUNG classification.

Keywords: heavy metals, soil pollution, *Bruchus rufimanus* Boh.

One of the ways of agricultural management of heavy metal polluted soils is their destination for seed crops. Soil concentrations of heavy metals such as zinc and nickel on the level of medium pollution in the IUNG classification [1] cause considerable decline in or total loss of broad bean seed yield. On the other hand, copper, lead and cadmium do not adversely affect the quantity or quality of broad bean seed yield and in the case of seeds injured by broad bean beetle (*Bruchus rufimanus* Boh.) even stimulate their germination ability. Soils contaminated with these elements may be thus designed

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for seed plantation of this crop [2]. A similar beneficial effect of lead and cadmium on broad bean plants was observed in the case when the soil pollution level was equal to I class of pollution in the IUNG classification [3]. Heavy metals which occur in soil jointly often have a different effect on a plant than when present separately [4]. Because among the five heavy metals (Pb, Cd, Cu, Zn and Ni) studied so far and applied separately, zinc and nickel revealed the strongest (negative) effect both on broad bean plant growth and the degree of injuries due to pests, it seems justified to study the effect of soil contamination with mixtures of these two heavy metals with cadmium, lead and copper regarding a possible reduction of this negative effect.

The work aimed at an assessment of soil contamination with mixtures of heavy metals with zinc and nickel on two levels of pollution on the broad bean seed yield, degrees of injuries caused by broad bean beetle, and the germination energy and ability.

Material and methods

Broad bean, White Windsor c.v. was cultivated in a control soil with natural heavy metal concentrations without (Control) and with mineral treatment (Control + NPK) and in the soil contaminated with the following mixtures of heavy metals:

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

The level of soil contamination corresponded to II and III class of pollution acc. to the classification suggested by IUNG in Pulawy [1]. The plants were cultivated in plastic pots with 9.8 kg d.m. of soil under field conditions. Detailed description of the methods of heavy metal supply into the soil was presented in another publication [5]. The experiment was conducted in 2008 on degraded chernozem developed from loess with acid reaction (pH in 1 mol · dm^{-3} KCl solution was 5.5 and in water 6.3) and organic carbon content 1.13 %. Harmfulness of broad bean beetle was estimated on the basis on injured seeds in relation to seed total mass. The assessment of germination energy and ability of broad bean seeds was tested in laboratory, according to generally used standards. The test was conducted in Petri dishes on filter paper as a medium. Germination energy was assessed after 4 days and germination ability after 14 days.

The significance of differences between the means were tested by means of an one-way ANOVA. Means were differentiated using the Duncan test on the significance level $p < 0.05$.

Results and discussion

Soil contamination with mixtures of most of the analysed heavy metals with zinc on a lower level of pollution caused a marked decline in the number of pods and seed weight per plant in comparison with the minerally fertilized control (Table 1). Only the mixture of zinc and cadmium did not significantly affect these features. The soil contamination with a mixture of zinc and lead most strongly reduced the number of developed pods and seeds. In comparison with the mixtures of zinc with other metals, the soil contamination with nickel mixtures with copper, cadmium and lead on the II pollution level limited pod and seed formation by broad bean to a smaller degree. Contamination of soil with a mixture of zinc and nickel and zinc and cadmium on a higher level of pollution weakened broad bean plant growth so that they were unable to form seeds. Contamination with the other analysed heavy metals on a higher level significantly decreased the number of formed pods and seeds in comparison with the minerally fertilized control. The effect was stronger than in soil contaminated with the mixtures on II pollution level. In the Author's former research, as a result of soil contamination only with zinc on III level of pollution the plants did not form seeds [2, 3, 5–7].

Table 1

Characteristics of broad bean seeds from plants cultivated in natural soil and in heavy metal contaminated soil, and degree of injuries due to *Bruchus rufimanus* Boh.

Object	Average number of pods perplant [percent in relation to Control + NPK]	Average seed weight per plant [percent in relation to Control + NPK]	Weight of seeds injured by *Bruchus rufimanus* Boh. [percent in relation to Control + NPK]
ZnII + CuII	25.00 abcd*	23.05 a	33.40 a
ZnII + NiII	25.00 abcd	15.84 a	40.63ab
ZnII + CdII	62.50 def	50.15 abc	47.13 ab
ZnII + PbII	16.67 ab	6.68 a	36.58 ab
NiII + CuII	54.17 cde	33.00 ab	72.05 ab
NiII + PbII	66.67 def	49.89 abc	61.13 ab
NiII + CdII	58.33 de	48.24 abc	108.53 b
NiIII + CdIII	15.63 abc	1.94 a	80.09 ab
NiIII + PbIII	25.00 abcd	18.34 a	69.05ab
ZnIII + PbIII	8.33 a	1.35 a	60.72 ab
ZnIII + CuIII	37.50 abcde	17.81 a	109.73 b
Control + NPK	100.00 f	100.00 c	100.00 ab
Control	76.04 ef	91.07 bc	93.38 ab

* Values for individual metals or control marked by different letters in columns are statistically different ($p < 0.05$).

However, nickel present in soil usually caused a marked decrease in the number and weight of formed seeds [2, 3] or prevented seeds being formed by broad bean [5,6]. Despite quite considerable diversification between the analysed objects as to the degree of seed injuring by broad bean beetle, statistical analysis did not reveal any significant differences between the objects where the soil was contaminated with heavy metals and the control soil. In the former research soil contamination with nickel on III level of pollution led to a decrease in the degree of seed injuries by broad bean beetle. Decreased seed attractiveness for this pest was also observed under conditions of soils contaminated with copper and lead [2]. *Bruchus* beetles proved less harmful for the seeds of plants cultivated under conditions of soils contaminated with single heavy metals on I level of pollution in comparison with the minerally fertilized control soil [3]. Also in the presented investigations on most contaminated soils the percentage of seeds injured by broad bean beetle was lower than in the minerally fertilized control. On the other hand, in the research where the above-mentioned metals were applied jointly on the level of elevated content [8] no significant effect was registered on the degree of seed injuring by *Bruchus* larvae. Some *Bruchidae* during larval development absorb and accumulate zinc and copper, whereas calcium, magnesium, iron or manganese are mostly excreted [9]. On the other hand, such metals as cadmium or mercury reveal an inhibitory effect on some enzyme activity in larvae of *Acanthoscelides obtectus* Say [10].

Too few seeds were collected from the objects with the soil contaminated by the mixture of lead and copper with zinc on III level of pollution to test the germination energy and ability. The other analyzed objects did not differ significantly with respect to germination energy (Fig. 1). Between 0 % and 41 % of seeds germinated 4 days after the test start, the most numerous in conditions of the soil contaminated with a mixture of nickel and lead on a higher level. On the other hand, in the object contaminated with

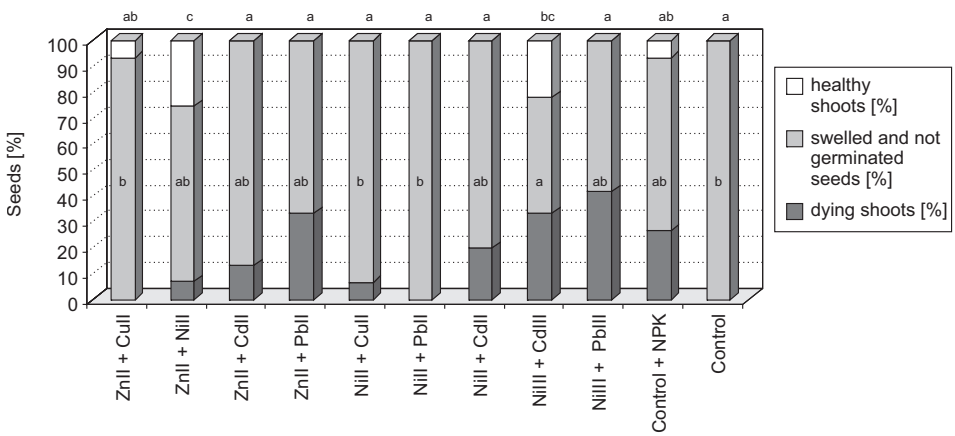

Fig. 1. Germination energy of broad bean seeds originating from plants growing in natural and heavy metal contaminated soil. Values for individual metals or control and for individual features, marked by different letters are statistically different ($p < 0.05$). Assessments were presented only if there was statistical differentiation between objects. In other cases differences were statistically insignificant

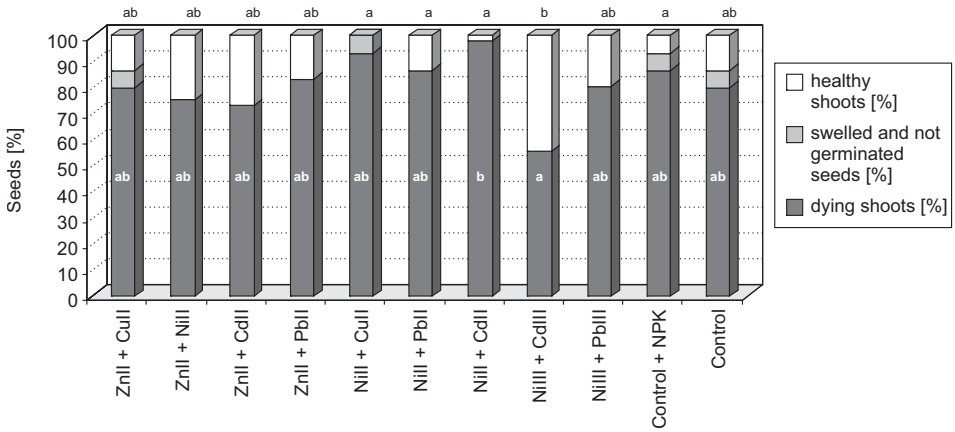

Fig. 2. Germination ability of broad bean seeds originating from plants growing in natural and heavy metal contaminated soil. Values for individual metals or control and for individual features, marked by different letters are statistically different ($p < 0.05$). Assessments were presented only if there was statistical differentiation between objects. In other cases differences were statistically insignificant.

zinc and nickel on II pollution level significantly more dead seeds were found than in the conditions of the control soil fertilized minerally.

Quite a few dead seeds were found also on the object with the soil contaminated with a mixture of nickel and cadmium on a higher level of pollution. On the latter object the share of dead seeds increased during the testing period (Fig. 2). Seeds from this object were characterized by the lowest germination ability. Also the number of lateral roots obtained from seedlings was low (Table 2).

Table 2

Characteristics of germinating broad bean seeds from plants cultivated in natural soil and in heavy metal contaminated soil.

Objects	Shoot length	Underground part length	Number of lateral roots 2 mm
ZnII + CuII	1.700 ab*	6.000 a	8.100 ab
ZnII + NiII	0.872 a	4.561 a	3.556 a
ZnII + CdII	2.194 ab	4.208 a	5.972 ab
ZnII + PbII	1.333 ab	4.417 a	8.417 ab
NiII + CuII	1.170 ab	6.375 a	8.183 ab
NiII + PbII	1.767 ab	6.583 a	9.650 ab
NiII + CdII	2.200 ab	5.733 a	11.667 b
NiIII + CdIII	4.333 b	4.333 a	6.500 ab
NiIII + PbIII	1.611 ab	4.861 a	11.806 b
Control + NPK	2.458 ab	4.925 a	11.167 b
Control	0.533 a	4.533 a	6.033 ab

* Values for individual metals or control marked by different letters in columns are statistically different ($p < 0.05$).

Quite a high number of dead seeds was also registered under conditions of soil contaminated with mixtures of zinc and nickel and zinc with cadmium on a lower level. The number of lateral roots in seedlings obtained from the seeds on the object contaminated with zinc and nickel on II level of pollution was significantly lower than in seedlings from seeds originating from the control plants receiving mineral fertilizers. On the other hand no apparent differences were found in the germination ability and condition of the obtained seedlings between the seeds from plants cultivated in the soil contaminated with mixtures of zinc with copper and lead and nickel with copper, lead and cadmium on II level of pollution and also nickel with lead on III level of pollution, and the seeds from the control plants fertilized minerally. In the Author's former investigations, soil contamination with cadmium, lead and copper applied separately on III level of pollution did not affect negatively the germination ability of broad bean seeds [2]. Soil contamination with single heavy metals (Cd, Pb, Cu, Zn, Ni) on I level of pollution acc. to the IUNG classification did not influence negatively germination energy or ability. Some of the tested metals (lead and cadmium) even revealed a positive effect on the number of formed seeds and their germination ability [3].

Conclusions

1. Soil contamination with mixtures of zinc and nickel with cadmium, copper and lead on III level of pollution acc. to the IUNG classification led to a significant decline in broad bean seed yield or its total loss, but the decrease in yield was lower than when the soil was contaminated by zinc or nickel used separately.

2. Soil contamination with mixtures of zinc with nickel, zinc with copper and zinc with lead and nickel with copper on II level of pollution in the IUNG classification also caused a notable decrease in broad bean seed yield.

3. Soil pollution with the tested mixtures of heavy metals did not affect the degree of seed injuries due to *Bruchus rufimanus* or their germination energy.

4. Soil contamination with a mixture of zinc and nickel in II class of pollution in the IUNG classification leads to a worsening of the seed quality (the percentage of dead seeds increased and the condition of obtained seedlings worsened). The above-mentioned features were also negatively affected by the soil pollution with a mixture of nickel and cadmium on III level of pollution in the IUNG classification.

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ODDZIAŁYWANIE SKAŻENIA GLEBY MIESZANINĄ METALI CIĘŻKICH NA JAKOŚĆ NASION BOBU (*Vicia faba* L.)

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Abstrakt: Celem pracy było określenie wpływu skażenia gleby mieszaninami metali ciężkich (kadmu, miedzi i ołowiu) z cynkiem i niklem na dwóch poziomach zanieczyszczenia (wg II i III klasy zanieczyszczenia zgodnie z klasyfikacją IUNG) na plon nasion bobu, stopień ich uszkodzenia przez strąkowca bobowego oraz energię i zdolność kiełkowania. Szkodliwość strąkowca bobowego oceniono na podstawie masy nasion uszkodzonych w stosunku do ogólnej masy nasion. Ocenę energii i zdolności kiełkowania nasion bobu przeprowadzono w warunkach laboratoryjnych, zgodnie z ogólnie przyjętymi normami. Skażenie gleby mieszaninami cynku i niklu z kadmem, miedzią i ołowiem na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG prowadzi do znacznego spadku plonu nasion bobu lub całkowitej jego utraty. Skażenie gleby mieszaninami cynku z niklem, cynku z miedzią i cynku z ołowiem oraz niklu z miedzią na poziomie II stopnia zanieczyszczenia wg klasyfikacji IUNG prowadzi także do znacznego spadku plonu nasion bobu. Skażenie gleby badanymi mieszaninami metali ciężkich nie wpływa na stopień uszkodzenia nasion przez strąkowca bobowego oraz ich energię kiełkowania. Skażenie gleby mieszaniną cynku i niklu na poziomie II klasy zanieczyszczenia zgodnie z klasyfikacją IUNG prowadzi do pogorszenia jakości nasion (wzrasta odsetek nasion martwych, pogarsza się kondycja uzyskanych siewek). Niekorzystnie na ww. cechy oddziałuje także skażenie gleby mieszaniną niklu i kadmu na poziomie III klasy zanieczyszczenia wg klasyfikacji IUNG.

Słowa kluczowe: metale ciężkie, zanieczyszczenie gleby, *Bruchus rufimanus* Boh.

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IMPACT OF TEMPERATURE ON VISCOSITY OF SEWAGE SLUDGE AFTER CONDITIONING

WPŁYW TEMPERATURY NA LEPKOŚĆ KONDYCJONOWANYCH OSADÓW ŚCIEKOWYCH

Abstract: The technology of wastewater treatment is always associated with utilization of sewage sludge. The process of sewage sludge utilization aims at transforming (biologically, physically or chemically) sewage sludge into a state which does not pose any threats for human health and natural environment. Conditioning – a process which has an impact on the structure and properties of sewage sludge – allows for more efficient removal of water from sewage sludge. Also, temperature is a crucial parameter in the final stage of utilization and transportation of sewage sludge.

The paper presents the results of the investigations on viscosity of chemically conditioned sewage sludge exposed to selected temperatures and variable sheer velocity gradients. Municipal sewage sludge and sludge from cellulose industry were used as substrates. The impact of temperature on viscosity of fermented sewage sludge and also the impact of a polyelectrolyte dose and a velocity gradient on sewage sludge viscosity were investigated. Viscosity of sewages sludge was determined in the temperature range of 20 to 36 ^{o}C for every 2 ^{o}C at variable sheer velocity gradients (60, 100, 200 r/min). The HAAKE Viscotester 7L/R plus and the Termostat DC 10 bathtube were used in the investigations.

Keywords: sewage sludge, conditioning, viscosity, temperature

The sewage treatment technology is closely associated with the disposal of sewage sludge. This process involves the transformation of sewage sludge (by biological, physical or chemical methods), which is intended to bring the sludge to a state that does not pose any hazards to the life or health of population or to the natural environment [1].

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Until recently, the sewage sludge treatment process went unnoticed. Unfortunately, the sewage sludge management in sewage treatment plants continues to be a topical and not completely resolved problem. Failures in this field bring about serious consequences and may be a source of risk to the natural environment of man. The construction of new sewage treatment plants and the intensification of treatment processes is accompanied also by the increase in the amount of sewage sludge which accounts for 1–2 % of the total volume of sewage flowing to the sewage treatment plant. Of the treatment plant's overall costs, the costs of the construction and operation of sewage sludge treatment equipment can account for as much as 50 %. By treating sewage sludge as a raw material of some fertilizer or energy value, at least a partial return on the incurred outlays could be obtained [2, 3].

The neutralization of sewage sludge in treatment plants involves the removal of water that it contains, whereby a reduction of the sewage sludge volume is achieved. The process whereby this effect is achieved is conditioning, that is one of the processes which have the effect of changing the structure and properties of sewage sludge, enabling a more efficient removal of water contained in the sewage sludge. Change in the form of sewage sludge is the change of its structure, which also influences its viscosity [4, 5]. For the majority of sewage sludges, physical and chemical conditioning processes are used, which can run either under natural conditions or with the use of mechanical devices. The selection of the sewage sludge conditioning method is most often influenced by the possibility of subsequent use and utilization of the sewage sludge, as the conditioning process determines the final products formed [6, 7]. One of the conditioning methods is by changing the temperature. The temperature is one of the important parameters in the final stage of neutralizing and transporting sewage sludge (rheological parameters) [8, 9].

The purpose of the studies undertaken was to determine the viscosity of chemically conditioned sewage sludges subjected to the action of temperature and variable magnitudes of the shearing velocity gradient.

Experimental

The substrate of the tests was the sludge from municipal wastewater treatment plant after aerobic stabilization process. Its sewage treatment capacity was 1000 m^3/d. The characteristic of sludge was as follow: dry matter 18.38 g/dm^3, initial hydration 97.3 %, *capillary suction time* (CST) was 154 s, resistivity $21.4 \cdot 10^{12}$ m/kg and viscosity was equal 132 mPa · s. Sludge was conditioned with low and high cationic polyelectrolytes. The capillary suction time was used to determine polyelectrolyte dose. Viscosity of sludge was measured in the range of temperatures from 20 to 36 ^{o}C with the step of 2 ^{o}C (20, 22, 24, 26, 28, 30, 32, 34, 36), for the variable shear velocity gradient (60, 100 and 200 rpm) and for different polyelectrolytes doses. The viscosity was measured with HAAKE Viscotester 7L/R plus – rotational viscosimeter designed to perform the fast determination of viscosity in accordance with the ISO 2555 standards (Fig. 1). To maintain the required temperature thermostat DC10 was used.

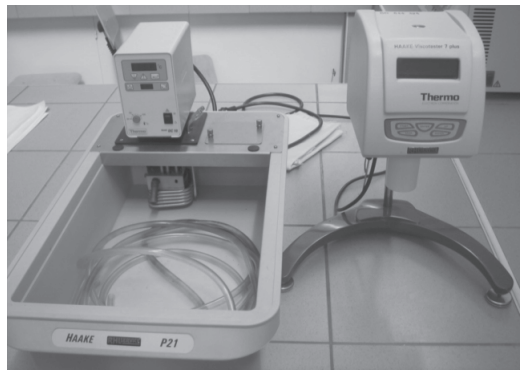

Fig. 1. Viscosity measurement set

Analysis of investigation results

The effect of temperature on the change in the viscosity of sewage sludges examined

By analyzing the curves of the viscosity of sewage sludges conditioned with polyelectrolytes versus temperature it was found that the sewage sludge viscosity decreased with increasing temperature. Already at a slight temperature increase by 2 ^{o}C, a drop in viscosity was noted. Variations in viscosity with increasing sewage sludge temperature at different shearing velocities (60, 100, 200 rpm) are illustrated in Figures 2 to 4. Similar relationships can be observed for sewage sludges conditioned with a weak-cationic polyelectrolyte (Praestol 650BC).

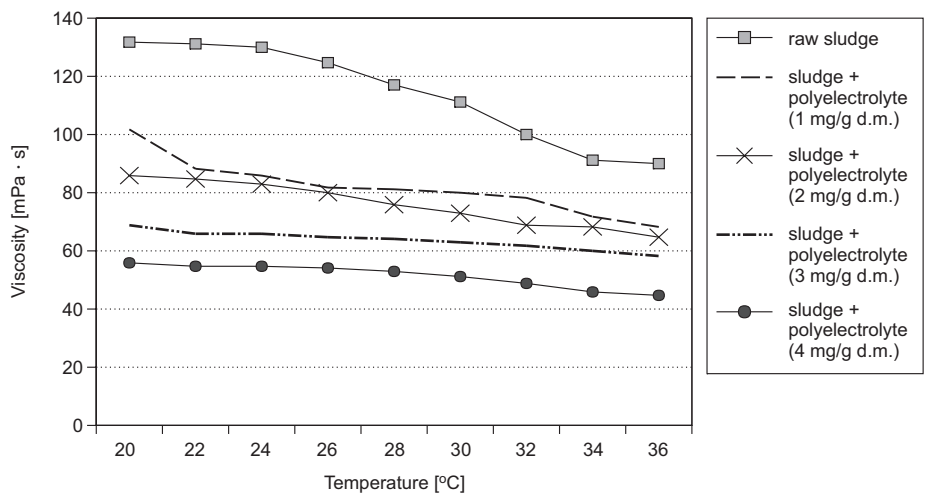

Fig. 2. Influence of temperature on the viscosity of the sludge conditioned with different doses of polyelectrolyte Praestol 658BC-S (rotational speed 60 rpm)

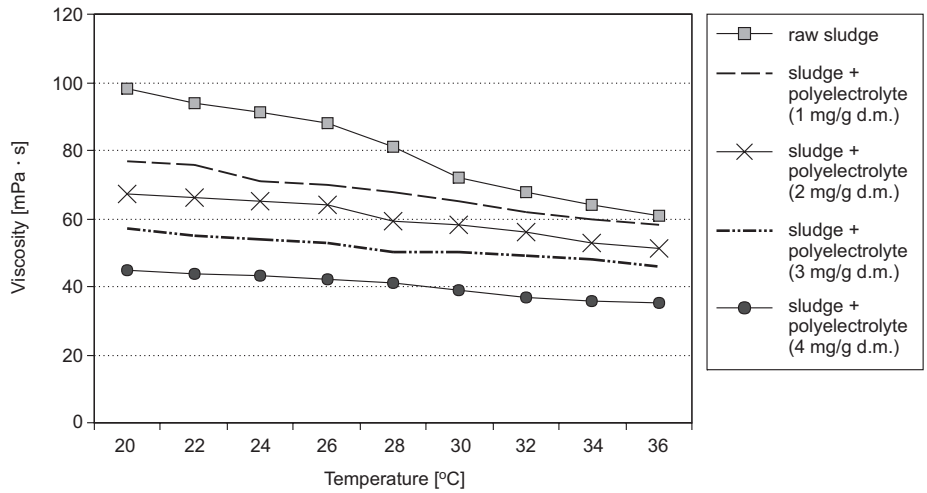

Fig. 3. Influence of temperature on the viscosity of the sludge conditioned with different doses of polyelectrolyte Praestol 658BC-S (rotational speed 100 rpm)

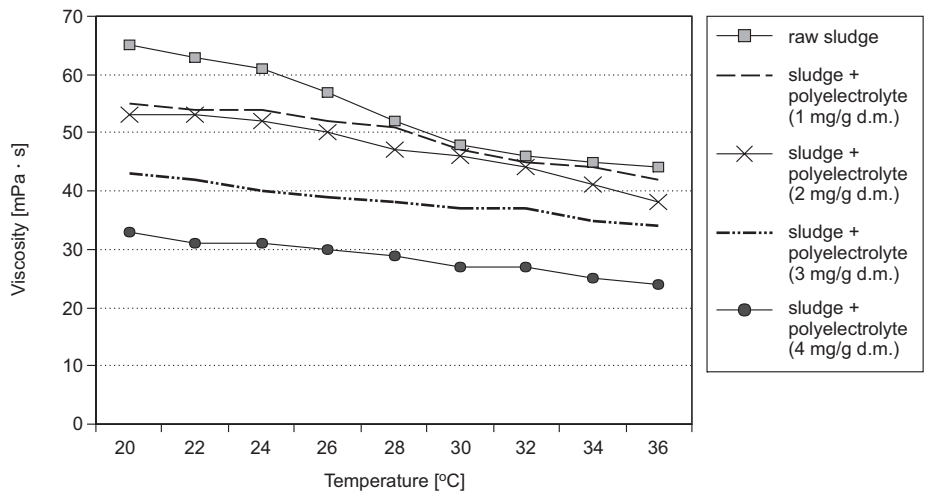

Fig. 4. Influence of temperature on the viscosity of the sludge conditioned with different doses of polyelectrolyte Praestol 658BC-S (rotational speed 200 rpm)

The effect of the dose of polyelectrolyte on the change in the viscosity of sewage sludges examined

From the analysis of the graphs showing the effect of the polyelectrolyte dose on sewage sludge viscosity it was found that the increase in the polyelectrolyte dose caused a decrease in sewage sludge viscosity (Figs. 5–7). With increasing polyelectrolyte dose, the sewage sludge viscosity decreased, reaching minimum values at the highest doses applied.

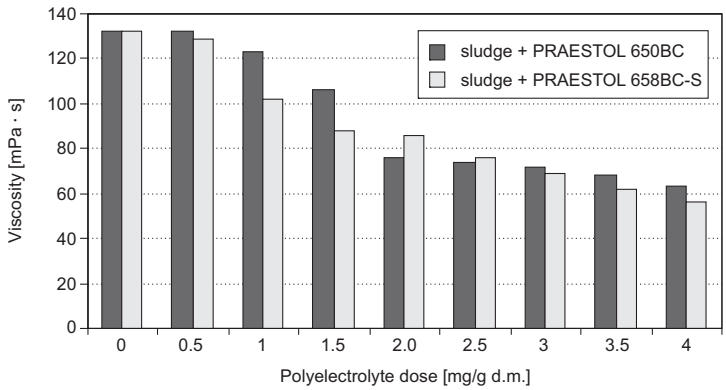

Fig. 5. Influence of polyelectrolytes dose on the viscosity of tested sludge (rotational speed 60 rpm; temperature 20 °C)

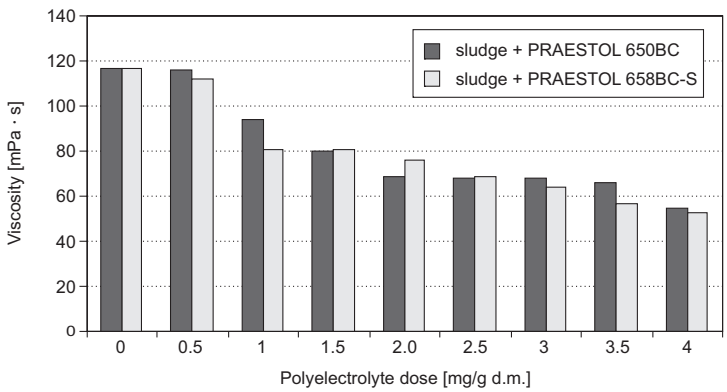

Fig. 6. Influence of polyelectrolytes dose on the viscosity of tested sludge (rotational speed 60 rpm; temperature 28 °C)

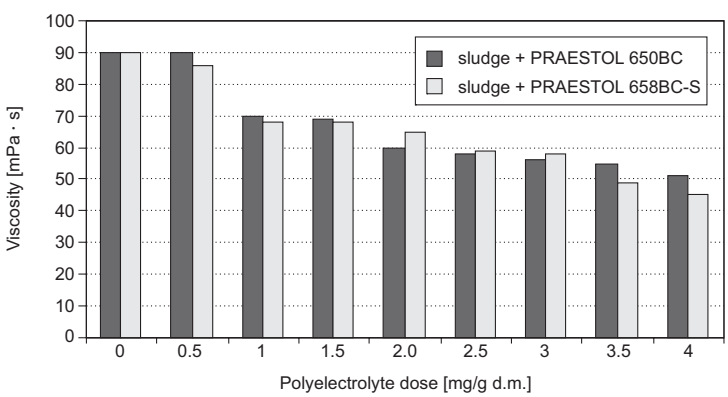

Fig. 7. Influence of polyelectrolytes dose on the viscosity of tested sludge (rotational speed 60 rpm; temperature 36 °C)

The effect of the velocity gradient on the change in the viscosity of sewage sludges examined

By analyzing the effect of the gradient of velocity on the change in the viscosity of sewage sludge it was found that the increase in shearing velocity was accompanied by a decrease in viscosity (Fig. 8). The viscosity curves show a drop in viscosity against the increase in shearing velocity. The decrease in viscosity with the increase in shearing velocity was noted for the entire of temperature examined (20–36 ^{o}C).

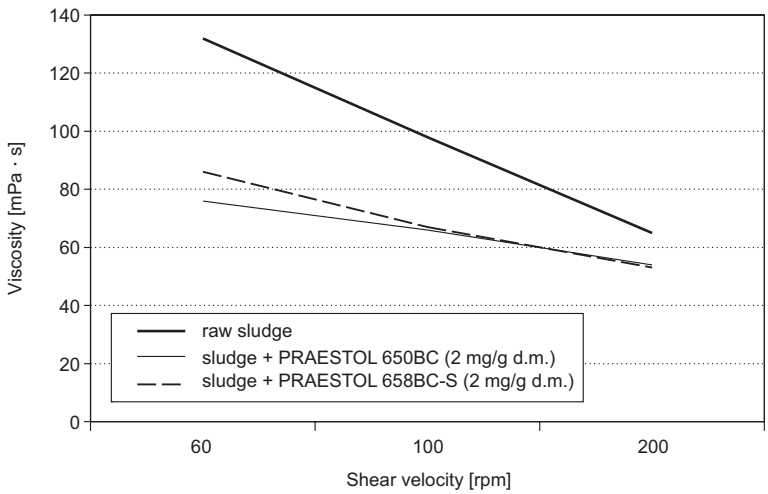

Fig. 8. Influence of shear velocity gradient on the viscosity of tested sludge in the temperature 20 ^{o}C

Conclusions

From the investigation carried out it was found that the viscosity of sewage sludge decreased with increasing temperature. Already at a slight temperature increase by 2 ^{o}C, a drop in the viscosity of sludges examined was noted.

By analyzing the effect of polyelectrolyte doses on the sewage sludge viscosity it was determined that the increase in the polyelectrolyte dose results in a decrease in sewage sludge viscosity. With the increase in the dose of polyelectrolytes used, the viscosity decreased, reaching minimum values at the highest doses applied. In most cases, introducing doses of a strong-cationic polyelectrolyte, Praestol 658BC-S, caused greater viscosity decreases compared with the weak-cationic polyelectrolyte Praestol 650BC with the identical doses. The viscosity of sludge treated with the strong-cationic polyelectrolyte Praestol 658BC-S at a temperature of 20 ^{o}C and a rotational speed of 100 rpm was 98 mPa · s, while with the highest doses of this polyelectrolyte and under the identical temperature and rotational speed conditions it was 45 mPa · s.

The investigation carried out indicates that the increase in shear velocity is accompanied by a decrease in the viscosity of sewage sludges examined. This applies to

untreated sludge, as well as to sludge conditioned with different doses of polyelectrolyte. The values on the viscosity curves decrease from the first examined velocity of 60 rpm, then consecutively increased to 100 rpm and 200 rpm, against the increase in shear velocity.

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WPŁYW TEMPERATURY NA LEPKOŚĆ KONDYCJONOWANYCH OSADÓW ŚCIEKOWYCH

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Abstrakt: Oczyszczanie ścieków jest ściśle związane z problemem unieszkodliwiania osadów ściekowych. Proces unieszkodliwiania jest przekształceniem, które ma doprowadzić osady do stanu, który nie stwarza zagrożeń dla życia lub zdrowia ludności oraz dla środowiska. Kondycjonowanie, czyli jeden z procesów mający wpływ na zmianę struktury i właściwości osadów, pozwala na bardziej skuteczne usuwanie zawartej w osadach wody. Również temperatura jest ważnym parametrem w końcowym etapie unieszkodliwiania i transportu osadów ściekowych. W artykule przedstawiono wyniki badań dotyczące wyznaczenia lepkości kondycjonowanych chemicznie osadów ściekowych, poddanych działaniu temperatury oraz zmiennym wartościom gradientu prędkości ścinania. Przeprowadzono badania komunalnych osadów ściekowych oraz osadów z przemysłu celulozowego. Ocenie poddano zarówno wpływ temperatury na lepkość przefermentowanych osadów ściekowych, jak i wpływ dawki polielektrolitów oraz gradientu prędkości na lepkość badanych osadów ściekowych. Lepkość osadów została wyznaczona w zakresie temperatur od 20 do 36 ^{o}C, co 2 ^{o}C, przy zmiennym gradiencie prędkości ścinania (60, 100, 200 obr/min). W badaniach wykorzystano wiskozymetr HAAKE Viscotester 7L/R plus oraz wannę Termostat DC10.

Słowa kluczowe: osady ściekowe, kondycjonowanie, lepkość, temperatura

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Teresa RAUCKYTE-ŻAK[1] and Sławomir ŻAK[1]

CHANGEABILITY OF THE SPECIATION FORMS OF HEAVY METALS IN SOIL SUBJECT TO MANY YEARS OF FERTILIZATION WITH WASTEWATERS FROM VEGETABLE FAT PRODUCTION

ZMIENNOŚĆ FORM SPECJACYJNYCH METALI CIĘŻKICH W GLEBIE PODDANEJ WIELOLETNIEMU NAWOŻENIU ŚCIEKAMI Z PRODUKCJI TŁUSZCZÓW ROŚLINNYCH

Abstract: Agro-utilization of pretreated industrial wastewaters from the vegetable fat production for irrigation and as fertilizers is, among others, limited by heavy metal concentrations in wastewaters and the soil where wastewaters are directed. This paper presents the results of 4-year research on the influence of the irrigation process and fertilization with wastewaters on the concentration and changeability of heavy metal (Cd, Cr, Cu, Hg, Ni, Pb, Zn) speciation forms in the superficial soil layer. The fractional composition of metals was determined in accordance with Tessier's methodology. The obtained results prove a significant stability of speciation loads of metals determined in the examined soil within the experimental period. Analysis of the fractional composition showed an insignificant decreasing tendency of the leached fraction percentage and an increase in the carbonate fraction share in the case of the majority of the analyzed heavy metals.

Keywords: heavy metals, metals speciation forms, agro-utilization of wastewaters from vegetable fat production

After physicochemical pretreatment, industrial wastewaters from vegetable fat production are free from heavy metals, and in the pollutant load there are mainly soluble low molecular substances of vegetable origin (ie lecithins, glycerols, proteins, aldehydes, ketones, lactones, sterols, tocopherols) and orthophosphates from refining processes [1, 2]. Composition qualifies wastewaters of this type to the group of biodegradable sectors, enabling them to be agriculturally used for irrigation and as fertilizers [3]. The fundamental limitation of agro-utilization is the limitation with nitrogen and heavy metal threshold concentrations in soils, where wastewaters are directed [3, 4]. The share of metal speciation forms in soil – the multi-phase polydispersion system – depends on

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a number of factors: minerals forming the solid phase, organic matter content and composition, content, type and water transfer, local climate, variety of the world of microorganisms, the level of energetic processes, etc. [5, 6].

With many various factors deciding about the quality of a given soil, the introduction of the outer substance load must result in significant changes in the past mechanisms of chemical, biochemical and biological transformations [7, 8]. It is like that also in the case of introducing pretreated wastewaters from the production of fats of vegetable origin [9–11]. Wastewaters, especially the pretreated ones additionally contain mineral substances – orthophosphates and the calcium ion (ie in the form of $Ca^{2+} \cdot aq$, $CaOH^{+}$ or ion pairs $Ca^{2+} \cdot CO_3^{2-}$ and $Ca^{2+} \cdot HCO_3^{-}$), which show definite reactivity in relation to soil components [2, 12].

Only the toxic chemical components which can occur in wastewaters are the subject to a limit, while the quantity of water does not include any limitations, and this factor significantly influences the dynamics of transformations and transport in soil [13]. Introducing a new load of organic and mineral substances with a significant amount of water can lead to significant transformations in soils and at the same time change the metal speciation form composition, especially in the long-term period of time when the wastewaters are used for agriculture [8, 18].

Materials and methods

Soil material (from the depth of surface layer 0.0–20.0 cm) for the analyses of the individual heavy metal fractions was collected for 4 years at regular intervals – one series every half a year (one in April, the second one in October) from the experimental field (of the area of 15.0 ares) irrigated and fertilized with pretreated wastewaters from the production of fats of vegetable origin. Analyses were made in accordance with Tessier's methodology, in the detailed form presented in Table 1 [14–18]. There were the following parameters [mg/dm^3] of the pretreated wastewaters during the time of running experiments: *total nitrogen* (TN) 10.9–53.9, *total phosphorus* (TP) 7.4–39.3, *etheric extract* (EE) 2.7–28.5, *total suspensions* (TSS) 38.9–105.2, *chemical oxygen demand* (COD) 1307.9–3095.7, *biochemical oxygen demand* (BOD_5) 448.9–2055.8 and *reaction* (pH) 7.8–8.8. The wastewater pretreatment process was carried with the use of coagulation with lime milk (at a dose of 0.75–1.0 kg CaO/m^3 of wastewaters), aided with flocculation (Praestol 2340 BC Stockhausen) at a dose of 15.0 g/m^3 of wastewaters and pressure flotation (saturation time 10.0 min, saturation pressure 500 kPa, level of pretreated waste recirculation 20 %), aided with hydrogen peroxide oxidation (at a dose of 150.0 $gH_2O_2/m^3$of wastewaters) [4].

Discussion

The aim of the research was to check the influence of four-year irrigation and fertilization with pretreated wastewaters from the vegetable fat production on the fractional composition and changeability of heavy metal fractional loads contained in

Table 1

Description of Tessier's methodology used to determine the heavy metal fraction in the examined soil, where agro-utilization of pretreated wastewaters from vegetable fats production was carried out[a]

Fraction number	Fraction	Details concerning preparation procedure[b]
I	exchangeable	Soil samples (100.0 g) were treated with 400.0 cm^3 (1.0 M) $CaCl_2$ and after intensive mixing (at pH 7.0) shaken for 1.0 hour at room temperature. Analyses were made after separating solid components with the use of filtration.
II	carbonate	Dried soil samples which remained after carrying out the procedure for fraction I were subject to extraction, adding 800.0 cm^3 (1.0 M) CH_3COONH_4 and acidifying 80 % CH_3COOH to pH 7.0. After mixing, the mixture was shaken for 5.0 hours at room temperature. During this operation the reaction was controlled: when pH > 7.0 – it was acidified. The analyses were made after separating solid components.
III	Fe/Mn oxides	800.0 cm^3 (0.04 M) of $NH_2OH \cdot HCl$ solution in 25 % CH_3COOH was added to the dried sample of the solid phase after applying the procedure to fraction II and mixed. Samples (of pH 2.0) were shaken for 5.0 hours at the temperature of 96 ± 3°C. Analyses were made after separating the solid components.
IV	organic	200.0 cm^3 (0.02 M) HNO_3 and 200.0 cm^3 30 % aqueous solution of H_2O_2 were added to the solid sample obtained from the procedure run for fraction III, then the mixture was mixed (pH 2.0) and shaken for 135.0 min at the temperature of 85 ± 2°C. Next, 200.0 cm^3 30 % H_2O_2 was added and shaken for 135.0 minutes. After the time, 400.0 cm^3 (3.0 M) CH_3COONH_4 in 20 % HNO_3 (v/v) was added and shaken for 30.0 min. at room temperature (pH 2.0).
V	residual	Shaken for 1.0 hour at boiling point, at the same time adding HNO_3 and H_2O_2 twice until releasing white ashes. Next, 400.0 cm^3 30 % H_2O_2 was added and shaken for 30.0 min at boiling point. Analyses were made after cooling.

[a] Based on the publications [14–18]; [b] filtrate for determination of fractions I – V were subject to AAS analyses (apparatuses: BUCK Scientific 210 VGP and Spectr AA Varian 220 SS).

the soil where wastewaters were directed. The problem of metal speciation in soil material is strictly related to the particular specificity of a given soil [5, 6]. On the basis of the analyses made, the insignificant periodical fluctuations of total concentrations for individual metals in the analyzed layer were found. The problem was presented and interpreted in detail in publication [4], and the present paper is just the extension of the problems discussed in the publication. Despite applying wastewaters of reaction pH 7.8–8.8 (after coagulation with lime milk) within 4 years of experiments, no change was found in soils from experimental field because the level of this parameter was within the range of pH $\approx 6.7 \pm 0.2$. Therefore, it is the reaction level at which the ionic metal forms (M) such as: chromium, zinc, copper, lead or mercury show particular preference to form difficult to solve hydroxide forms of type $M(OH)_{n(s)}$ [19, 20]. Also, in the case of these metal carbonates, the values of their solubility products are at a very low level (below $1.0 \cdot 10^{-10}$), which shows that these forms are favorable to form difficult to solve $MCO_{3(s)}$ carbonates in conditions of the subject soil reaction [21]. At the significant participation of chemical and biochemical reactions which lead to mineralization of some organic contaminations directed to soil, the stability of soil reaction can also result from forming a strong carbonate–hydrocarbonate buffer system. On the basis of the present research within four years of experiment, it was found that the total average metal concentrations during this period were not subject to significant changes [4]. In the case of chromium and nickel, an insignificant growth tendency was registered for total concentration in relation to initial concentrations (C_0) [4]. Analyzing the fractional composition of individual metals in the subject soil during the experimental period, the limited phenomenon of fractional load percentage fluctuation was found: exchangeable (fraction I) and carbonate (fraction II) for most of the analyzed metals (Table 2). However, the registered changes for exchangeable fraction showed a long-term decreasing tendency, and for carbonate fraction the increasing percentage tendency. For instance, there were the following exchangeable fraction (fraction I) percentages for the examined elements before the experiment [%]: Cd – 4.8, Cr – 3.4, Cu – 3.9, Hg – 4.9, Ni – 3.1, Pb – 4.5, Zn – 1.7, and after four years of wastewaters agro-utilization they were respectively [%]: Cd – 0.6, Cr – 0.2, Cu – 0.5, Hg – 0.1, Ni – 0.2, Pb – 0.1, Zn – 0.2. In the case of carbonate fraction these changes were respectively: before the experiment [%]: Cd – 2.6, Cr – 1.7, Cu – 0.5, Hg – 1.7, Ni – 1.7, Pb – 2.7, Zn – 0.9, and after the experiment [%]: Cd – 7.3, Cr – 6.6, Cu – 2.5, Hg – 6.6, Ni – 4.9, Pb – 6.6, Zn – 4.9 (Table 2). It should be emphasized that the registered changes in the percentages of these fractional loads in both cases are connected with the values not exceeding 5 %. Analyzing the residual fractional composition, definitely the highest percentage was registered in the case of so called residual fraction, exceeding 70 % for each metal (fraction V, Table 2). In the case of a fraction related to hydrated Fe/Mn oxides, a stabile tendency to maintain a similar level of this fraction content for all examined metals in the experimental period was observed. The organic fraction (fraction IV) was characterized by periodical changes of its percentage with time. The obtained results of the analyses showed a significant speciation load stability in the case of metals which were in the examined soil within four years of agro-utilization of wastewaters from vegetable fat production.

Table 2

Changeability of heavy metal fractional load percentages determined during the four-year experiment for soil where wastewaters from vegetable fats production were agro-utilized

No.	Heavy metal	Fractions	% participation of fraction in subsequent measurements								
			P0	P1	P2	P3	P4	P5	P6	P7	P8
1	Chromium (Cr)	I	3.4	2.0	2.8	1.4	1.8	1.4	1.1	0.3	0.2
		II	1.7	2.7	2.4	2.9	3.3	3.7	4.6	4.1	6.6
		III	7.4	8.7	5.1	6.9	8.1	10.0	10.1	10.0	8.2
		IV	12.0	12.1	14.0	14.0	10.1	13.2	11.2	11.5	12.3
		V	75.5	74.5	75.7	72.8	76.7	71.7	73.0	74.1	72.7
2	Cadmium (Cd)	I	4.8	4.2	4.3	3.6	3.3	3.5	2.7	1.3	0.6
		II	2.6	3.2	4.4	4.7	4.1	4.7	5.7	6.9	7.3
		III	6.7	9.6	9.5	8.2	11.9	10.6	9.1	8.4	8.9
		IV	13.0	11.5	10.4	13.1	10.6	11.1	10.6	13.7	12.4
		V	72.9	71.5	71.4	70.4	70.1	70.1	71.9	69.7	70.8
3	Nickel (Ni)	I	3.1	3.4	2.8	1.8	1.8	1.5	1.4	0.4	0.2
		II	1.7	2.2	2.7	3.4	3.3	3.7	4.4	4.1	4.9
		III	11.0	9.2	9.1	10.8	12.9	10.5	9.8	11.9	13.2
		IV	12.7	9.7	10.7	13.2	10.3	14.6	14.3	13.9	13.5
		V	71.5	75.5	74.7	70.8	71.7	69.7	70.1	69.7	68.2
4	Lead (Pb)	I	4.5	4.5	3.5	3.6	2.6	1.6	1.7	0.9	0.1
		II	2.7	3.6	3.4	3.7	4.6	5.0	5.4	6.6	6.6
		III	10.9	10.0	11.5	10.0	10.8	10.5	11.5	10.4	11.8
		IV	8.0	8.1	8.8	10.8	10.2	11.7	10.8	11.4	11.0
		V	73.9	73.8	72.8	71.9	71.8	71.2	70.6	70.7	70.5
5	Copper (Cu)	I	3.9	3.1	1.9	1.0	1.1	0.9	0.6	0.7	0.5
		II	0.5	0.8	0.7	0.7	0.9	0.9	1.5	1.5	2.5
		III	8.9	10.7	15.8	15.9	13.5	17.2	13.5	13.5	12.9
		IV	14.6	15.2	11.7	12.3	14.5	11.2	13.5	14.1	12.5
		V	72.1	70.2	69.9	70.1	70.0	69.8	70.9	70.2	71.6
6	Mercury (Hg)	I	4.5	4.5	3.5	2.0	2.6	1.7	1.6	0.9	0.1
		II	1.7	3.4	3.7	3.9	4.7	5.4	5.0	6.6	6.6
		III	9.9	10.0	9.5	10.0	9.8	9.5	8.7	10.4	9.8
		IV	9.0	8.3	9.5	11.2	9.1	8.2	8.4	9.4	9.0
		V	74.9	73.8	73.8	72.9	73.8	75.2	76.3	72.7	74.5
7	Zinc (Zn)	I	1.7	1.9	1.7	1.3	1.0	1.1	0.6	0.7	0.2
		II	0.9	2.2	2.3	2.9	2.9	3.2	3.3	3.2	4.9
		III	12.4	14.3	14.2	14.6	13.9	12.6	9.8	12.3	10.5
		IV	11.2	11.6	10.6	11.3	11.7	11.2	14.0	13.2	13.2
		V	73.8	70.0	71.2	69.9	70.5	71.9	72.3	70.6	71.2

Where: P0 – zero sample (before agro-utilization of pretreated wastewaters) – total initial concentrations (C_0) were respectively [mg/kg d.m.]: Cd – 0.19, Hg – 0.16, Cr – 5.29, Ni – 5.02, Cu – 8.11, Pb – 9.28 and Zn – 20.35; P1–P8 measurement series carried every six months within four years of analyses.

Conclusions

The obtained results of the analyses show a significant stability of metals speciation loads present in the subject soil within four years of carrying out agro-utilization of wastewaters from vegetable fat production. Therefore, it can be expected that in the case of further many-year irrigation and fertilization of the examined soil in the same process pretreatment conditions and agro-technical ones, no significant changes in individual shares of heavy metal speciation loads will be found.

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ZMIENNOŚĆ FORM SPECJACYJNYCH METALI CIĘŻKICH W GLEBIE PODDANEJ WIELOLETNIEMU NAWOŻENIU ŚCIEKAMI Z PRODUKCJI TŁUSZCZÓW ROŚLINNYCH

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Abstrakt: Agroutylizacja podczyszczonych ścieków technologicznych z produkcji tłuszczów roślinnych na cele nawadniające oraz nawozowe jest m.in. limitowana stężeniem metali ciężkich w ściekach oraz glebie, na którą kieruje się odpady. W pracy przedstawiono wyniki czteroletnich badań nad wpływem procesu nawadniania oraz nawożenia ściekami na stężenie oraz zmienność form specjacyjnych metali ciężkich (Cd, Cr, Cu, Hg, Ni, Pb, Zn) w przypowierzchniowej warstwie gleby. Skład frakcyjny metali był oznaczany zgodnie z metodyką Tessiera. Uzyskane wyniki wskazują na występowanie istotnej stabilności pól specjacyjnych metali oznaczanych w badanej glebie w okresie doświadczalnym. Analizując skład frakcyjny, stwierdzono występowanie nieznacznego trendu zmniejszania udziału procentowego frakcji wymywanej oraz wzrostu udziału frakcji węglanowej w przypadku większości badanych metali ciężkich.

Słowa kluczowe: metale ciężkie, formy specjacyjne metali, agroutylizacja ścieków z produkcji tłuszczów roślinnych

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EFFECT OF NITROGEN FERTILIZATION ON SEED PRODUCTION OF *Lolium perenne* L. TURFGRASS CULTIVARS

NAWOŻENIE AZOTEM W PRODUKCJI NASIENNEJ GAZONOWYCH ODMIAN *Lolium perenne* L.

Abstract: The paper contains the results of field and laboratory experiments on three *Lolium perenne* L. turfgrass cultivars (Gazon, Nira and Sandra) at Malopolska Plant Breeding Station (HBP) in Skrzeszowice near Krakow (220 m a.s.l.) conducted in the years 2006–2008. The analyses assessed the effect of doses of mineral fertilization with nitrogen (60, 90 and 120 kg N · ha^{-1}) applied at early spring, the start of the earing stage and autumn on the seed yield. The highest yield was monitored by the inflorescence shoot density of cv. Nira (1895 shoots/m^2), cv. Gazon (1976 shoots/m^2) and cv. Sandra (2098 shoots/m^2). Fertilization dose of 90 kg N · ha^{-1} applied three times effected the most considerably the number of spikelets per ear and the efficiency of seed settling on the ear: from 73 % (cv. Nira) to 76 % (cv. Sandra). The highest seed yield was obtained as a result of the 120 kg N · ha^{-1} fertilizations applied three times.

Keywords: *Lolium perenne*, cultivars, fertilization, seed yield

Perennial ryegrass (*Lolium perenne* L.) belongs to the species which still raise interest of researchers, as evidenced by the most numerous collection of cultivars in Poland. It is also apparent as the ever growing market demand for this species. It has been estimated that the European Union's demand for perennial ryegrass seeds is the greatest among grasses and on average reaches 60 000 Mg a year [1]. In Poland it occupies a prominent position in the seed production, accounting for ca 35–45 % of the whole seed crop acreage. According to Martyniak [2] and Svensson and Boelt [3] seed plantations of perennial ryegrasses may provide an alternative to cereal cultivation, the overproduction of which will make farmers seek new sources of income. It is commonly known that in order to improve the profitability of seed crop production it is necessary to increase seed yield and the main element determining the productivity the most is nitrogen fertilization. It turns out that optimisation of nitrogen fertilization of perennial ryegrass plantations involves not only determining the dose in the production

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cycle but also its proper division into parts and setting proper dates of application [4, 5]. Therefore, the aim of the Author's investigations was an attempt at assessment of the nitrogen fertilization dose and its application date on the amount of the seed yield of three lawn cultivars of perennial ryegrass.

Materials and methods

The research was conducted in 2006–2008 at the Malopolska Plant Breeding Experimental Station – HPB in Skrzeszowice (220 a.s.l.) near Krakow, on degraded chernozem developed from loess.

The soil revealed the following chemical properties: pH_{KCl} – 6.5; available P – 56, K – 132 and Mg – 39 g · kg^{-1}; organic N – 1.5 and total carbon – 16.1 g · kg^{-1} of soil.

The experiment was set up in the autumn 2005 using the randomised block method in four replications. The area of each plot was 10 m^2 (1 × 10).

The object of the investigation was a seed plantation of three fodder cultivars of perennial ryegrass (*Lolium perenne*): Gazon, Nira and Sandra.

Phosphorus, dosed 30 kg P · ha^{-1} as triple superphosphate (46 % P_2O_4), was used for fertilization conducted once in spring. Potassium, in the amount of 66 kg K · ha^{-1} as high grade potassium salt (60 % K_2O), was applied once, also in spring.

Nitrogen fertilization in the doses of 60, 90 and 120 kg N · ha^{-1} was applied on the following dates:

– once in the early spring;

– twice in two equal portions (in the early spring and at the beginning of the earing stage);

– three times in three equal parts (in the early spring, at the beginning of earing and in autumn).

Chemical weed control on the seed plantation, with a dose of 1 dm^3 · ha^{-1} of Aminopielik Gold, was conducted each year at the beginning of April (when the vegetation started) and in September. Before the seed harvesting single weeds were hand removed.

In each year of the investigations, field observations and biometric plant measurements were conducted at the beginning of August. The number of plants was counted on individual plots on the area of 1 m^2, as well as the number of spikelets per spike, and seed setting efficiency in spikes was assessed. The harvesting of the seed plantations was carried out in one stage by means of Wintersteiger plot combined harvester. The harvested seed material was dried in a storeroom and subsequently cleaned on the winnower. The cleaned grains were weighed, the obtained yield was calculated per 1 ha and a thousand grain weight was determined.

The obtained results were verified statistically by means of the analysis of variance. The differences between means were estimated using the Student test at the significance level $p = 0.05$ and the correlation coefficient was calculated for selected features.

The annual precipitation total for the period of investigations ranged from 463.8 to 683.4 mm, whereas the precipitation total for the six months (April–September) from

345.2–561.7 mm. Average annual temperature reached from 6.4 to 6.9 ^{o}C and between 11.9 and 12.8 ^{o}C during the vegetation season.

The work presents the mean results for the 3 years of the investigations.

Results and discussion

The level of nitrogen fertilization is the factor affecting generative shoot formation on plantations of perennial ryegrass [6]. This thesis has been confirmed also by the results of the Author's own investigations, where nitrogen fertilization significantly modified the number of generative shoots per area unit (Table 1). The highest shoot density in Gazon cv. – 2018 and Nira cv. – 1953 shoots/m^2 was registered after a single application of nitrogen (in early spring) in the dose of 120 kg N · ha^{-1}. On the other hand in Sandra cv. the largest number of generative shoots (2098 shoots/m^2) was calculated on the object fertilized with 120 kg N · ha^{-1} applied on three dates: early in spring, at the beginning of the earing stage and in autumn.

Table 1

The mean number of generative stems of three *Lolium perenne* cultivars [units · m^{-2}]

Specification	cv. Gazon	cv. Nira	cv. Sandra
Control	1 354	1 119	1 543
N_{60} – in one dose + $P_{30}K_{66}$	1 741	1 527	1 792
N_{60} – in two doses + $P_{30}K_{66}$	1 527	1 485	1 761
N_{90} – in one dose + $P_{30}K_{66}$	1 963	1 751	1 957
N_{90} – in two doses + $P_{30}K_{66}$	1 934	1 728	1 934
N_{90} – in three doses + $P_{30}K_{66}$	1 972	1 895	2 015
N_{120} – in one dose + $P_{30}K_{66}$	2 018	1 953	2 086
N_{120} – in two doses + $P_{30}K_{66}$	1 853	1 796	2 041
N_{120} – in three doses + $P_{30}K_{66}$	1 976	1 895	2 098
LSD (p = 0.05)	101.7	127.5	148.9

Perennial ryegrass is a nitrophilous species, visibly responding to nitrogen supplied with fertilizers by showing the changes of eg morphological properties determining the amount of seed yield. In the opinion of Young et al [7] and Golinski [6], nitrogen fertilization influences the number of kernels formed per spikelet in this species. Proper plant nutrition with nitrogen determines the effectiveness of their setting in the ear [8–11]. On the basis of the Author's own research, a favourable effect of nitrogen fertilization on this feature value was observed only to the level of 90 kg N · ha^{-1} (Table 2). In conditions of nitrogen application in the dose of 120 kg N · ha^{-1} a worsening of kernel setting in ear efficiency was registered in each of the three tested cultivars. According to Marshall [12], the reason for a decline in the efficiency of kernel setting in the ear when larger nitrogen doses were applied was the competition for assimilates between the vegetative and generative shoots which follows the flowering stage and may affect a dieback of embryos and developing seeds.

Table 2

Effectiveness of embedding of seeds in spikes [%] and mean number of spikelets per spike of three *Lolium perenne* cultivars

Specification	cv. Gazon		cv. Nira		cv. Sandra	
	effectiveness of embedding of seeds in spikes	number of spikelets per spike	effectiveness of embedding of seeds in spikes	number of spikelets per spike	effectiveness of embedding of seeds in spikes	number of spikelets per spike
Control	379	2.15	306	2.28	463	2.71
N_{60} – in one dose + $P_{30}K_{66}$	527	2.32	752	2.37	759	2.79
N_{60} – in two doses + $P_{30}K_{66}$	518	2.24	746	2.34	741	2.85
N_{90} – in one dose + $P_{30}K_{66}$	764	2.45	893	2.51	885	2.83
N_{90} – in two doses + $P_{30}K_{66}$	752	2.40	875	2.31	867	2.78
N_{90} – in three doses + $P_{30}K_{66}$	781	2.28	898	2.36	894	2.80
N_{120} – in one dose + $P_{30}K_{66}$	936	2.48	974	2.52	1 026	2.94
N_{120} – in two doses + $P_{30}K_{66}$	923	2.39	961	2.46	997	2.79
N_{120} – in three doses + $P_{30}K_{66}$	948	2.40	985	2.49	1 031	2.85
LSD (p = 0.05)	23.9	0.16	21.8	0.17	27.9	0.17

Table 3

The mean yields [kg · ha^{-1}] and mass of a thousand seeds [g] of three *Lolium perenne* cultivars

Specification	cv. Gazon		cv. Nira		cv. Sandra	
	yield	mass of a thousand seeds	yield	mass of a thousand seeds	yield	mass of a thousand seeds
Control	379	2.15	306	2.28	463	2.71
N_{60} – in one dose + $P_{30}K_{66}$	527	2.32	752	2.37	759	2.79
N_{60} – in two doses + $P_{30}K_{66}$	518	2.24	746	2.34	741	2.85
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N_{120} – in three doses + $P_{30}K_{66}$	948	2.40	985	2.49	1 031	2.85
LSD (p = 0.05)	23.9	0.16	21.8	0.17	27.9	0.17

Young et al (1996) state that there is an optimal nitrogen dose for each cultivar which allows for obtaining a maximum seed yield; these doses are diversified and may range from 60 to 180 kg N · ha^{-1}. In the conducted experiments, fertilization with each dose of nitrogen significantly diversified seed yield (Table 3). The highest seed yield from each cultivar was obtained as a result of 120 kg N · ha^{-1} applied in the early spring term, at the beginning of earing and in autumn. About thrice higher yields, ranging from 985 (Nira cv.) to 1031 kg · ha^{-1} (Sandra cv.) were produced on the unfertilized objects. An increase in the total nitrogen dose was usually accompanied by an increase in one-thousand-seed weight. However, these tendencies were not stabilized. A crucial importance of early spring fertilization was noted in the assessment of the seeding value of the seeds by means of one-thousand-seed weight. A single nitrogen dose applied in early spring increased one-thousand-seed weight in comparison with the control: in Gazon cv. from 7 % (60 kg N · ha^{-1}) to 15 % (120 kg N · ha^{-1}), in Nira cv. from 3 % (60 kg N · ha^{-1}) to 10 % (90 and 120 kg N · ha^{-1}) and in Sandra cv. between 2 % (60 kg N · ha^{-1}) and 8 % (120 kg N · ha^{-1}).

Conclusions

1. The highest seed yield of *L. perenne* cvs per 1 m^2 was obtained at the following flower shoot densities: 1895 in Nira cv., 1976 in Gazon cv. and 2098 in Sandra cv. at dose of the 120 kg N · ha^{-1} fertilizations applied three times.

2. Nitrogen fertilization dose of 90 kg N · ha^{-1}, applied three times (at the early spring, the beginning of earing and in autumn) increased the number of spikelets per ear and seed setting efficiency in ear to 73 % (Nira cv.) and to 76 % (Sandra cv.).

3. The increment of seed setting efficiency as a result of nitrogen fertilization at the use of 90 kg N · ha^{-1}, which decrease after treatment.

4. The highest seed yield was obtained under the influence of the 120 kg · ha^{-1} nitrogen dose.

5. The crucial importance of a single fertilization dose applied in early spring was registered in the assessment of the sowing value of seeds by means of one-thousand--seed weight.

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NAWOŻENIE AZOTEM W PRODUKCJI NASIENNEJ GAZONOWYCH ODMIAN *Lolium perenne* L.

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Abstrakt: Praca zawiera zestawienie wyników badań polowych i laboratoryjnych przeprowadzonych w Stacji Małopolskiej Hodowli Roślin – HBP w Skrzeszowicach koło Krakowa (220 m n.p.m.) w latach 2006–2008 z trzema gazonowymi odmianami życicy trwałej (Gazon, Nira, Sandra). W badaniach poddano ocenie wielkość dawki nawożenia mineralnego azotem (60, 90, 120 kg N · ha^{-1}) oraz termin jego stosowania (wczesnowiosenny, początek kłoszenia i jesienny), na wielkość plonu nasion. W przeliczeniu na 1 m^2 uzyskanie największego plonu nasion zapełniała obsada pędów kwiatowych: 1895 u odmiany Nira, 1976 u odmiany Gazon oraz 2098 u odmiany Sandra. Nawożenie azotem w dawce 90 kg N · ha^{-1} zastosowanej w trzech terminach: wczesnowiosennym, na początku kłoszenia i jesiennym najbardziej zwiększało liczbę kłosków w kłosie oraz efektywność osadzania nasion w kłosie od 73 % u odmiany Nira do 76 % u odmiany Sandra. Największy plon nasion uzyskano pod wpływem dawki 120 kg · ha^{-1}, zastosowanej w trzech terminach: wczesnowiosennym, na początku kłoszenia i jesiennym.

Słowa kluczowe: *Lolium perenne*, odmiany, nawożenie, plon nasion

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KITCHEN WASTES AS A SOURCE OF NITROGEN AND OTHER MACROELEMENTS ACCORDING TO TECHNOLOGY OF VERMICULTURE

ODPADY KUCHENNE JAKO ŹRÓDŁO AZOTU I INNYCH MAKROPIERWIASTKÓW ZALEŻNIE OD TECHNOLOGII PROWADZENIA WERMIKULTURY

Abstract: The proper utilization of organic wastes has become an important environmental problem (in relation to economic and social aspect of sustainable development). In case of selected organic kitchen wastes, the process of vermicomposting on site where they are produced (in innovative "earthworm ecological box") could be a successful method of their neutralization.

The aim of this work was to specify the fertilizing qualities of vermicompost from kitchen wastes depending on the technology of its production (vermicomposting took place in "the earthworm ecological boxes" by means of a condensed substrate and a population of earthworms technology (Z) or their dividing every 4 weeks (R)).

The qualities of the vermicomposts produced this way were determined in a dynamic system (three times). pH was specified potentiometrically, the concentration of salt – by means of the conductometric method, the $N\text{-}NO_3$ content in the extract of 0.03 moles of acetic acid – potentiometrically, selected macroelements: assimilable P, K, Mg and calcium Ca were determined in the extract of 0.03 moles of acetic acid CH_3COOH.

It has been proven that the content of obtained products depended on the way of treatment of the boxes. A significantly bigger growth of nitrate nitrogen content $N\text{-}NO_3$, assimilable phosphorus, potassium and magnesium was the result of the application of the (Z) technology – the intense vermicomposting without dividing the substrates and earthworm population.

Vermicomposting organic kitchen wastes by a more frequent dividing of the substrates and earthworm population – had a more favourable influence on the reduction of the average concentration of salt in the vermicompost.

Keywords: organic kitchen waste, vermicompost, macroelements, pH, salinity

The organic wastes produced by a vast array of business sectors related to agriculture have become an increasingly significant problem. Their proper utilization has become of utmost importance not only from the environmental point of view (the replenishment

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of the organic substances in soil, the threat of air, soil and water degradation) [1], but also in relation to the continuously growing prices of land located next to urban and rural areas [2]. Depending on the features of such wastes, the method of their neutralization could be the process of fermentation or composting and vermi-composting. In case of composting, the main problem is the relative length of this process and the necessity to aerate the material, while the advantage could be the high temperature of the process (about 70 ^{o}C), which causes the death of all the pathogens in the composted material [3, 4]. Because of the above-mentioned factors, a considerable interest has been aroused by the integration of the process of composting and vermicomposting, which, on the one hand, allows to dispense with heavy equipment used to aerate heaps [2], and, on the other, prevents the created products from the lack of hygienizing temperature. The technology of vermicomposting facilitates a more rapid transformation especially of nitrogen, potassium, phosphorus and calcium compounds into forms, which are more easily dissolved and more available for plants. This is connected with the fact that each day an earthworm absorbs a large amount of organic material from soil, yet only 5–10 % of the digested material is assimilated by its organism, the remainder is excreted in the form of coprolites rich in nitrogen, phosphorus, potassium and microorganisms which are beneficial for soil [5–8].

Nitrogen accounts for only 0.5–4 % of dry mass in plants, yet it plays a critical part in their growth as a constituent of proteins as well as other important compounds such as nucleic acids, phytohormones, and nucleotides. Mineral nitrogen in the form of $N\text{-}NH_4$ and $N\text{-}NO_3$ is most readily available for plants of higher order, ie those not in symbiotic relationships with microorganisms binding elemental nitrogen [9]. Nitrogen is one of the chemical elements controlling the growth of organisms, both plants and animals, in accordance with Liebig's Law of the Minimum. Shortage of this element severely hinders the growth and normal development in plants. The most significant symptoms of nitrogen deficiency include: leaves falling off early, deteriorating over and underground organs, and irregular shape of fruit [10, 11].

Of great importance is the interaction between nitrogen and potassium. Even with the most favourable contents of nitrogen in forms which are readily available to plants, a deficiency of potassium in soil is a disturbing factor for the transformation of mineral nitrogen into proteins. N/K imbalance leads to increased content of nitrates in plants which adversely impacts their nutritional value. Adequate content of potassium in plants improves their response to nitrogen fertilizers, and similarly potassium fertilization is more effective in plants adequately supplied with nitrogen. On the other hand, overfertilization with potassium causes magnesium deficiency because K is antagonistic in relation to Mg and hinders both its assimilation and its movement in the plant [10].

As a result of the long-lasting exploitation, the soils of Europe contain at present from 2 to 3.5 % of organic substances [12]. As a consequence, the European Strategy for Soil Protection [13] contains the conclusion that the exploitation of land in regions, where the content of organic substances in soil is lower than 2 %, should be necessarily connected with actions aimed at soil stabilization or absolutely indispensably connected with the gradual increase of nitrogen-rich organic substances in soil. The search for nitrogen sources in wastes of various origins is well justified.

A citizen with a proper awareness of this issue can segregate organic kitchen wastes on site where they are produced in order to obtain fertilizing material of high quality, with no pollution. The aim of this work was to specify the fertilizing qualities of vermicompost from kitchen wastes depending on the technology of its production in the ecological boxes.

Materials and methods

The research was conducted in the laboratory conditions (temp. 20 ± 5 ^{o}C) of Department of Natural Theories of Agriculture and Environmental Education at the Faculty of Agriculture and Biology of the University of Rzeszow, over the period of 4 months. In order to improve the conditions of vermicomposting, kitchen wastes were mixed with cellulose in the ratio of 4:1 [14] and the vermicomposting took place in "the earthworm ecological boxes" by means of the technology of a condensed substrate and a population of earthworms (Z) and their dividing every 4 weeks (R) (each type in 3 repetitions) (Table 1).

Table 1

Arrangement of the experiment

Containers	**Containers** 1–3 (***type (Z)***): the earthworms stay there over the period of 4 months without dividing their population **Containers** 4–6 (***type (R)***): the substrates and the earthworms are divided every 4 weeks and put into other containers, which simulates the frequent division of breeding beds
Initial substrate	In each case 1 dm^3 of gardening soil with a specific chemical composition*
Earthworms	In each case 50 specimens of mature earthworms *E. fetida* of known biomass (with the density of 25 specimens · dm^{-3} and 0.541 ± 0.006 g · dm^{-3})
Wastes	Bread and pasta leftovers, apple and potato peelings + cellulose (in the ratio of 4:1)
Feeding / utilization in the process of vermicomposting	In each case 0.2 dm^3 of the above-mentioned wastes, each time in the same way, into each container, during the analysis of the conditions of the earthworm population performed every four weeks (in the same way in all the boxes)
Watering	In each case the same amount of tap water**

* pH in H_2O – 6.2, salinity NaCl – 0.5 g · dm^{-3}, N-NO_3 – 180 mg · dm^{-3}, P – 63 mg · dm^{-3}, K – 186 mg · dm^{-3}, Ca – 1027 mg · dm^{-3}, Mg – 141 mg · dm^{-3}; ** pH – 7.6, conductivity – 542 μS · cm^{-1}, nitrates(V) – 8.9 mg · dm^{-3}, Mg – 15.7 mg · dm^{-3}, hardness – 257 mg $CaCO_3$ · dm^{-3}.

The qualities of the vermicomposts produced this way were determined in a dynamic system (three times), while: pH in the water was specified by means of the potentiometric method, the concentration of salt – by means of the conductometric method, the N-NO_3 content in the extract of 0.03 moles of acetic acid – potentiometrically, selected macroelements: assimilable P, K, Mg and calcium Ca were

determined in the extract of 0.03 moles of acetic acid CH_3COOH using the Spurway method, as modified by Nowosielski [15].

The results were analysed in the Statistica PL program, with the application of two-factor analysis of variance (the time and technology of vermicomposting).

Results and discussion

In the conducted experiment it has been proven that kitchen wastes are a valuable source of nutrients for plants. The levels of macroelements in the form available for plants in the vermicomposting products under research exceeded the average nutritional requirements of most garden plants [16].

The composition of the obtained vermicomposts depended on the technology applied to the compost box (Table 2). The statistical analysis showed differences in the average content of macroelements (except for calcium Ca) present in the substrates created by vermicomposting (over the period of 16 weeks) the 1 dm^3 of the initial substrate and 4 dm^3 of organic wastes.

The content of nitrate nitrogen ($N\text{-}NO_3$) depended on the way of treating the boxes. In the containers (Z) (where earthworms with the initial density of 25 specimens $\cdot$ dm^{-3} and the mass 0.541 ± 0.006 g $\cdot$ dm^{-3} stayed without dividing their population over the period of 4 months) the level of the element was determined to be over ten times higher than the nutritional requirements of plants. In the containers (R) (where similar substrates and earthworm populations were divided every 4 weeks and put into other containers, which simulated the frequent separation of breeding beds) the content exceeded the requirements only over five times ($p < 0.001$).

The changes in the content of assimilable phosphorus P, potassium K and magnesium Mg were similar. While in the non-separated containers (Z) the potassium content should be diluted depending on the requirements of the garden plants, from eight to four times (in case of phosphorus from ten to five times and in case of magnesium from six to three times, respectively), the content of all these elements in the divided containers (R) was significantly lower; however, in this case it was also too high (Table 2) ($p < 0.001$).

The content of calcium Ca was different from the elements described so far. The calcium content was close to the nutritional requirements of garden plants and remained unchanged regardless of the different technologies applied to the boxes.

After four months' period of utilizing kitchen wastes, pH_{H2O} of the produced vermicomposts decreased and was included in the range from 4.90 to 5.58. It was slightly lower than the one obtained by Kostecka [14] (6.0–8.6) and Garczynska [17] (5.9–6.1) while vermicomposting the same wastes.

Under the influence of vermicomposting in the technology of dividing (R) the obtained salinity of vermicompost was significantly lower – 3.79 ± 0.63 g NaCl $\cdot$ dm^{-3}. In case of the divided containers this parameter fluctuated more slowly during the first 16 weeks of the research. Vermicomposting organic kitchen waste in the containers with a condensed earthworm population triggered an immediate increase in salinity to 5.5 ± 0.21 g NaCl $\cdot$ dm^{-3} (by 45 %) ($p < 0.001$) (Table 2). The issue of the significantly

Table 2

The qualities of vermicompost obtained as a result of different technologies of the vermicomposting process in a dynamic system

Measure-ment	pH_{H_2O}		The concentration of salt NaCl [g · dm^{-3}]		$N\text{-}NO_3$		P *assimilable*		K *assimilable*		Ca		Mg *assimilable*	
					[mg · dm^{-3}]									
Initial substrate	6.16–6.19		0.5		18 ± 1		63 ± 2		186		1027 ± 18		141 ± 2	
1	Z	R	Z	R	Z	R	Z	R	Z	R	Z	R	Z	R
	5.11^{min} 5.32^{max}	5.19^{min} 5.48^{max}	4.09 ± 0.12	3.48 ± 0.27	688 ± 50	573 ± 90	170 ± 10	145 ± 7	731 ± 106	605 ± 41	1674 ± 56	2061 ± 125	187 ± 7	177 ± 9
2	4.96^{min} 5.27^{max}	5.05^{min} 5.31^{max}	5.34 ± 0.62	4.32 ± 0.55	962 ± 117	757 ± 107	202 ± 21	159 ± 17	877 ± 162	682 ± 206	1796 ± 91	1990 ± 195	207 ± 11	181 ± 8
3	5.44^{min} 5.47^{max}	4.90^{min} 5.58^{max}	5.5^{a} ± 0.21	3.79^{b} ± 0.63	1217^{a} ± 58	659^{b} ± 142	315^{a} ± 12	120^{b} ± 34	1359^{a} ± 8.5	649^{b} ± 190	2098^{a} ± 60	1932^{a} ± 190	349^{a} ± 3	179^{b} ± 8
The optimal level for plants*	6.0–7.5		about 1.0		50–120		40–80		125–250		1000–2000		60–120	

* According to Konczak-Konarkowska [16]; a, b – statistically significant differences ($p < 0.05$); Z – research containers – technology without dividing the substrate; R – research containers – technology of dividing the substrate and the population of earthworms: 1 – measurement after 8 weeks of vermicomposting; 2 – vermicomposting over the period of 12 weeks; 3 – vermicomposting over the period of 16 weeks.

increased salinity of the vermicomposts from household waste has already been highlighted in previous publications [18,19], pointing out the limitations concerning their use.

The discussed problem has to be considered important because at present non--segregated organic wastes fill about 30–50 % of the volume of our dumps. However, they could become a material to produce composts and vermicomposts – important from the point of view of replenishing organic substances in the soils.

Vermicomposts produced in both technologies were not suitable to be used directly as substrates for plant growth because most of the researched qualities highlighted the necessity to dilute them. The positive influence of dilution can also solve the problem of excessive concentration of salt in the analysed substrate.

Conclusions

1. Organic kitchen wastes can be vermicomposted in ecological boxes on site where they were produced.

2. It has been proven that the content of obtained products depended on the way of treatment of the boxes. From the point of view of the content of the basic nutrients for plants, a significantly bigger growth of nitrate nitrogen content $N\text{-}NO_3$, assimilable phosphorus, potassium and magnesium was the result of the application of the (Z) technology – the intense vermicomposting without dividing the substrates and earthworm population.

3. Technology (R) – vermicomposting organic kitchen wastes by a more frequent dividing of the substrates and earthworm population – had a more favourable influence on the reduction of the average concentration of salt in the vermicompost.

4. When analysing the obtained results from the point of view of the growing need for natural fertilizers and the necessity for pro-environmental utilization of huge amounts of organic wastes without placing them in dumping sites, another advantage of (R) technology (with the more frequent dividing of the substrates and the population of earthworms) is the fact that over the period of 16 weeks three times more organic kitchen wastes were neutralised using this method.

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ODPADY KUCHENNE JAKO ŹRÓDŁO AZOTU I INNYCH MAKROPIERWIASTKÓW ZALEŻNIE OD TECHNOLOGII PROWADZENIA WERMIKULTURY

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Abstrakt: Prawidłowe unieszkodliwianie odpadów organicznych stało się ważnym problemem ekologicznym (w powiązaniu z gospodarczym i społecznym aspektem zrównoważonego rozwoju). W odniesieniu do selekcjonowanych organicznych odpadów kuchennych możliwe jest ich wermikompostowanie na miejscu powstawania (w innowacyjnych „dżdżownicowych skrzynkach ekologicznych").

Celem prezentowanej pracy było określenie właściwości nawozowych wermikompostu z odpadów kuchennych zależnie od technologii jego produkcji (wermikompostowanie prowadzono w „dżdżownicowych skrzynkach ekologicznych" w technologii zagęszczonego podłoża i populacji dżdżownic (Z) albo ich rozdzielania co 4 tygodnie (R)). Cechy tak produkowanych wermikompostów określono w układzie dynamicznym przy czym: pH określano metodą potencjometryczną, stężenie soli metodą konduktometryczną, zawartość $N\text{-}NO_3$ w wyciągu 0,03 mol kwasu octowego potencjometrycznie, wybrane makroelementy – przyswajalny P, K, Mg i Ca, oznaczano w wyciągu 0,03 mol kwasu octowego CH_3COOH.

W prowadzonym doświadczeniu uzyskano wermikomposty o składzie zależnym od technologii wermikompostowania. Znacznie większy wzrost zawartości azotu azotanowego $N\text{-}NO_3$, przyswajalnego fosforu, potasu, magnezu oraz wapnia zapewniała technologia intensywnego wermikompostowania bez podziału podłoża i populacji dżdżownic.

Technologia wermikompostowania kuchennych odpadów organicznych przez częste rozdzielanie podłoża i populacji dżdżownic była korzystniejsza dla obniżenia średniego zasolenia wermikompostu.

Słowa kluczowe: organiczne odpady kuchenne, wermikompost, makropierwiastki, pH, zasolenie

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INFLUENCE OF 1,3,4-THIADIAZOLE DERIVATIVES ON THE BIOLOGICAL ACTIVITY OF THE SELECTED ENVIRONMENTAL BACTERIA

WPŁYW POCHODNYCH 1,3,4-TIADIAZOLI NA AKTYWNOŚĆ WYBRANYCH BAKTERII ŚRODOWISKOWYCH

Abstract: The paper presents the results of microbiological research carried out with the living microorganisms participation and in the domestic sewage and sludge from municipal sewage treatment plants environment. The influence of the selected heterocyclic compounds – the derivatives of 1,3,4-thiadiazole on susceptibility of aerobic Gram-positive and Gram-negative bacteria was investigated. For the research of biological activity the following environmental bacterial strains were selected: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg,* and *Salmonella typhimurium.* For the group of the tested bacteria, 2-amino-1,3,4-thiadiazole turned out to be the most active chemical compound. It was found that evenly matched concentrations of this compound allow for the selective elimination of such microorganisms as: *Salmonella senftenberg*, *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecium*. In the case of *Escherichia coli*, the minimum inhibitory concentration (MIC) was detected at the level of 12.5 mg/cm^3 (determined in relation to 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide), and for 2-amino-1,3,4-thiadiazole: 0.5 mg/cm^3. Similar results were observed in case of *Enterococcus faecium* bacterium: MIC for 2-amino-1,3,4-thiadiazole was less than 0.25 mg/cm^3, and MIC for bis(2-acetylamino--1,3,4-thiadiazole)-5,5'-disulfonamide this level was equal to 25.0 mg/cm^3.

Keywords: 1,3,4-thiadiazole derivatives, microbiological research, sanitary status indicator

The interest in derivatives of 1,3,4-thiadiazole has increased since phenylhydrazine and hydrazine were found. Thus, the research widened our knowledge concerning the preparation and chemical properties of 1,3,4-thiadiazoles. Derivatives of this compound showing biological activity are known as medical preparations [1]. A lot of research is

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being carried out concerning the issue of the application of heterocyclic derivatives in viral and bacterial diseases treatment [2–4]. It is known that 1,3,4-thiadiazole derivatives possess such biological activity as: antibacterial [5, 6] antimycobacterial [7, 8], antidepressive [9] and cardiotonic [10]. Resent investigation has also detected the analgesic [11] and anti-inflammatory [12–14] activity for these heterocyclic compounds. The compositions containing this heterocyclic ring form reactive centres in many enzymes and coenzymes, and are included in the composition of structures such as nucleic acids, chlorophyll, haemin – red blood pigment, pesticides, medicines (such as: 4-dimethylamino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, penicillin G, diazepam, resirpin, acridine), hormones (serotonin, melatonin), amino acids (eg: tryptophan, histidine) and many others. Other 1,3,4-thiadiazole derivatives are practically obtained by the application of different methods and their preparation technologies are strictly related to water and the pollution of its derivative compounds as well as to sewage and solid waste formation. In the pharmaceutic industry, diuramid is mostly prepared from 2-acetylamine-5-mercapto-1,3,4-thiadiazole by the process of chlorination involving gaseous chlorine and the ammonolysis of the semi-product [15]. The compounds such as: 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (known under the trade name of diuramid, acetazolamide, diamox, diacarb, diluran), 2-amino-1,3,4-thiadiazole and bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide are found in the wastes and wastewaters from different diuramid production technologies [16]. These compounds also occur in nature and show high biological activity [17, 18].

The aim of the work is investigating the influence of the 1,3,4-thiadiazole derivatives on the activity of the selected environmental bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg,* and *Salmonella typhimurium.*

Experimental part

The following heterocyclic compounds were applied in the biological research: *2-acetylamino-1,3,4-thiadiazole-5-sulfonamide* (ATS), *2-amino-1,3,4-thiadiazole* (AT) and *bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide* (BATD). They were prepared with the use of the procedures described below. Reagents of the analytically pure class produced by Sigma-Aldrich and POCh Gliwice were used for syntheses.

Preparing 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (ATS)

For the purpose of the biological study, concerning the subject of our research, we prepared acetazolamide with the use of hydrogen peroxide – hydrochloric acid. The synthesis was carried out as follows: First, 50.0 cm^3 (1.62 mole) of concentrated 36.0 % hydrochloric acid (d = 1.18 g/cm^3) was introduced into the reactor equipped with the reflux condenser, the mixer and the thermometer. Then, holding temperature within the range of: 5 to 10 ^oC, 80 cm^3 (2.64 mole) of 30.0 % hydrogen peroxide (d = 1.11 g/cm^3) was added in small doses. After introducing the whole amount of the oxidizer, 5.0 g (0.029 mole) of 2-acetylamino-5-mercapto-1,3,4-thiadiazole was added in doses. The

last stage included the introduction of 99.5 % (d = 1.05 g/cm^3) acetic acid in the total volume of 50.0 cm^3 (0.88 mole). All the components were stirred for 120 min in temperature ranging from 5 to 10 ^{o}C. The obtained precipitate of 2-acetylamino-5-sulfochloro-1,3,4-thiadiazole (7.7 g in the form of the moist substance) was filtered and washed with ice-cold distilled water and submitted to ammonolysis by applying 9-time surplus of 25 % aqueous ammonia (d = 0.90 g/cm^3). Later, 2-acetylamino-5-sulfochloro-1,3,4-thiadiazole was introduced into 68.6 cm^3 of ammonia with the temperature not exceeding 20 ^{o}C. Then, the reaction mixture was heated for 120 minutes at 50 ^{o}C in order to remove the excess of ammonia. After that, charcoal was added and heated for 10 minutes. The hot mixture was filtered and cooled down to 2–3 ^{o}C. Later it underwent acidification with 36.0 % hydrochloric acid up to pH = 1. The obtained acetazolamide was separated and recrystallised from hot, distilled water. 2.05 g (yield: 32.0 %) of 2-acetylamino-5-sulfonamido-1,3,4-thiadiazole at melting point (m.p.) 257 ^{o}C (lit. m.p. 258–259 ^{o}C [15]) was prepared.

Preparing 2-amino-1,3,4-thiadiazole (AT)

The next compound: 2-amino-1,3,4-thiadiazole (AT) was prepared from thiosemicarbazide and ethyl orthoformate using the method described in [19]. A mixture containing 4.5 g (0.05 mole) of thiosemicarbazide and 8.0 cm^3 (0.05 mole) of ethyl orthoformate was introduced into the reactor equipped with a reflux condenser, a mixer and a thermometer. Then the mixture was heated on a water bath for 6 hours. Then, 250 cm^3 (4.3 mole) of boiling ethyl alcohol was added. The hot mixture was filtered in order to remove *N,N'*-bis-(1,3,4-thiadiazole-2) formamide. The filtrate was concentrated in a vacuum evaporator until approx. 50 cm^3 and then cooled. As much as 2.31 g (yield: 32.6 %) of 2-amino-1,3,4-thiadiazole was obtained with the melting point ranging from 190 to 191 ^{o}C (lit. m.p. 191 ^{o}C [19]).

Preparing bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide (BATD)

Bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide (BATD) (dimer) was obtained according to the method of Sarbu and co-workers described in item [20]. First, 0.40 g (0.01 mole) of sodium hydroxide was diluted in 100 cm^3 of water. Then, 2.2 g (0.01 mole) of technical acetazolamide was introduced at temperature 5–10 ^{o}C. Within the time of 1 hour, wet 2-acetylamino-5-sulfochloro-1,3,4-thiadiazole was added. After introducing sulfochloride, the mixture was kept for 90 minutes at the temperature ranging from 5 to 10 ^{o}C. Residual, unreacted acetazolamide and sulfochloride were filtered out. Afterwards, the filtrate was treated with the 40 % aqueous solution of sodium hydroxide until it reached pH = 12. After 24 hours, a white precipitate was found in the filtrate, then it was separated and dissolved in the minimum amount of water, heated and acidified with 36.0 % hydrochloric acid (d = 1.18 g/cm^3) up to 1 to 3 pH. After cooling, white crystals were separated from the solution, which were filtered and dried in the ambient temperature to the constant weight. 0.645 g of the product was obtained (yield: 15.3 %); melting point: 307–311 ^{o}C (lit.: m.p. 318 ^{o}C [20]).

The solutions of the synthesised compounds of concentration from 0.5 to 100.0 mg/cm^3, presented above, were prepared with the use of the dimethylsulfooxide (DMSO) solvent. A control sample with bactericidal properties was made for every strain of bacteria. The effect of tested heterocyclic compounds concerning their sensitivity to Gram-positive and Gram-negative aerobic bacteria, such as: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg* and *Salmonella typhimurium* was determined. Bacterial strains were received from Hohenheim University in Stuttgart. The above microorganisms were multiplied within 24 hours and diluted in liquid substratum: Standard-I Broth and added to the solid medium: Standard-I Agar (Standard I-Nhragar, Merck No. 7881) in the amount equal to 0.5 cm^3 of suspended microbes per 250.0 cm^3 of agar. Then, Agar with vaccinated microbes was spilled on the Petri dishes and left to solidify. Afterwards, cylindrical wells with the radius of 4 mm were cut in the solidified bases, and filled with solutions of the tested compounds (this activity was performed twice each time and the amounts used were, respectively: 50.0 and 100.0 mg/cm^3; 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 dissolvent itself was acting as the control. The samples were incubated in the temperature of 37 ^{o}C for 24 hours [21]. After the incubation, the areas of microbes growth inhibition (in mm) were measured and the *minimal inhibition concentrations* (MIC) were defined.

Results and discussion

The bactericidal properties of sulfonamides with free amine group in para-position in benzene ring [22] are discussed in the specialist literature. Seeking effective substances to eliminate pathogenic bacteria and, at the same time, the bacteria present in different types of pharmaceutical waste, a group of heterocyclic compounds was typed: *2-amino--1,3,4-thiadiazole* (AT) and *2-acetylamino-1,3,4-thiadiazole-5-sulfonamid* (ATS), and *bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide* (BATD). In order to achieve this aim, studies on biological activity of all the derivatives of 1,3,4-thiadiazole for selected environmental bacteria: *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis, Enterococcus faecium*, *Salmonella senftenberg*, *Salmonella typhimurium*, *Escherichia coli* and *Staphylococcus aureus* were carried out. Pure compounds contained in solid wastes and waste water from diuramid production were used in the research. Tables 1–3 present the determined activities of the tested chemical compounds for different concentrations reacting with the individual bacteria and values necessary to determine their biological activity. The values presented in the form of a table describe: R – half of the zone of bacteria growth inhibition and r – radius of the well equal to 4.0 mm, respectively. Basing on R/r, the activity of a given compound was determined for individual tested bacteria strains within the range of 1,3,4-thiadiazole concentration. As the results clearly illustrate: if the relation $R/r < 1$ occurs, it means low activity of the studied chemical substance. The relation $R/r < 2$ indicates medium and $R/r \geq 2$ high activity [21]. As the data demonstrate, after 24 hours of incubation at temperature 37 ^{o}C, the zone of bacteria growth inhibition expressed in the form of minimum inhibitory concentration (MIC) depended on the type of the tested compound as well as on the

Table 1

Activity of 2-acetamide-1,3,4-thiadiazole-5-sulfonamide (ATS) for chosen bacterial species

Bacterial species	Concentration [mg/cm^3]											
	12.5			25.0			50.0			100.0		
	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity
Escherichia coli	4.0	1.0	++	4.0	1.0	++	4.0	1.0	++	4.0	1.0	++
Staphylococcus aureus	4.0	1.0	++	3.0	0.8	+	3.5	0.9	+	4.0	1.0	++
Bacillus subtilis	—	—	—	—	—	—	—	—	—	—	—	—
Bacillus cereus	3.0	0.8	+	3.5	0.9	+	4.0	1.0	++	n.s.	n.s.	n.s.
Proteus mirabilis	2.5	0.6	+	3.0	0.8	+	3.5	0.9	+	n.s.	n.s.	n.s.
Enterococcus faecium	3.5	0.9	+	2.5	0.6	+	2.5	0.6	+	n.s.	n.s.	n.s.
Salmonella senftenberg	3.0	0.8	+	3.5	0.9	+	3.5	0.9	+	n.s.	n.s.	n.s.
Salmonella typhimurium	2.5	0.6	+	3.0	0.8	+	3.0	0.8	+	n.s.	n.s.	n.s.

Where: n.s. – non-studied; +++ – high activity; ++ – middle activity; + – low activity; — – deficiency of activity.

Table 2

Activity of 2-amide-1,3,4-thiadiazole (AT) for chosen bacterial species

Bacterial species	Concentration [mg/cm^3]																	
	0.5			1.0			12.5			25			50			100		
	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity
Escherichia coli	3.0	0.8	+	4.0	1.0	++	5.0	1.3	++	6.0	1.5	++	6.5	1.6	++	7.0	1.8	++
Staphylococcus aueus	3.5	0.9	+	7.5	1.9	++	14.5	3.6	+++	11.5	2.9	+++	11.0	2.8	+++	12.0	3.0	+++
Bacillus subtilis	4.0	1.0	++	7.5	1.9	++	11.0	2.8	+++	10.0	2.5	+++	11.0	2.8	+++	n.s.	n.s.	n.s.
Bacillus cereus	3.0	0.8	+	4.0	1.0	++	6.5	1.6	++	5.5	1.4	++	8.0	2.0	+++	n.s.	n.s.	n.s.
Proteus mirabilis	—	—	—	2.5	0.6	+	2.5	0.6	+	5.5	1.4	++	7.0	1.8	++	n.s.	n.s.	n.s.
Enterococcus faecium	3.0	0.8	+	3.5	0.9	+	4.0	1.0	++	7.5	1.9	++	13.0	3.2	+++	n.s.	n.s.	n.s.
Salmonella senftenberg	2.5	0.6	+	3.0	0.8	+	4.0	1.0	++	9.0	2.2	+++	11.0	2.8	+++	n.s.	n.s.	n.s.
Salmonella typhimurium	2.5	0.6	+	2.5	0.6	+	3.5	0.9	+	16.0	4.0	+++	17.0	4.2	+++	n.s.	n.s.	n.s.

Where: n.s. – non-studied; +++ – high activity; ++ – middle activity; + – low activity; — – deficiency of activity.

Table 3

Activity of bis(2-acetamide-1,3,4-thiadiazole)-5,5'-disulfonamide (BATD) for chosen bacterial species

Bacterial species	Concentration [mg/cm^3]											
	12.5			25.0			50.0			100.0		
	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity
Escherichia coli	2.5	0.6	+	—	—	—	2.5	0.6	+	3.0	0.8	+
Staphylococcus aueus	—	—	—	3.0	0.8	+	3.5	0.9	+	6.5	1.6	++
Bacillus subtilis	—	—	—	—	—	—	3.0	0.8	+	n.s.	n.s.	n.s.
Bacillus cereus	2.5	0.6	+	3.5	0.9	+	4.0	1.0	++	n.s.	n.s.	n.s.
Proteus mirabilis	3.0	0.8	+	3.0	0.8	+	3.5	0.9	+	n.s.	n.s.	n.s.
Enterococcus faecium	—	—	—	2.5	0.6	+	3.0	0.8	+	n.s.	n.s.	n.s.
Salmonella senftenberg	2.5	0.6	+	2.5	0.6	+	3.5	0.9	+	n.s.	n.s.	n.s.
Salmonella typhimurium	—	—	—	3.0	0.8	+	3.5	0.9	+	n.s.	n.s.	n.s.

Where: n.s. – non-studied; +++ – high activity; ++ – middle activity; + – low activity; — – deficiency of activity.

bacteria strain. Table 1 puts together values referring to determined activities of ATS for the tested species of bacteria. Table 2 contains the results obtained for AT and Table 3 – these for BATD. Tables 1 and 3 present the results for the following concentrations applied in the tests: 12.5, 25.0, 50.0, and 100.0 mg/cm^3. Additionally, Table 2 – presents concentrations 0.5 and 1.0 mg/cm^3 for the most biologically active AT compound. Biological activity for all the derivatives of 1,3,4-thiadiazole with the concentration of 100.0 mg/cm^3 were not tested for: *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis, Enterococcus faecium*, *Salmonella senftenberg* and *Salmonella typhimurium*.

On the basis of the research carried out it was found that ATS shows medium activity of identical values in relation to *Escherichia coli* in all tested concentrations (Table 1). Low activity of ATS with concentrations: 12.5, 25.0, and 50.0 mg/cm^3 was found for: *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg* and *Salmonella typhimurium*. It was found that for *Staphylococcus aureus* and *Bacillus cereus* low activity of ATS prevailed. No activity was detected for *Bacillus subtilis* in the whole range of concentrations. It could be the result of ATS chemical structure and the difference between its functional groups and reactive groups of *Bacillus subtilis*. Therefore, ATS is characterised by low biological activity in relation to the tested strains of bacteria.

The next tested compound: 2-amino-1,3,4-thiadiazole (AT) demonstrates: high activity for *Staphylococcus aureus* with concentrations within the range 12.5–100.0 mg/cm^3 (determined MIC = 12.5 mg/cm^3), medium activity with concentration 1.0 mg/cm^3, and low activity with concentration 0.5 mg/cm^3 (Table 2). Diversified activity was found for *Bacillus cereus*, where high activity for concentration 50.0 mg/cm^3, medium activity – for 1.0 mg/cm^3 and low activity – for 0.5 mg/cm^3; for *Enterococcus faecium* these are, respectively: high activity – MIC = 50.0 mg/cm^3, medium – MIC = 12.5 mg/cm^3, low MIC = 0.25 mg/cm^3 (the only activity value possible to be determined at the concentration of 0.25 mg/cm^3 is not included in the table); *Salmonella senftenberg*: MIC for high activity – 25.0 mg/cm^3, MIC for medium activity – 12.5 mg/cm^3, and MIC for low activity – 0.5 mg/cm^3. In case of *Escherichia coli*, medium activity of AT was found within the range of concentrations from 1.0 to 100.0 mg/cm^3 (MIC of medium activity: 1.0 mg/cm^3) and low activity for 0.5 mg/cm^3. Only *Bacillus subtilis* showed high and medium activities (MIC, respectively: 12.5 mg/cm^3 and 0.5 mg/cm^3) while *Salmonella typhymurium* showed high and low activities (MIC, respectively: 25.0 and 0.5 mg/cm^3). The relation between AT and *Proteus mirabilis* appeared to be an interesting case in which medium activity was determined for concentrations 25.0 and 50.0 mg/cm^3, low activity – for 1.0 and 12.5 mg/cm^3, and zero activity for 0.5 mg/cm^3. It was found that AT was characterised by the most diversified activity amongst the tested 1,3,4-thiadiazole derivatives in relation to *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecium* and *Salmonella senftenberg* bacteria, which could be determined by a suitable selection of MIC.

Dimer bis(2-acetamido-1,3,4-thiadiazole)-5,5'-disulfonamide (BATD) is characterised by its lack of activity or low activity in relation to the tested strains of bacteria. As in the case of ATS towards *Bacillus subtillis*, the dimer shows no activity and low

activity for the concentration of 50.0 mg/cm^3. The lack of BATD activity in relation to *Bacillus subtilis* may be interpreted by similarity of chemical structure of functional groups to ATS.

It was found that the low activity of dimer occurred for *Escherichia coli*, *Proteus mirabilis*, *Salmonella senftenberg*, *Enterococcus faecium,* and *Salmonella typhimurium.* Interestingly enough, the lack of activity was observed for the concentration of 12.5 mg/cm^3. The most diverse activities were observed for *Staphylococcus aureus*: from medium activity for the concentration of 100.0 mg/cm^3, through low activity for the concentrations of 25.0 and 50.0 mg/cm^3, and the lack of activity for the concentration of 12.5 mg/cm^3. Medium activity was found for *Bacillus cereus* for the concentration of 50.0 mg/cm^3, and the deficiency of activity for the concentrations of 12.5 and 25.0 mg/cm^3.

Figures 1–4 present the values' dependence on the growth zone inhibition [in mm] for the given individual bacterial strains as a function relating to the concentration of 1,3,4-thiadiazole derivatives selected for the tests.

Figure 1 shows the values' dependence on the size of growth zone inhibition for *Proteus mirabilis*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella senftenberg*, *Salmonella typhimurium* bacteria towards concentrations of biologically most active compound 2-amino-1,3.4-thiadiazole (AT). For all the tested strains of bacteria with the increase of AT concentration there is the increase in size of growth zone inhibition. The most steady increase of the growth zone inhibition was observed for *Proteus mirabilis, Bacillus cereus* and *Bacillus subtilis*. The difference between values of the growth zone inhibition was observed in case of: *Salmonella senftenberg* and *Salmonella typhimurium* for AT concentration of 25.0 mg/cm^3, which for the concentration of 12.5 mg/cm^3 was higher than the preceding value by 2.25 and 4.6 times, respectively. The values of growth zone inhibition of *Staphylococcus aureus* are worth considering. In this case a significant increase the trend was observed up to AT

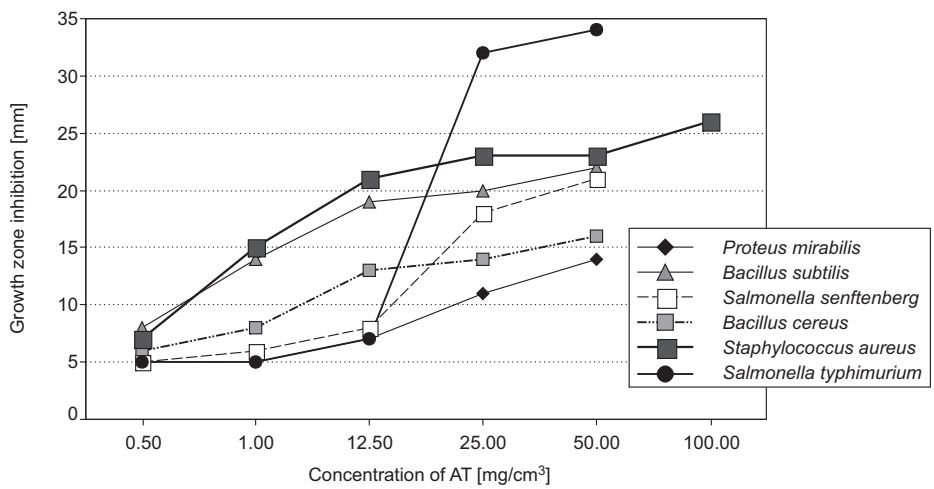

Fig. 1. Effect of AT concentration on the level of the growth zone inhibition for selected strains of bacteria

concentration of 12.5 mg/cm^3, and then the values of this parameter were kept on the same level.

Figures 2–4 present the characteristics for *Escherichia coli* and *Enterococcus faecium* bacteria as its dependence on the growth zone inhibition in the function of ATS (Fig. 2), AT (Fig. 3), and BATD (Fig. 4) concentrations. These bacteria are considered to be an environmental sanitary indicator.

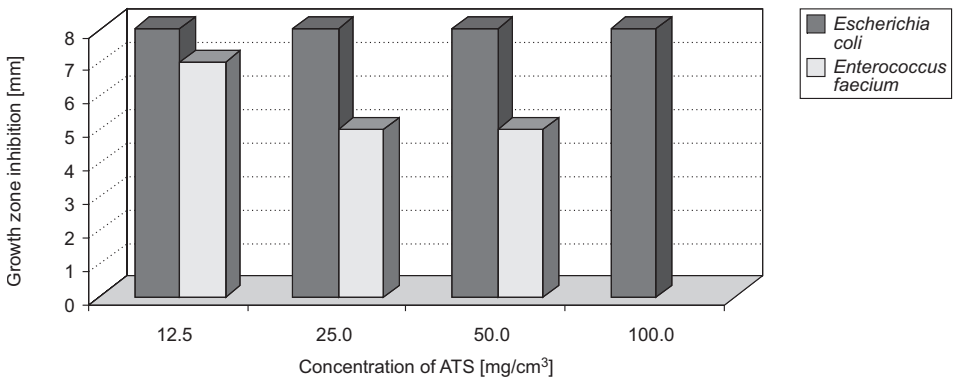

Fig. 2. Effect of ATS concentration on the level of the growth zone inhibition for *E. coli* and *Enterococcus faecium*

Together with the increase of ATS concentration, values of the growth zone inhibition for *Escherichia coli* remained unchanged at 8.0 mm. As far as the case of *Enterococcus faecium* is concerned, a different fact was observed: the value of the growth zone inhibition increased with the decrease of ATS. It might be determined that the optimal ATS concentration which inhibits the *Enterococcus faecium's* growth zone is 12.5 mg/cm^3.

Figure 3 indicates a very steady increase of *Escherichia coli* growth zone inhibition with the increase of AT concentration within the range of 0.5–100.0 mg/cm^3. A more

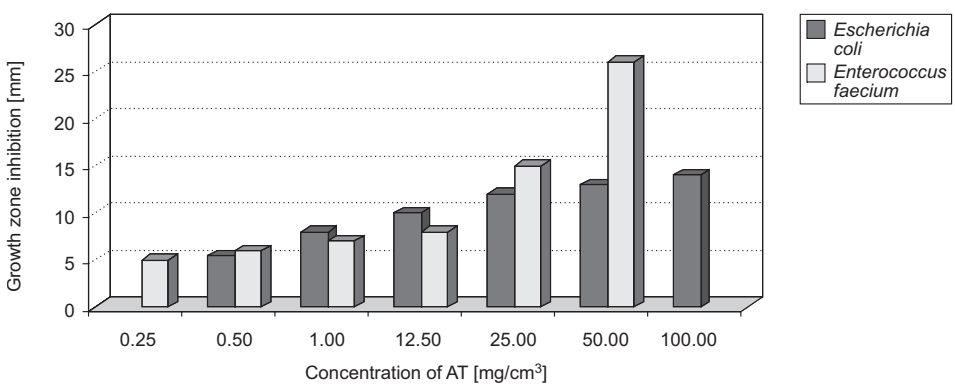

Fig. 3. Effect of AT concentration on the level of the growth zone inhibition for *E. coli* and *Enterococcus faecium*

rapid increase of the *Enterococcus faecium* growth zone inhibition (even up to 25.5 mm) is observed with the increase of AT concentration within the range from 0.25 to 50.0 mg/cm^3.

A small increase in the value of *Escherichia coli* and *Enterococcus faecium* growth zone inhibition (by one unit only) is characterised by the increase of BATD concentration within the range from 25.0 to 50.0 mg/cm^3 for *Enterococcus faecium*, and from 12.5 mg/cm^3 (50.0) to 100.0 mg/cm^3 for *Escherichia coli* (Fig. 4).

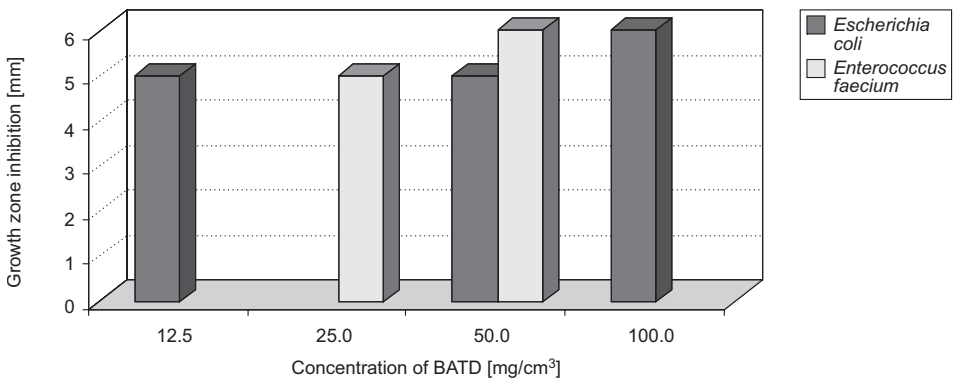

Fig. 4. Effect of BADT concentration on the level of the growth zone inhibition for *E. coli* and *Enterococcus faecium*

For each strain of the tested bacteria, a control test of organic solvent used for dissolving the chemical compounds was carried out. The tests were conducted in order to define a possible effect of the solvent used on the tested microorganisms. The studied microbes are the representatives of pathogenic microorganisms that are generally identified in the environment [23] and belong to the group of the most thermo-tolerable bacteria. Only in case of *Proteus mirabilis* and *Salmonella senftenberg* bacteria an occasional minimum bright area of diameter not exceeding the sensitivity of the method was found.

Conclusion

On the basis of the research it was found out that 2-amino-1,3,4-thiadiazole (AT) presented the highest bacteriological activity in relation to all the tested micro-organisms. It was found that the activity inhibiting the growth of the tested bacteria in this case amounted to 0.25 mg/cm^3. However, bis(2-acetylamino-1,3,4-thiadiazole--5,5'-disulfonamide) (BATD) was characterized by the lowest bacteriological activity, because MIC for this compound was 12.5 mg/cm^3.

2-amino-1,3,4-tiadiazol (AT) turned up to be the most effective as far as inhibiting the growth of the *Salmonella typhimurium* and *Salmonella senftenberg* bacteria is concerned. These microbes belong to the *Enterobacteriaceae* family which contains numerous species and strains living on different organisms and environments [24].

Salmonella typhimurium is a representative of the particularly dangerous microbes causing many pathogenic infections. The data indicate that in order to inhibit the growth of microbes belonging to *Salmonella* type, AT should be applied in the dosage of up to 25.0 mg/cm^3 due to its high activity in this range of concentration. Also, the activity of this reagent with the *Bacillus* type microorganisms appeared to be quite effective, especially in the case of the tested *Bacillus subtilis* species. As it results from the data, its high activity was observed even in the case of the 12.5 mg/cm^3 concentration, and medium activity was found for the concentration of 0.25 mg/cm^3. The tested species of *bacilli,* which belong to the *Bacillus* type are the examples of the microorganisms living in the environment rich in organic substance characteristic for soil. Furthermore, from the clinical point of view they are a source of allergic chronic diseases and alimentary toxicoses [24]. The obtained results indicate that in order to eliminate *Bacillus subtilis* bacteria, high bactericidal activity was observed for the optimal AT concentration of 12.5 mg/cm^3, but medium activity was observed even for 0.25 mg/cm^3. It should be noticed that in case of determining bactericidal activity for this group of bacteria, the deficiency of activity was found in case of *Bacillus subtilis* for ATS and low activity in case of BATD for 50.0 mg/cm^3.

Escherichia coli and *Enterococcus faecium* bacteria were used as indicators of the environmental microorganisms (Fig. 2–4). The activity of ATS and AT, as far as their interaction with *Escherichia coli* is concerned, was found at the medium level for each tested concentration ranging respectively: from 12.5 to 100.0 and from 0.5 to 100.0 mg/cm^3. The presence of these bacteria in the environment, especially in the surface waters, is a basic quality indicator of the biologically clean waters and allows for classifying them into their respective quality classes. However, *Enterococcus faecium* bacteria which belong to *Enterococcus* type are the microbes characteristic for the environment as far as the pollution with *fecal streptococci* is concerned [23]. Furthermore, their presence points at different types of the environmental pollution with the substances of organic origin [25]. As far as their influence on *Enterococcus Faecium* microorganisms activity is concerned the tested compounds were characterized by low effectiveness in relation to ATS and BATD. It was found that AT activity is concentration-based and demonstrates high, medium and low activity with the concentrations: 50 mg/cm^3, 12.5 to 25.0 mg/cm^3 and 0.25 to 0.5 mg/cm^3, respectively. These data proved that the tested thiadiazole derivative could be selectively used to eliminate pollutions of fecal origin.

Furthermore, it can be found that properly selected AT concentrations allow to eliminate selectively such microorganisms as *Salmonella senftenberg*, *Bacillus cereus* and *Staphylococcus aureus*.

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WPŁYW POCHODNYCH 1,3,4-TIADIAZOLI NA AKTYWNOŚĆ WYBRANYCH BAKTERII ŚRODOWISKOWYCH

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Abstrakt: Przedstawiono wyniki badań mikrobiologicznych, prowadzonych z udziałem mikroorganizmów, występujących m.in. w środowisku biologicznym ścieków gospodarczo-bytowych oraz w osadach ściekowych z komunalnych oczyszczalni. Badano wpływ wybranych związków heterocyklicznych pochodnych 1,3,4-tiadiazolu na wrażliwość bakterii aerobowych Gram-dodatnich i Gram-ujemnych. Do badań aktywności biologicznej wytypowano szczepy bakterii środowiskowych: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg* i *Salmonella typhimurium.* Dla badanej grupy bakterii najbardziej aktywnym związkiem chemicznym okazał się 2-amino-1,3,4-tiadiazol. Stwierdzono, że odpowiednio dobrane stężenia tego związku pozwalają na selektywną eliminację takich drobnoustrojów, jak: *Salmonella senftenberg, Bacillus cereus, Staphylococcus aureus* i *Enterococcus faecium*. W przypadku *Escherichia coli* najniższe stężenie hamujące wzrost bakterii (MIC) stwierdzono na poziomie 12,5 mg/cm^3 (wyznaczone względem 2-acetyloamino-1,3,4-tiadiazolo-5-sulfonamidu i bis(2-acetyloamino-1,3,4-tiadiazolo)-5,5'-disulfonamidu), a dla 2-amino-1,3,4-tiadiazolu 0,5 mg/cm^3. Podobne wyniki uzyskano w przypadku bakterii *Enterococcus faecium* z tym, że dla 2-amino-1,3,4-tiadiazolu MIC < 0,25 mg/cm^3, a dla bis(2-acetylamino-1,3,4-tiadiazolo)-5,5'-disulfonamidu MIC = 25,0 mg/cm^3.

Słowa kluczowe: pochodne 1,3,4-tiadiazolu, badania mikrobiologiczne, wskaźnik sanitarny środowiska

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Krystyna PRZYBULEWSKA[1], Anna STOLARSKA[2]
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CHANGE OF PROLINE CONTENT IN SELECTED SOIL FUNGI AS AFFECTED BY OSMOTIC STRESS

ZMIANA ZAWARTOŚCI PROLINY U WYBRANYCH GRZYBÓW GLEBOWYCH POD WPŁYWEM STRESU OSMOTYCZNEGO

Abstract: The study aimed at demonstrating intracellular proline synthesis under osmotic stress conditions in selected soil fungi on the example of *Trichoderma* sp. and *Trichotecium roseum*. Effect of the increase of sodium chloride (NaCl) salinity, in concentrations of 1 to 1000 mmol · dm^{-3} PDA medium, on proline content in the fresh matter of mycelium cultured on medium was examined.

The increase of medium salinity affects production of osmoregulatory substances in the form of proline in selected soil fungi. Its content in mycelium depends on salt (NaCl) concentration in medium as well as on species. Intracellular proline synthesis increased starting with the least salinity. As osmotic stress increased, proline content in mycelium almost quadrupled in *Trichoderma* sp. and quintupled in *Trichotecium roseum*.

Keywords: fungi, salinity, proline

Fungi play an important role in decomposition of organic matter and humification processes in soil. Their number and activity depend largely on different factors, both natural and anthropogenic ones, such as temperature, pH or osmotic pressure. Salinity induces increase of osmotic pressure, which limits water uptake or even makes it impossible and can be a reason of growth arrest or even of cell destruction [1–6].

Microorganisms show certain adaptative mechanisms and adaptabilities which allow them to outlast unfavourable conditions in environment [7–9]. Microorganisms are capable of intracellular accumulation of osmoregulatory substances which allow preserving a turgor indispensable for proper cell functioning [1, 10–12]. These substances include different organic compounds such as amino acids, peptides,

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saccharides, alcohols and others [13–14]. One of these compounds is proline which can be absorbed from environment by microorganisms or synthesised intracellularly by them to tolerate easily growth inhibition [1, 15–19].

The study aimed at demonstrating intracellular proline synthesis on the example of selected soil fungi under osmotic stress conditions.

Material and methods

Two soil fungi, ie *Trichderma* sp. and *Trichotecium roseum* (Photo 1 and 2) were used in the study. Species affinity was determined with microscopic methods and was done by gas chromatography fatty acid methyl ester (FAME) analysis performed by Microbial ID (Newark, DE, USA). Effect of the osmotic stress resulting from sodium chloride (NaCl) salinity in concentrations of 1, 10, 100 and 1000 mmol · dm^{-3} PDA medium was examined. Mycelium cultured on salt-free medium was a control. Experiment was set up in three replications. Culture incubation was carried out on solid media at 24 ^{o}C for 2 weeks. Mycelium was homogenised in 10 % aqueous solution of sulfosalicylic acid in order to determine proline content.

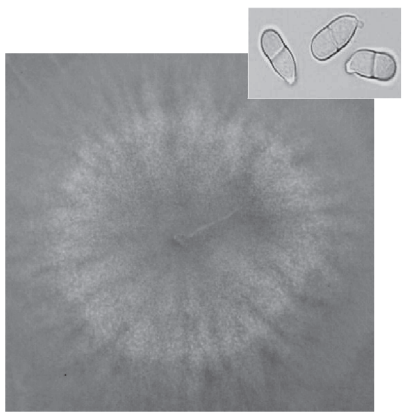

Photo 1. *Trichotecium roseum*

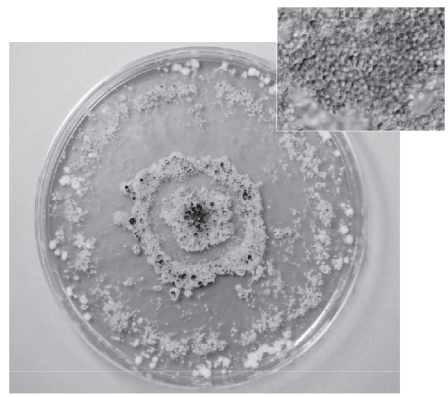

Photo 2. *Trichderma* sp.

Homogenate was filtered through Whatman No. 2 filter paper and filled up to a volume of 10 cm^3. Proline content was determined with the method of Bates et al [20].

Data were converted and given in μg of proline · g^{-1} of mycelium fresh matter. The obtained results were analysed statistically, using analysis of variance and testing the factors with the Duncan's test. Also a Pearson's correlation coefficient was calculated between medium salinity increase and mycelium proline content.

Results and discussion

The number and activity of fungi depends largely on various factors, both natural and anthropogenic ones [7–9]. Osmotic stress, resulting from the increase of medium salinity, has a significant effect on their development and enzymatic activity [4–6].

Based on the analysis of results, it is possible to state that the increase of medium salinity with sodium chloride (NaCl) significantly affected intracellular proline content in the mycelium of examined species. Increase in its content as affected by osmotic stress differed depending on the fungi examined (Fig. 1).

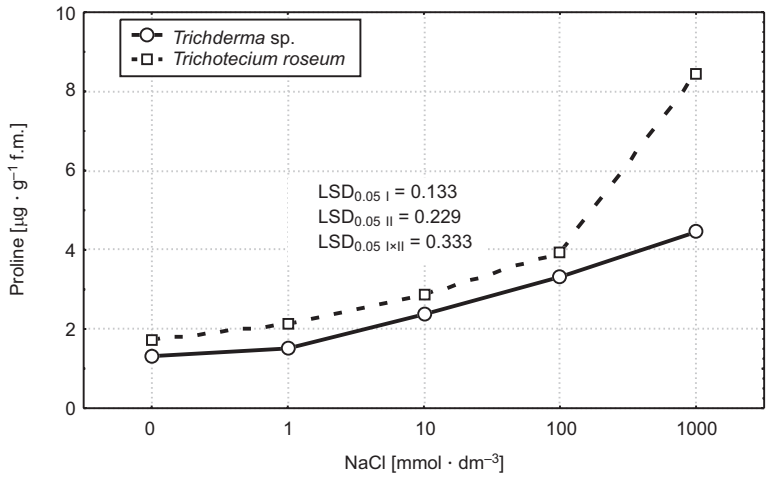

Fig. 1. Proline content in mycelium under sodium chloride (NaCl) medium salinity conditions

Proline content in the mycelium cultured on control medium without NaCl addition amounted to 1.32 µg · g^{-1} f.m. in *Trichoderma* sp., whereas its content was by 39 % larger in *Trichotecium roseum*. Salt addition to medium in the form of NaCl induced a significant increase of the content of examined amino acid irrespective of the species of examined fungus. The least amount of salt introduced into medium, ie 1 mmol · dm^{-3}, increased proline content by 15 % in *Trichoderma* sp. and by 24 % in *Trichotecium roseum* in relation to control (Photo 2). A tenfold and hundredfold increase of medium salinity increased proportionally its content in the mycelium of both examined species. Proline content in mycelium fresh matter was by 70–80 % larger at medium salinity with 10 mmol NaCl · dm^{-3}, while it exceeded its amount two- and a half-fold in relation to its content in the mycelium cultured on control medium. The largest proline content, ie 8.45 µg · g^{-1} f.m., was found in *Trichotecium roseum* cultured on the most salinated medium (1000 mmol NaCl · dm^{-3}). Significantly smaller proline content (33 %) under largest osmotic stress conditions was found in case of the second examined fungus, ie *Trichoderma* sp. Magnitude of the synthesis of osmoregulatory substances of that sort depends on the type of microorganisms and in case of this study depends even on fungus species. On average, its content was almost 20 % larger in *Trichotecium roseum* than in *Trichoderma* sp. (Fig. 2).

The carried out study confirms information that also soil fungi, as exampled by *Trichoderma* sp. and *Trichotecium roseum*, are capable of intracellular accumulation of specific osmoregulatory substances which are stress proteins, in this case proline, under

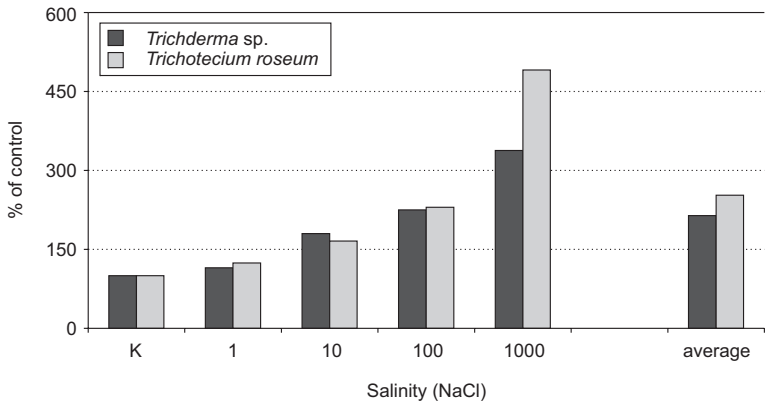

Fig. 2. Proline content in mycelium under sodium chloride (mmol NaCl · dm^{-3}) medium salinity conditions expressed as percent of control

medium salinity increase conditions. These results are corroborated by other authors in studies on bacteria or other eukaryotic organisms [1, 15–18].

In addition, the obtained regression equation, ie y = 0.0043x + 2.252, confirmed by significant correlation coefficient r = 0.84, points to the presence of dependence between medium salinity increase and mycelium proline synthesis.

Conclusions

1. Increase of medium salinity affects production of osmoregulatory substances in the form of proline in selected soil fungi. Its content in mycelium depends on salt (NaCl) concentration in medium as well as on fungus species.

2. Intracellular proline synthesis increased starting with the least medium salinity (1 mmol NaCl · dm^{-3}) and amounted on average to 20 % when compared with control. As salinity increased, its content increase in mycelium almost quadrupled in *Trichoderma* sp. and quintupled in *Trichotecium roseum* under the strongest osmotic stress conditions.

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ZMIANA ZAWARTOŚCI PROLINY U WYBRANYCH GRZYBÓW GLEBOWYCH POD WPŁYWEM STRESU OSMOTYCZNEGO

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Abstrakt: Celem badań było wykazanie syntezy wewnątrzkomórkowej proliny w warunkach stresu osmotycznego u wybranych grzybów glebowych na przykładzie *Trichoderma* sp. i *Trichotecium roseum*. Badano wpływ stresu osmotycznego w wyniku zasolenia chlorkiem sodu (NaCl), w stężeniu od 1 do 1000 mmol · dm^{-3} pożywki PDA na zawartość proliny w świeżej masie grzybni wyrosłej na podłożu.

Wzrost zasolenia podłoża wpływa na wytwarzanie substancji osmoregulacyjnych w postaci proliny u wybranych grzybów glebowych. Jej zawartość w grzybni jest zależna od stężenia soli NaCl w podłożu oraz od gatunku. Synteza proliny wewnątrzkomórkowej zwiększała się począwszy od najmniejszego zasolenia. W miarę wzrostu stresu osmotycznego zawartość proliny w grzybni zwiększyła się prawie czterokrotnie u *Trichoderma* sp. i pięciokrotnie *Trichotecium roseum*.

Słowa kluczowe: grzyby, zasolenie, prolina

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OCCURRENCE OF ENTOMOPATHOGENIC FUNGI AND NEMATODES ON PASTURES IN CENTRAL AND NORTHERN POLAND

WYSTĘPOWANIE GRZYBÓW I NICIENI ENTOMOPATOGENNYCH NA PASTWISKACH W CENTRALNEJ I PÓŁNOCNEJ POLSCE

Abstract: Entomopathogenic fungi and nematodes as important elements of the natural environment focussed the attention of researchers. Both groups of organisms possess an ability to infect and reduce the number of insects at various stages of their growth. This ability was used in the production of biopreparations. For this reason, the studies were aimed at estimating species composition and population density of entomopathogenic fungi and nematodes living in a given ecosystem. Experiments were performed in soils obtained from pastures. Fungi: *Metharisum anisopliae, Bauveria basianna, Pecylomyces fumosoroseus* as well as and nematodes of the family *Steinernematidae* were isolated from soil samples.

Keywords: entomopathogenic fungi and nematode, soil

Entomopathogens comprise a very numerous group of microorganisms including fungi and nematodes. Under natural conditions these organisms were found in various ecosystems. They are an important factor decreasing population density of eg plant pests. Arthropods are their main food base though they do not show distinct food preferences [1–4]. The most frequent entomopathogenic fungi in Poland are the species of the genera: *Peacilomyces*, *Bauveria*, and *Metarhizium*. Apart from entomopathogenic nematodes, these organisms are a very efficient factor controlling arthropod populations and the first discovered pathogens of insects [2, 5, 6]. They were used to control pests already at the end of the 19th century. Intensification of agricultural production and common use of plant protection chemicals inclined researchers to search for alternative methods of plant protection. The last decade has brought many studies on these groups

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of organisms which contributed to the commercial use and production of biopreparations based on local isolates. Species composition and density of entomopathogenic fungi and nematodes depends on factors like: soil type and moisture, human impact and land use in a given area [7–11].

Material and methods

Soil samples collected in 2007 and 2008 from variously managed pastures were used in the experiment. Mixed samples were taken with the soil cane to the depth of 20 cm. The first sample originated from barren pasture in Niekursk which was extensively grazed by sheep. The second study area was a pasture in Konradow grazed by slaughter cattle of the Hereford race and the third pasture was situated in Spala. Since 1985 this pasture has been unmanaged.

Entomopathogenic fungi and nematodes were isolated from soil samples with the method of Zimmermann (1986) [12] using trap insects. The trap organisms were the *G. mellonella* caterpillars from the culture bred in the Department of Zoology, WULS. The experiment was carried out at 25 ^{o}C for 25 days for each soil sample. The first control was performed 5 days after the set up of the experiment and later the samples were controlled every 2–3 days. Dead insects were removed from soil samples and replaced by live ones. Dead insects were transferred to Petri dishes to estimate the reason of their death, to obtain complete growth of mycelium on the skin surface or to multiplicate entomopathogenic nematodes for estimation of their species composition. The obtained results were statistically processed with the SPSS 11 statistical software.

Results and discussion

The use of pastures affected species composition of isolated pathogens and intensity of their occurrence. Three most frequent species of entomopathogenic fungi were isolated from the studied samples were: *P. fumosoroseus, B.bassiana,* and *M. anisopliae* (Table 1). Apart from fungi, entomopathogenic nematodes of the family *Steinernematidae* were also found in studied area. *M. anisopliae* was the most frequent in pastures in Spala and Niekursk. The second frequent fungal species in these pastures was *P. fumosoroseus*. The least frequent species was *B. bassiana* (Table 1).

Table 1

The occurrence of entomopathogenic fungi and nematode in the soil [%]

		Niekursk	Konradow	Spala
Entomopathogenic fungi	*B. bassiana*	2.0	0.7	10.0
	P. fumosoroseus	6.2	24	36.7*
	M. anisopliae	17.4*	0	31.1
Saprofic fungi		10.0	43.3	6.7
Entomopathogenic nematode (*Steinernematidae*)		48.6	27.3*	7.8
Other biotic factor		16.1	4.7	7.8

$p < 0.05$ for the test ANOVA.

The rate of insect infection by nematodes is largely determined by the size of the nematode population in soil and by their ability to infect the host. Studies of three soils from different pastures showed high variability of trap insect infection by nematodes (Table 1). Most entomopathogenic nematodes were isolated from the soil from Niekursk in the first week of the experiment.

Isolating entomopathogenes from soil presents problems with rich soil microflora (saprophagous fungi, plant pathogens, various bacteria and saprophytes). The use of trap insects enables isolation of most frequent species and their further culture under laboratory conditions [1–4, 12, 13] to estimate their species composition. Many authors underline that the occurrence of entomopathogens varies seasonally and their composition depends on sampling date. Entomopathogenic nematodes attack their prey most often in summer and late autumn. This was confirmed in the present experiment. Autumn samples from Niekursk contained more nematodes which infected insects before fungal infection.

According to Ropek (2005) [14] the occurrence of spores of entomopathogenic fungi in soil may significantly decrease nematode infections. Fungal spores infecting an insect produced enzymes that inhibit the growth of mutualistic bacteria living in symbiosis with nematodes. Another factor affecting differentiation of pathogens was the type of pasture used. Pastures in Niekursk and Konradow are used permanently in contrast with that in Spala. Organic fertilisation affects mainly the occurrence of entomopathogenic nematodes [3, 9–11] and less intensively the abundance of fungi [2, 5, 15, 16].

Conclusions

Entomopathogenic fungi were mainly isolated from the soil from pastures in Spała and Niekursk. The dominating species was *M. anisopliae*. Nematodes of the family *Steinernematidae* were less frequent. No representatives of *Heterorhabditis species* were isolated.

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WYSTĘPOWANIE GRZYBÓW I NICIENI ENTOMOPATOGENNYCH NA PASTWISKACH W CENTRALNEJ I PÓŁNOCNEJ POLSCE

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Abstrakt: Polska jako kraj o zróżnicowanej topografii stwarza dogodne warunki do rozwoju oraz zróżnicowania fauny. Do niej zaliczamy m.in. grzyby i nicienie entomopatogenne, które są ważnym elementem środowiska. Ważną rolą jest ich zdolność do infekowania różnych stadiów rozwojowych owadów i ich redukcja. Te umiejętności są wykorzystywane w produkcji biopreparatów. Z tego powodu prowadzone badania miały na celu określenie składu gatunkowego oraz wielkość populacji grzybów i nicieni entomopatogennych występujących w określonym ekosystemie. Doświadczenie przeprowadzono na glebach pozyskanych z pastwisk. Z badanych próbek glebowych wyizolowano takie grzyby, jak: *M. anisopliae, B. basianna, P. fumosoroseus* oraz nicienie z rodziny *Steinernematidae.*

Słowa kluczowe: nicienie entomopatogenne, grzyby entomopatogenne, gleba

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SUCCESSIVE INFLUENCE OF FLUIDAL ASHES ON GENERAL NUMBER OF BACTERIA, ACTINOMYCETES AND FUNGI IN POT EXPERIMENTS

NASTĘPCZY WPŁYW POPIOŁÓW FLUIDALNYCH NA OGÓLNĄ LICZBĘ BAKTERII, PROMIENIOWCÓW I GRZYBÓW W BADANIACH WAZONOWYCH

Abstract: The aim of conducted studies was to determine the impact of fluidal ashes obtained from coal combined with fermented sewage sludge and straw and using effective microorganisms, on the overall abundance of bacteria, fungi, actinomycetes and *coli* bacteria. The experiments were conducted in a pot environment. An increase in the number of bacteria and actinomycetes in the samples containing fluidal ashes and various organic components was observed in comparison with the first year of studies. However, the number of *coli* bacteria, compared with the first two years of the experiment, was significantly reduced.

Keywords: fluidal ash, bacteria, fungi, actinomycetes, *coli* bacteria

Because of physicochemical properties such as very high pH and low organic matter content, slag waste is not a suitable environment for the development of microorganisms. Therefore, when it is used in agriculture, it becomes necessary to enrich it with various types of organic materials, such as composted sewage sludge, straw or effective microorganisms (ceramic EM-X and EM-1), which would also be a source of microflora [1, 2]. The aim of this study was to compare the successive effect of fluidal ashes from CHP Zeran combined with fermented sewage sludge and straw, using effective microorganisms, on the number of bacteria, fungi, actinomycetes and *coli* bacteria.

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Material and methods

In the pot experiment the granulometric composition of loamy and slightly acidic soil (pH_{KCl} 5.13) was used. The experiment was set up by the method of complete randomization in four replications. The scheme of the experiment included eight variants of fertilizing: 1 – control (soil), 2 – control II (fluidal ash), 3 – soil + sewage sludge + straw (4:2:1), 4 – fluidal ash + sludge + straw (4:2:1), 5 – soil + sewage sludge + straw (4:2:1) + microbiological formulation EM-1 (15 $dm^3 \cdot ha^{-1}$), 6 – fluidal ash + sludge + straw (4:2:1) + microbiological formulation EM-1 (15 $dm^3 \cdot ha^{-1}$), 7 – soil + sewage sludge + straw (4:2:1) + microbiological formulation EM-1 (15 $dm^3 \cdot ha^{-1}$) + EM ceramic powder-X (40 $dm^3 \cdot ha^{-1}$), 8 – fluidal ash + sludge + straw (4:2:1) + microbial formulation EM-1 (15 $dm^3 \cdot ha^{-1}$) + EM ceramic powder-X (40 $dm^3 \cdot ha^{-1}$). Soil samples for microbiological analysis were taken after harvesting the crop (test plant *Festulolium*, variation *Felopa*). In the collected samples of ash and soil, the following parameters were determined: the number of bacteria on Bunte-Roviry's base [3], the number of actinomycetes on Cyganow's base [4] and the number of fungi on Martin's base [5]. Because of the fact that digested sludge was used, the number of *coli* bacteria of faecal form was determined according to PN-77, C – 04615, Sheet 07, on Endo nourishment [6].

Cultures incubated at room temperature (20 ^oC) for 3 to 7 days. The number of *coli* bacteria was determined after 24 h of incubation at 37 ^oC. The results were subjected to univariate analysis of variance and then on the basis of Tukey's test $LSD_{0.05}$ values were calculated and homogenous groups were distinguished at significance level of P = 0.05.

Results and discussion

The obtained results are presented in Figs. 1, 2, 3 and 4, homogeneous groups are given. Analyzing the obtained results it should be noted that in comparison with the first year of studies a gradual increase in the number of bacteria in subsequent years was observed. The increase was from 2 to 10 times higher in comparison to the number of bacteria in 2007. Most of these microorganisms were found in samples containing fluidal ash, sewage sludge, straw and microbiological formulations.

The number of actinomycetes also increased in comparison with the first year of study in all examined soil and ash samples. The largest, statistically significant, increase in the number of these bacteria was observed in samples with the addition of sewage sludge, straw and microbiological formulations: EM-1 and EM-X. The significant increase in the number of these microorganisms could be related to the antiacidic influence of the added ashes. The reaction is, in fact, one of the factors influencing the dynamics of the development of soil actinomycetes [7, 8].

The number of microscopic fungi in comparison to the year 2007 increased significantly in the control soil, in the soil with sewage sludge and straw and in the sample with fluidal ash, sediment, straw and microbiological formulations. In other trials the number of fungi, in most cases, was lower in comparison to the amount measured in the second year of the experiment.

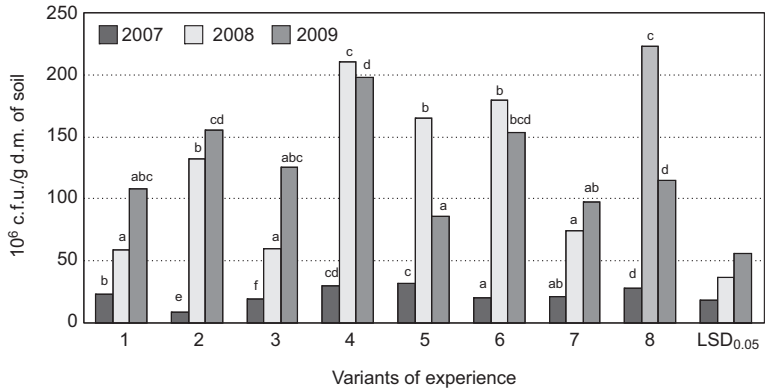

Fig. 1. The number of bacteria in examined samples of soil and fluidal ash
Variants of experiments: 1 – control (soil), 2 – control (fluidal ash), 3 – soil + sewage sludge + straw (4:2:1), 4 – fluidal ash + sewage sludge + straw (4:2:1), 5 – soil + sewage sludge + straw (4:2:1) + microbial formulation EM-1 (15 $dm^3 \cdot ha^{-1}$), 6 – fluidal ash + sewage sludge + straw (4:2:1) + microbial formulation EM-1 (15 $dm^3 \cdot ha^{-1}$), 7 – soil + sewage sludge + straw (4:2:1) + microbial formulation EM-1 (15 $dm^3 \cdot ha^{-1}$) + EM-X Ceramic Powder (40 $dm^3 \cdot ha^{-1}$), 8 – fluidal ash + sewage sludge + straw (4:2:1) + microbial formulation EM-1 (15 $dm^3 \cdot ha^{-1}$) + EM-X Ceramic Powder (40 $dm^3 \cdot ha^{-1}$)

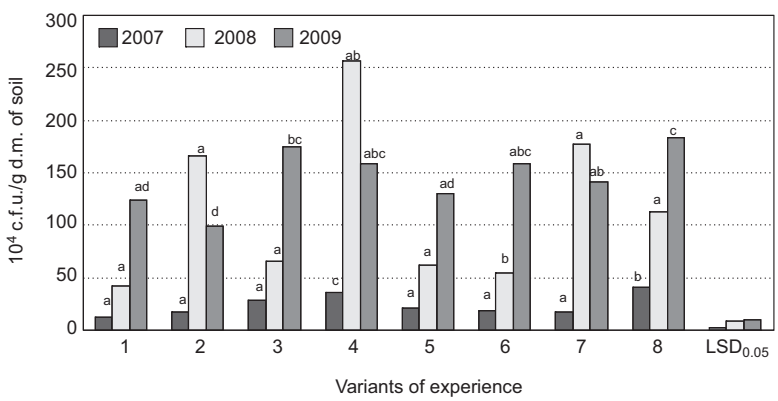

Fig. 2. The number of actinomycetes in examined samples of soil and fluidal ash
Explanations: see Fig. 1.

The number of *coli* bacteria, as an indicator of sanitary contamination, ranged from about 250 to 1200 in the first year of studies, up to about 2–20 cells in 1 g d.m. of soil and fluidal ash sample in the third year of research (Fig. 4). Compared with the control soil, statistically most of these bacteria were observed in the soil with sewage sludge and straw and in the combination of soil, sewage sludge, straw and microbiological formulation EM-X. The statistically lowest content of *coli* occurred in the control soil and fluidal ash.

Summarizing the results of three-year studies on the fitness of fluidal ashes for agricultural purposes and soil recultivation, it should be noted that the addition of

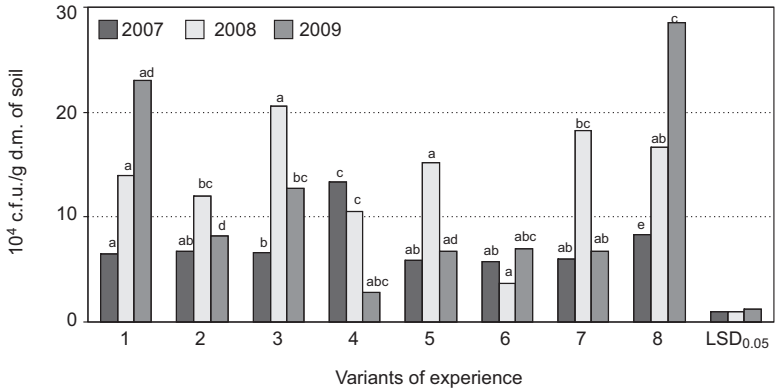

Fig. 3. The number of fungi in examined samples of soil and fluidal ash
Explanations: see Fig. 1.

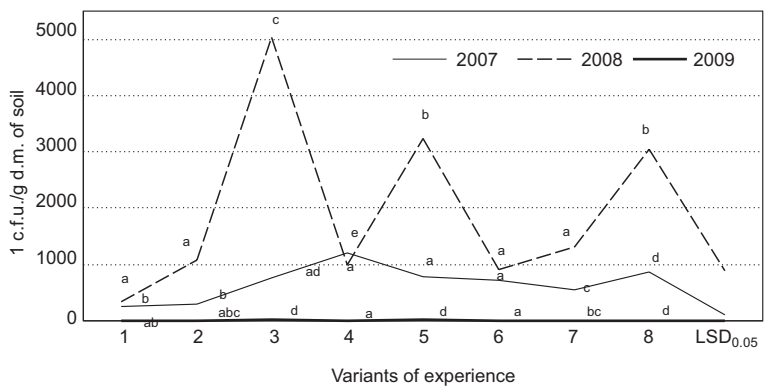

Fig. 4. The number of *coli* bacteria in examined samples of soil and fluidal ash
Explanations: see Fig. 1.

organic materials, such as composted sewage sludge, which is rich in microorganisms or microbial preparations EM-1 or EM-X to ashes or soils with ashes, caused an statistically significant increase in the number bacteria and actinomycetes. Studies by other authors [9–11] confirm the stimulative effect of fermented sewage sludge on the number and activity of many groups of microorganisms. Also the addition of microbiological formulations EM-1 or EM-X, caused in particular the increase in the number of bacteria and actinomycetes. As indicated in a few studies, the EM formulations, containing several strains of microorganisms isolated from soil, classified in different taxons, and having different physiological and biochemical features [12] have demonstrated the beneficial influence both on the functioning of a soil ecosystem and the intensity of growth and development of plants. The results of conducted studies indicate that the addition of effective microorganisms to soil or fluidal ash increased the number of microorganisms, and hence, caused the intensification of metabolic and biochemical processes in soil, and, indirectly, affected pedogenetic abilities of micro-

organisms. Enrichment of ash in the microbiological formulation (effective micro-organisms) accelerated the mineralization of soil organic compounds and thereby increased the biological activity of soil [13, 14]. The obtained results indicate that coal ashes can be used for fertilizing purposes.

Conclusion

1. Compared with the first year of the experiment an increase in the overall number of bacteria and actinomycetes in soil and ash samples was found.
2. There was a significant decrease in the number of *coli* bacteria in comparison to the first year of studies.

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NASTĘPCZY WPŁYW POPIOŁÓW FLUIDALNYCH NA OGÓLNĄ LICZBĘ BAKTERII, PROMIENIOWCÓW I GRZYBÓW W BADANIACH WAZONOWYCH

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Abstrakt: Celem przeprowadzonych badań było określenie następczego wpływu popiołów fluidalnych z węgla kamiennego w połączeniu z przefermentowanym osadem ściekowym i słomą, przy użyciu efektywnych mikroorganizmów, na ogólną liczebność bakterii, grzybów, promieniowców oraz bakterii z grupy *coli*. Badania prowadzono w warunkach wazonowych. Stwierdzono wzrost liczebności bakterii

i promieniowców w próbkach zawierających popiół fluidalny oraz różne komponenty organiczne w porównaniu z pierwszym rokiem badań. Natomiast liczebność bakterii z grupy *coli* w porównaniu z dwoma pierwszymi latami doświadczenia uległa istotnemu zmniejszeniu.

Słowa kluczowe: popiół fluidalny, bakterie, grzyby, promieniowce, bakterie z grupy *coli*

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Katarzyna GLEŃ

EFFECT OF FOLIAR FERTILIZERS AND THEIR MIXTURES ON PHYTOPATHOGENIC *FUSARIUM* FUNGI

WPŁYW NAWOZÓW DOLISTNYCH I ICH MIESZANIN NA GRZYBY FITOPATOGENNE Z RODZAJU *FUSARIUM*

Abstract: The paper focuses on the response of phytopathogenic *Fusarium* fungi to various concentrations of foliar fertilizers: Mikrovit Fe, Mikrovit Zn, urea, magnesium sulphate, and the mixtures of Mikrovit Fe + urea + magnesium sulphate, and Mikrovit Zn + urea + magnesium sulphate added to the medium. Under in vitro conditions, the analysis determined the influence of the foliar fertilizers on linear growth, biomass increment and sporulation of the following fungi: *Fusarium poea*, *Fusarium sulphureum* and *Fusarium culmorum*.

Mikrovit Zn revealed the strongest fungistatic properties among the tested foliar fertilizers. Applied to the medium in 1.0 mm^3/cm^3 concentration, it very strongly inhibited the linear growth (91.93–94.17 %) and sporulation of all tested fungi and most strongly limited biomass increments in *F. poea* and *F. sulphureum*, whereas mixtures of Mikrovit Zn and Mikrovit Fe with urea and magnesium sulphate revealed slightly weaker fungistatic effect. Urea applied in 1.0 mm^3/cm^3 concentration reduced increments of the test fungi biomass in the range from 56.73 to 64.03 %, while magnesium sulphate, as the only one among the fertilizers used for the experiment, stimulated surface growth, biomass increment and sporulation process in all tested fungi. It should be remembered that in the agrocenoses the effect of foliar fertilizers on fungi infecting plants is more complex and conditioned by many factors. Therefore, it is necessary to conduct further research on the influence of foliar application of fertilizers on plant healthiness.

Keywords: *Fusarium*, foliar fertilizers, linear growth, biomass, sporulation

Among phytopathogenic organisms fungi of *Fusarium* genus deserve special attention, since they are the most common in the environment where they infect many plant species causing various diseases which are difficult to control, such as fusarium wilts, take-all diseases or dry rot [1–2]. In the opinion of many authors [3–5] fusarioses are particularly dangerous in cereal crops since they not only contribute to yield losses, but infect grains with mycotoxins. Chemical plant protection is the most efficient method to combat these diseases, but it may negatively affect the quality of raw plant materials and pollute the environment. Several authors reported that increasingly

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common foliar fertilization not only positively affects the crop yield and its quality but may also efficiently prevent and protect plants against infectious diseases [6–12].

The investigations were conducted to test whether commonly applied foliar fertilizers Mikrovit Fe, Mikrovit Zn, magnesium sulphate, urea and their mixtures (Mikrovit Fe + magnesium sulphate + urea, and Mikrovit Zn + magnesium sulphate + urea) may limit the development of the following phytopathogenic fungi: *Fusarium poea* (Peck) Wollenw., *Fusarium culmorum* (W.G. Smith) Sacc. and *Fusarium sulphureum* Schlecht. under in vitro conditions.

Materials and methods

The fertilizers were tested on the phytopathogenic fungi selected from the collection owned by the Agricultural Environment Protection Department: *Fusarium poea*, *Fusarium culmorum* and *Fusarium sulphureum*, isolated from infected wheat kernels. Each of the studied fertilizers, ie Mikrovit Fe (Fe – 3 %, N – 4.50 %, pH – 3.2), Mikrovit Zn (N – 4.50 %, Zn – 3.5 %, pH – 2.0), magnesium sulphate (Mg – 15.65 %, S – 17.20 %, pH – 6.8) and urea (N – 46 %) and Mikrovit Fe + magnesium sulphate + urea, and Mikrovit Zn + magnesium sulphate + urea) were added to PDA medium with pH = 6.32 to obtain their medium concentrations of 0.1 mm^3/cm^3 (field dose) and 1.0 mm^3/cm^3. Subsequently, the tested fungus inoculum was supplied to Petri dishes containing the consolidated medium with added tested fertilizers. The experiment was conducted in five replications for each fertilizer combination and for each individual tested fungus. Petri dishes with the medium without fertilizers provided the control. The fungi were cultured in a thermostat at 23 ^{o}C. Daily increments of the fungi colonies served to compute the coefficient of the tested fungi linear growth rate in each fertilizer combination and on the control.

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \ldots + \frac{b_x}{d_x}$$

where: T – linear growth rate coefficient,
A – mean of colony diameter measurements [mm],
D – number of days since the experiment outset,
$b_1, \ldots, b_x$ – increment of colony diameter since the last measurement [mm],
$d_1, \ldots, d_x$ – number of days since the last measurement.

After three weeks of fungi culturing on PDA media with added foliar fertilizers and on the control, the number of spores was assessed in the Thome hemocytometer. The fungi biomass growth was maintained in 300 cm^3 Erlenmayer flasks on 100 cm^3 of the modified PDA medium (without agar-agar) with the foliar fertilizers added in the same concentrations as in the experiment on Petri dishes. The effect of individual foliar fertilizers on the linear growth of the studied phytopathogenic fungi was presented as a difference between fungus colony diameter on the control Petri dishes and fungus colony diameter on the Petri dishes with individual fertilizer concentrations, and converted into the inhibition-stimulation coefficient acc. to Abbot [3]. Biomass

increments were assessed in the same way. The results were elaborated statistically using ANOVA and the significance of differences was assessed by means of the t-Student test.

Results and discussion

For their development, fungal organisms need macroelements and microelements which they use for hyphae formation and production of numerous biologically active compounds and enzymes [14–15]. Conducted experiments allowed to determine that surface growth, biomass increment and sporulation of the tested fungus species were modified by the kind of foliar fertilizer and its concentration. Moreover within the same *Fusarium* genus, the tested fungi species differ with their sensitivity to the applied fertilizer preparations. The fact was confirmed by the Authors' and other previous investigations [16–21]. Among the tested foliar fertilizers, Mikrovit Zn applied in 1.0 mm^3/cm^3 concentration revealed the strongest fungistatic effect, almost completely (91.94–94.12 %) inhibiting the linear growth and blocking sporulation process in all tested fungal organisms (Tables 1, 3).

Table 1

Coefficients of rate (T) and inhibition of tested fungi linear growth

Foliar fertilizers	Concentration [mm^3/cm^3]	*F. poea*		*F. sulphureum*		*F. culmorum*	
		[T]	[%]	[T]	[%]	[T]	[%]
Urea	1.0	23.77	56.74	18.45	64.03	21.33	61.20
	0.1	55.45	6.35	44.80	20.79	52.28	16.80
Magnesium sulphate	1.0	73.06	+5.07*	81.01	+26.02	49.44	21.17
	0.1	60.40	1.98	49.77	16.07	89.50	+38.30
Mikrovit Fe	1.0	79.48	0.95	39.80	26.70	61.46	15.02
	0.1	80.60	+0.43	70.98	20.50	77.30	+11.02+
Mikrovit Zn	1.0	1.00	94.17	1.00	91.93	1.55	94.18
	0.1	61.29	35.94	62.90	+6.05	37.70	43.22
Mikrovit Fe + urea + magnesium sulphate	1.0	9.95	84.17	24.16	54.43	6.10	86.75
	0.1	59.58	31.18	63.09	+9.69	32.64	49.65
Mikrovit Zn + urea + magnesium sulphate	1.0	29.68	58.90	29.8	41.90	10.58	80.46
	0.1	80.10	0.20	52.1	8.62	34.03	48.34
Control		80.25		59.63		70.75	
$LSD_{0.05}$ for fertilizer kind		1.67		2.11		2.31	
$LSD_{0.05}$ for fertilizer concentration		1.13		1.14		1.24	

* + denotes linear growth stimulation.

On the other hand inhibition of *F. culmorum* and *F. sulphureum* biomass growth reached 67.88 % and 68.83 %, respectively (Table 2).

Table 2

Coefficient of biomass increment coefficient depending on foliar fertilizer [%]

Foliar fertilizers	*F. poea*		*F. sulphureum*		*F. culmorum*	
	Concentration [mm^3/cm^3]					
	1.0	0.1	1.0	0.1	1.0	0.1
Urea	44.60	5.35	53.25	41.48	22.92	10.91
Magnesium sulphate	17.85	+7.14	+17.46	4.36	+21.83	+11.64
Mikrovit Fe	73.21	73.21	46.58	58.22	52.40	55.31
Mikrovit Zn	91.07	55.35	68.33	39.30	67.88	50.22
Mikrovit Fe + urea + magnesium sulphate	69.64	75.00	60.00	56.77	66.96	64.05
Mikrovit Zn + urea + magnesium sulphate	73.21	70.53	56.77	53.31	71.32	65.50

+ denotes biomass increment stimulation.

A strongly inhibiting effect on *Fusarium* fungi was also observed after the application of English foliar fertilizer Yeald to the medium, which similar as Mikrovit Zn contained zinc [21]. Combined application of Mikrovit Zn with urea and magnesium sulphate in the Authors' own experiments more weakly affected the studied phytopathogenic fungi species. In the conducted experiment magnesium sulphate was the only fertilizer whose supplement in the medium, particularly in the higher concentration (1.0 mm^3/cm^3), stimulated hyphae surface growth and biomass increment in all test fungi species. Moreover it favoured sporulation, primarily in *F. poea* and *F. culmorum* (Table 3).

Table 3

The impact of foliar fertilizers on test fungi sporulation (quantity in 1 $cm^3 \times 10^6$)

Foliar fertilizers	*F. poea*		*F. sulphureum*		*F. culmorum*	
	Concentration [mm^3/cm^3]					
	1.0	0.1	1.0	0.1	1.0	0.1
Urea	0.16	0.30	8.96	2.78	1.10	0.59
Magnesium sulphate	12.04	17.23	1.55	3.24	2.10	2.80
Mikrovit Fe	0.30	0.35	13.87	1.52	14.60	3.27
Mikrovit Zn	—	0.22	—	1.55	—	2.12
Mikrovit Fe + urea + magnesium sulphate	0.05	0.20	0.15	0.4	0.87	2.30
Mikrovit Zn + urea + magnesium sulphate	3.00	0.10	0.17	2.45	0.30	1.00
Control	9.90		2.17		1.72	

Therefore, it may be assumed that the availability in the medium of other elements, such as magnesium or sulphur alleviates the fungistatic effect of zinc. On the other hand, on media with added 1.0 mm^3/cm^3 (field dose) of urea, limited surface growth of the tested fungi in the range from 56.73 % to 64.03 % and their biomass increment (22.93–53.255) were observed (Tables 1, 2). Irrespective of which concentration of this

fertilizer was applied, a considerable reduction of the produced spore number was observed in *F. poea* and *F. culmorum* (Table 3), as well as modified colour of aerial mycelium (naturally pink became white). Mikrovit Fe revealed weak fungistatic properties, particularly in a short experiment on Petri dishes. No matter which concentration was applied, this fertilizer limited the linear growth of *F. sulphureum* by 23.6 % and *F. culmorum* only by 2.0 %.

A variable effect of iron on *Fusarium* fungi was demonstrated also by other authors [16, 22], since iron may inhibit growth of fungal pathogens at limited element availability and stimulate at the element excess in the environment. On the other hand, Mikrovit Fe mixture with urea and magnesium sulphate had stronger effect, which may have resulted from accumulation of the fungistatic properties of its components (Tables 1–3). The number of *F. sulphureum* macroconidia on the medium containing 1.0 mm^3/cm^3 of this fertilizer mixture was thirteen times lower than in the control (Table 3), while the components of this mixture, ie urea and Mikrovit Fe applied separately in 1.0 mm^3/cm^3 concentration, strongly stimulated spore formation in *F. sulphureum*. The total number of spores produced by the phytopathogens evidences their infection potential, therefore, at reduced spore number the risk of plant infection diminishes [14].

Conclusions

Strong fungistatic properties, particularly of Mikrovit Zn and its mixtures with commonly used fertilizers, ie urea and magnesium sulphate and urea used separately, as presented in the paper, may find applications in the agricultural practice. It should be expected that application of these foliar fertilizers in cultivation of plants requiring zinc feeding, ie vegetables and fruit trees may result in less frequent occurrence of fungal diseases, especially when they are caused by fungi of *Fusarium* genus. Moreover, fungistatic properties of foliar fertilizers may undoubtedly contribute to reduce the amount of herbicides used for plant protection. Therefore, it is necessary to undertake research on the effect of foliar fertilizers on plant healthiness.

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WPŁYW NAWOZÓW DOLISTNYCH I ICH MIESZANIN NA GRZYBY FITOPATOGENNE Z RODZAJU *FUSARIUM*

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Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Badano reakcję grzybów chorobotwórczych z rodzaju *Fusarium* na dodatek do podłoża hodowlanego różnych stężeń nawozów dolistnych: Mikrovit Fe, Mikrovit Zn, mocznik, siarczan magnezu oraz mieszanin: Mikrovitu Fe + mocznik + siarczan magnezu i Mikrovitu Zn + mocznik + siarczan magnezu. W warunkach *in vitro* oceniano wpływ nawozów dolistnych na wzrost liniowy, przyrost biomasy i zarodnikowanie grzybów: *Fusarium poea*, *Fusarium sulphureum* i *Fusarium culmorum*.

Spośród badanych nawozów dolistnych Mikrovit Zn odznaczał się najsilniejszymi właściwościami fungistatycznymi. Zaaplikowany do podłoża hodowlanego w stężeniu 1.0 mm^3/cm^3 bardzo silnie hamował rozrost powierzchniowy (91.93–94.17 %) i zarodnikowanie wszystkich testowanych grzybów oraz najsilniej ograniczał przyrost biomasy *F. poea* i *F. sulphureum*. Natomiast nieco słabszą efektywność fungistatyczną wykazywały mieszaniny: Mikrovitu Zn oraz Mikrovitu Fe z mocznikiem i siarczanem magnezu. Mocznik zastosowany w stężeniu 1.0 mm^3/cm^3 ograniczał przyrosty biomasy grzybów testowych w zakresie od 56.73 do 64.03 %. Z kolei siarczan magnezu, jako jedyny spośród zastosowanych w doświadczeniu nawozów, stymulował wzrost powierzchniowy, przyrost biomasy oraz proces sporulacji wszystkich grzybów testowych. Należy pamiętać, że w agrocenozach oddziaływanie nawozów dolistnych na grzyby porażające rośliny jest bardziej złożone i uwarunkowane wieloma czynnikami. Dlatego istnieje potrzeba przeprowadzania badań nad wpływem aplikacji nalistnej nawozów na zdrowotność roślin.

Słowa kluczowe: *Fusarium*, nawozy dolistne, wzrost liniowy, biomasa, zarodnikowanie

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INFLUENCE OF THE CULTURE CONDITIONS ON LIPOLYTIC ACTIVITY OF *Bacillus cereus* AND *Bacillus mycoides*

WPŁYW WARUNKÓW ŚRODOWISKA NA AKTYWNOŚĆ LIPOLITYCZNĄ SZCZEPÓW *Bacillus cereus* I *Bacillus mycoides*

Abstract: The aim of the research was the evaluation of lipolytic activity of *B. cereus* and *B. mycoides* strains, in reference to carbon source, pH and the temperature. In the research, two strains of *Bacillus cereus* and *Bacillus mycoides* each, isolated from the soil and water, were applied. The sources of carbon in culture media were fatty substrates: tributyrin, Tween 40, Tween 60, Tween 80 and glucose. The lipolytic activity was measured by means of titration at pH ranging from 5 to 8 and the temperature ranging from 30 °C to 60 °C. The results were presented in the units of lipolytic activity [U cm^{-3}]. In the conducted research, the amount of liberated lipolytic activity depended on the type of fatty substrate in the medium, pH and the temperature. The strains under study showed the lowest activity at pH 5 and 6, and the highest at pH 7 and 8. In these conditions, most of the strains showed the lipolytic activity, even in case of the lack of fatty substrate in the medium. The highest amount of lipolytic activity was liberated at pH 8 in the medium with Tween 40, and the highest results (0.88 [U cm^{-3}]) were noted for the soil strain *B. cereus*. When analysing the influence of the temperature on the lipolytic activity, it was stated that the highest amount of lipolytic activity was noted at 30 and 40 °C, and the lowest at 50 and 60 °C. The best results were obtained for most of the strains at 30 °C, in medium with Tween 40, and the most active was the soil strain of *B. mycoides* (0.88 [U cm^{-3}]). The exception is *B. cereus*, as it liberated 1.38 [U cm^{-3}], in the medium with glucose. Taking into account all analysed sources of carbon and parameters, it seems that the most active were *B. mycoides* strains.

Keywords: *Bacillus cereus, Bacillus mycoides*, lipases, tributyrin, Tween

Lipases are an important group of biotechnologically valuable enzymes. They are defined as hydrolases of glycerol esters EC 3.1.1.3, and are the enzymes of high catalytical potential. They are produced by plants, animals and microorganisms, of which the last group remains in the centre of attention. Many kinds of bacteria possess

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the ability to produce them, among others bacteria of *Bacillus* kind [1, 2]. A common interest in bacterial lipases is connected with their role as biocatalysts in many biochemical processes. They have diverse applications in a wide variety of industries ranging from detergent, oleochemical, organic synthesis, dairy, fat and oil modification to pharmaceutical. They have many applications for stereospecific hydrolysis and synthesis of a wide variety of technologically valuable esters [1, 2].

The interest in microbial lipase production has risen in the last decades, because of its large potential in environmental protection as wastewater treatment as well as decomposition and removal of oil substances. Effective breakdown of solids and the clearing and prevention of fat blockage or filming in waste systems are important in many industrial operations. Bacterial lipases are also involved in solution of such environmental problems as the breakdown of fats in domestic sewage and anaerobic digesters [3].

As shown by data in literature [2, 4–6], they are varied in terms of their enzymatic activity, which depends on the species of microbes and the culturing conditions (eg pH of the growth medium, temperature, source of nitrogen and presence of lipids in the medium). Therefore, screening of microorganisms with lipolytic activities could facilitate the discovery of novel lipases.

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

Materials and methods

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

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

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

The inoculum was produced in the basal medium consisted of 1.0 g · dm^{-3} yeast extract, K_2HPO_4 3.0 g · dm^{-3}, KH_2PO_4 2.0 g · dm^{-3}, $(NH_4)_2SO_4$ 2.0 g · dm^{-3} and $MgSO_4 \cdot 7H_2O$ 0.5 g · dm^{-3}. The sources of carbon in culture media were the following fatty substrates: tributyrin, Tween 40, Tween 60, Tween 80 and glucose. The cultures were maintained in Erlenmeyer flasks of 250 cm^3 capacity containing 50 cm^3 of respective growth medium with an inoculum of density equal to E = 2, obtained from the 48-hour culture on a nutrient broth. Incubation was conducted on a rotary shaker for 2 days at 30 ^oC.

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

a subtraction between the proper treatment and the control treatment results. The results were presented in the units of lipolytic activity. The unit was expressed as the amount of μmoles of 0.05 M NaOH required to neutralize fatty acids liberated by the lipases contained in 1 cm^3 of post-culture liquid within 1 minute. The lipolytic activity was expressed in the unit U cm^{-3}.

Results and discussion

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

In conducted tests, lipolytic activity depended on the carbon source in the medium, pH and the temperature, while individual *B. cereus* and *B. mycoides* strains showed varied activity in exocellular lipases production.

For the soil strain *B. cereus* A96, a reverse correlation between their lipolytic activity and the temperature has been observed (Fig. 1) and it has been stated that the temperature growth caused a decrease in lipolytic activity U. The highest values were obtained at 30 °C in the presence of glucose as carbon source – 1.38 U cm^{-3}, and the lowest at 60 °C in the medium of Tween 80 and glucose – 0.13 U cm^{-3}. The highest, 10-fold, decrease in the lipolytic activity, has been recorded on the medium with glucose, while Tween 40 seemed to be the most stable and favourable source of the fatty substrate for *B. cereus* A96 (Fig. 1).

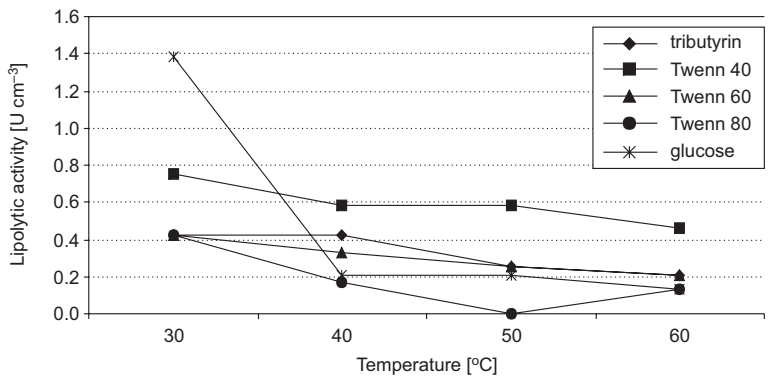

Fig. 1. Influence of temperature on the lipolytic activity of *Bacillus cereus* A96

In the presented paper, there has been no clear relationship found between the lipolytic activity and the temperature in case of *B. cereus* G10 strain isolated from water (Fig. 2). However, the highest activity has been recorded at 30 °C and the lowest at 60 °C, regardless of the carbon source. The most favourable source of the fatty substrate was Tween 40, where the highest values of the activity have been noted, regardless of the temperature. The highest value of 0.71 U cm^{-3} was noted at 30 °C. The least favourable source of carbon was Tween 80 and glucose, as irrespective of the

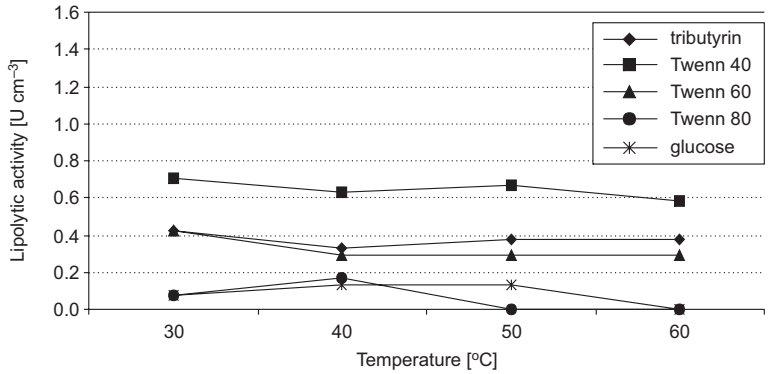

Fig. 2. Influence of temperature on the lipolytic activity of *Bacillus cereus* G10

temperature, obtained results were the lowest. Also, the activity of exocellular hydrolases has not been noticed at 50 °C and 60 °C in case of Tween 80, and at 60 °C in case of medium containing glucose (Fig. 2).

Growing temperature did not promote exocellular production of lipases in case of *B. mycoides* A134 and G3 strains (Fig. 3 and 4).

The exception has been noted for *B. mycoides* A134 strain on the medium with glucose, where the lipolytic activity grows from the initial value of 0.83 U cm^{-3} at 30 °C to 1.46 U cm^{-3} at 40 °C, to reach the value of 0.46 U cm^{-3} at the temperature 60 °C (Fig. 3).

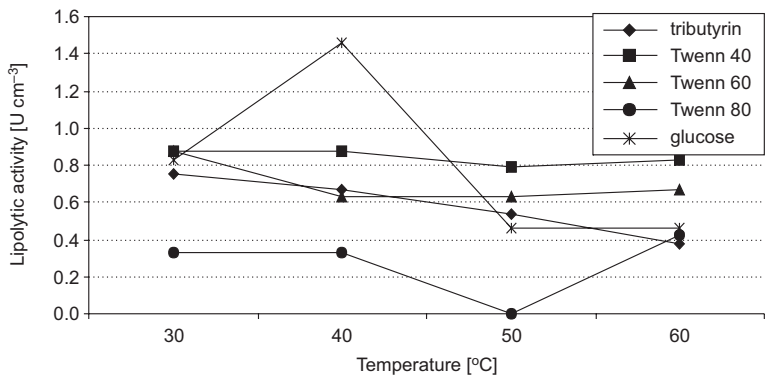

Fig. 3. Influence of temperature on the lipolytic activity of *Bacillus mycoides* A134

The most favourable medium for *B. mycoides* A134 strain, among media containing fatty substrate as the source of carbon, is medium with Tween 40. Recorded values of the lipolytic activity were the highest at all temperatures, when compared with other fatty substrates. The lipolytic activity at 30 °C amounted 0.88 U cm^{-3} and decreased slightly to the value of 0.83 U cm^{-3} at 60 °C. The least favourable fatty substrate was Tween 80, as at 30 °C measured activity amounted only 0.33 U cm^{-3}, and at 50 °C the strain did not show exocellular activity of hydrolases (Fig. 3).

In case of *B. mycoides* G3 strain the most favourable was the medium with Tween 40, similarly to *B. mycoides* A134 (Fig. 4). The highest values of the lipolytic activity have been obtained in the temperature ranging between 30 and 60 °C and amounted 0.83–0.67 U cm^{-3}. Similar changes, to the aforementioned, have been noted in reference to the medium with glucose, but recorded values of the lipolytic activity have been significantly lower. At the temperature of 30 °C the lipolytic activity amounted 0.58 U cm^{-3}, grew up to the value of 0.71 U cm^{-3} at 40 °C and reached the value of 0.33 U cm^{-3} at the highest temperature. The lowest difference in the lipolytic activity, at respective temperatures, have been obtained for the media with the following fatty substrates: tributyrin, Tween 40 and Tween 60 (Fig. 4).

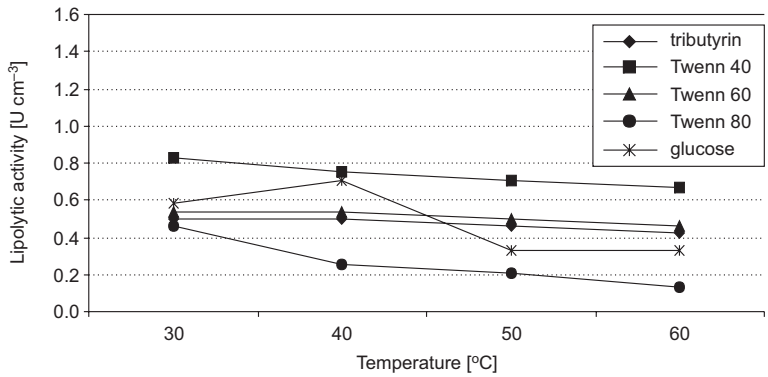

Fig. 4. Influence of temperature on the lipolytic activity of *Bacillus mycoides* G3

Presented paper considers also the influence of pH on the lipolytic activity of tested *B. cereus* and *B. mycoides* strains, with reference to the source of carbon in the culture medium. Some correlation has been observed in most cases, regardless to the strain tested and the culture medium, namely pH increase in the range between 5–8 promotes the increase in the lipolytic activity (Figs. 5–8).

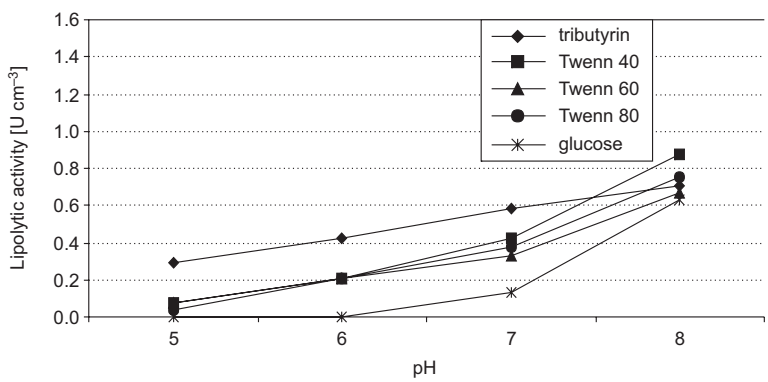

Fig. 5. Influence of pH on the lipolytic activity of *Bacillus cereus* A96

The most favourable culture media for the soil strain *B. cereus* A96 are those containing tributyrin and Tween 40, as the source of the fatty substrate. The gradual growth of the lipolytic activity from the initial amount of 0.29 U cm^{-3} at pH 5, up to the amount of 0.71 U cm^{-3} at pH 8 was observed in case of tributyrin. The lipolytic activity on Tween 40 was initially lower, when compared with tributyrin, and finally obtained the highest recorded value for the strain under study, equal to 0.88 U cm^{-3} at pH 8.

B. cereus A96 did not show the lipolytic activity at pH between 5–6 on the culture medium with glucose. It was recorded only at pH 7 and amounted 0.13 U cm^{-3}, growing up to the value of 0.63 U cm^{-3} at pH 8 (Fig. 5).

In case of *B. cereus* G10 strain, the most effective source of carbon in the process of exocellular lipases biosynthesis, were also fatty substrates under study (Fig. 6), for which recorded values of the lipolytic activity did not differ significantly at pH range between 5–7. The differences were observed at pH 8, where the highest value of 0.71 U cm^{-3} was obtained in the medium with Tween 40, and the lowest value of 0.38 U cm^{-3} in the medium with Tween 60.

Similarly to the soil strain, the lipolytic activity was not recorded if the medium did not contain the fatty substrate and glucose was the source of carbon. Such case was observed at pH between 5–6. At pH 7 and 8 measured values of the activity amounted 0.17 and 0.46 U cm^{-3} respectively (Fig. 6).

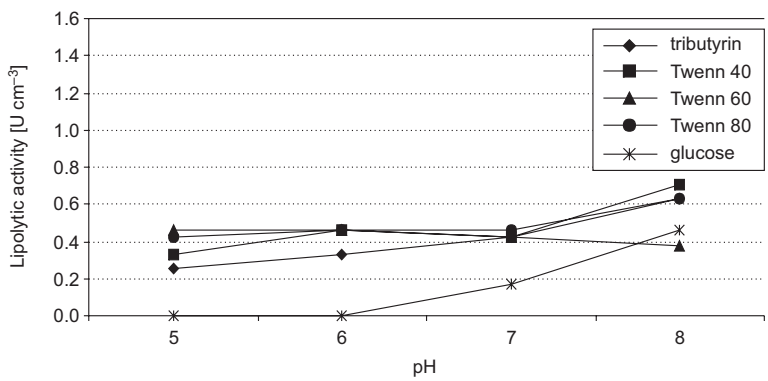

Fig. 6. Influence of pH on the lipolytic activity of *Bacillus cereus* G10

In own research, when analysing the influence of pH on the lipolytic activity, it has been noted that bacterial strains *B. mycoides* A134 and G3 preferred different sources of fatty substrates in comparison with *B. cereus* strains. The highest values of the lipolytic activity have been obtained on the culture media with addition of Tween 80, Tween 60 and Tween 40 (Fig. 7 and 8).

B. mycoides A134 strain revealed the highest lipolytic activity at pH 5–7 on the medium with Tween 80, and the recorded values ranged between 0.21–0.54 U cm^{-3}. However, in the presence of Tween 40 lipolytic activity of *B. mycoides* A134 was the highest and amounted 0.75 U cm^{-3}.

The strain did not show the ability to produce exocellular lipases at pH between 5–7, with no fatty substrate in the culture medium. Only at pH 8, the activity was noted at the level of 0.75 U cm^{-3} in case of the medium with glucose and it was one of the highest values obtained for the strain (Fig. 7).

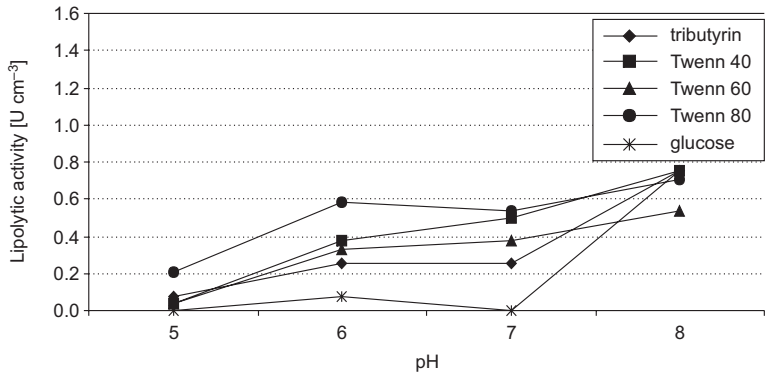

Fig. 7. Influence of pH on the lipolytic activity of *Bacillus mycoides* A134

The last strain under study – *B. mycoides* G3*,* preferred Tween 60 and Tween 80 as the sources of the fatty substrate (Fig. 8). The value of lipolytic activity on Tween 60, was growing from the amount of 0.54 U cm^{-3} at pH 5 to 1.0 U cm^{-3} at pH 8, which was the highest value recorded in the experiment. In Tween 80 case, the initial value of lipolytic activity at pH 5 and 6 was slightly higher and amounted 0.63 U cm^{-3} and 0.75 U cm^{-3}, respectively. At pH 8, it amounted 0.92 U cm^{-3} and was a bit lower when compared to the amounts obtained on the medium with Tween 60. It seems, that *B. mycoides* G3 strain, as the only one among all tested, uses Tween 60 as the most effective source of the fatty substrate in the process of extracellular lipases biosynthesis.

When analysing the influence of pH on the lipolytic activity of *B. mycoides* G3 strain, it has been noted, that it was the only active strain even if the medium did not

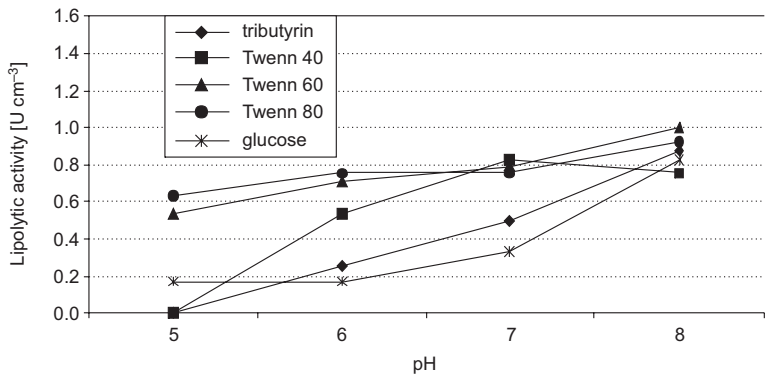

Fig. 8. Influence of pH on the lipolytic activity of *Bacillus mycoides* G3

contain fatty substrate. The value of its lipolytic activity in the presence of glucose increased from the value of 0.17 U cm^{-3} at pH 5 to 0.83 U cm^{-3} at pH 8 (Fig. 8).

Lipase production is influenced by temperature, pH and medium composition. The process of lipases biosynthesis conducted by strains *B. cereus* and *B. mycoides* was the most intensive at the temperature ranging between 30–40 ^{o}C. Hasan et al [7], Shaoxin et al [8] and Alkan et al [9] have also found similar results in case of *B.* sp. FH5, *B. cereus* C71 and B. *coagulans*. For *B. cereus* C71 [8] the lipase was active at temperature ranging between 30–45 ^{o}C with its most intensive activity at 33 ^{o}C. Whereas, for *B. coagulans* [9] and *B.* sp. FH5 [7], maximum activity have been observed at 37 ^{o}C.

The protein nature of enzyme under study means that pH will affect the ionization state of the amino acids which determines the primary and secondary structure of the enzyme and hence, controls its overall activity [10]. The optimum activity of lipase was observed at pH between 7.0–8.0. Isolates of *B.* sp. have been found to produce lipolytic enzymes under alkaline conditions [9]. Lipase from *B. subtilis* and *B. licheniformis* have been of particular interest because they exhibit optimal activity and stability at extreme alkaline pH values greater than 9.5 [11]. These enzymes, however are thermolabile. Another lipase produced by *B.* sp. RSJ-1 has shown maximum activity at pH between 8.0–9.0 [10]. These results are in contrast to those of lipase from the *B. cereus* and *B. mycoides* under study, which are thermotolerant but display maximum activity at moderate alkaline pH between 7.0–8.0. Hasan et al [7] and Alkan et al [9] found similar results from *B.* sp. FH5 and *B. coagulans*, respectively.

Lipases were defined as the enzymes hydrolyzing long-chain acyglycerols (≥ 10 carbon atoms). However, it is known that most of lipases are also active on short-chain fatty acid esterase [8]. In own research, the biosynthesis of exocellular lipases in most cases, was more effective in medium with fatty substrate than with the addition of glucose. This may prove, that the synthesis of these enzymes is induced by the lipids. In case of fatty substrates, the highest values of lipolytic activity have been noted mostly in the presence of Tween 40 (C10) as the source of carbon, and noted values of lipolytic activity were higher in case of *B. mycoides* strains. Similarly, lipase from *B. cereus* C71 [8] showed higher activity toward substrate with C12 than C16 and C18. These results are in contrast to the ones obtained for the lipase from *B.* sp. FH5 tested by Hasan et al [7]. In the present study it has been found that maximum lipase levels were obtained when Tween 80 (C18) was used as a source of lipid which served both as a carbon source and an inducer for a lipase production. A low lipase level in a medium with glucose has also been reported by Hasan et al [7].

Actually, the variation in enzyme production at different temperatures or pH values and medium composition resulted from bacterial strains specificity.

Conclusions

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

1. The highest amount of lipolytic activity were liberated by the strains at 30 ^{o}C and pH equal to 8. Under these conditions the most favourable medium was with the addition of Tween 40, as the source of fatty substrate.

2. Strains under study were active even if there was no fatty substrate in the growth medium.

3. The most active were *B. mycoides* strains. Additionally, because of its wide substrate specificity, the enzyme can be used not only for short-chain fatty acids (contained by tributyrin) but also for medium-chain fatty acids (contained in Tween 40), which will greatly broaden its environmental applications.

4. Individual strains of *B. cereus* and *B. mycoides* showed diversity in their lipolytic activity, which was influenced by the environment from which they were isolated.

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WPŁYW WARUNKÓW ŚRODOWISKA NA AKTYWNOŚĆ LIPOLITYCZNĄ SZCZEPÓW *Bacillus cereus* I *Bacillus mycoides*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej
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Abstrakt: Celem podjętych badań była ocena aktywności lipolitycznej szczepów *B. cereus* oraz *B. mycoides* w zależności od źródła węgla, pH oraz temperatury. Do badań wykorzystano 2 szczepy *Bacillus cereus* oraz 2 szczepy *Bacillus mycoides* wyizolowane z gleby i wody. Źródłem węgla w pożywkach były substraty tłuszczowe: tributyryna, Tween 40, Tween 60, Tween 80 oraz glukoza. Aktywność lipolityczną oznaczono w zakresie pH od 5 do 8 oraz temperaturach od 30 do 60^{o}C metodą miareczkową, a wyniki podano w jed-

nostkach aktywności enzymatycznej [U cm^{-3}]. W przeprowadzonym doświadczeniu aktywność lipolityczna uzależniona była od rodzaju substancji tłuszczowej zawartej w podłożu, pH oraz temperatury. I tak, badane szczepy *B. cereus* oraz *B. mycoides* wykazywały najmniejsza aktywność przy pH 5 oraz 6. Natomiast największą aktywność stwierdzono przy pH 7 i 8. W tych warunkach większość szczepów wykazywała aktywność lipolityczną nawet przy braku substratu tłuszczowego w podłożu. Największe wartości aktywności lipolitycznej uzyskano przy pH 8 na podłożu z dodatkiem Tween 40, a największe wartości (0,88 U cm^{-3}) uzyskano dla glebowego szczepu *B. cereus*. Analizując wpływ temperatury na aktywność lipolityczną stwierdzono, iż najwyższą aktywność odnotowano w temperaturze 30 i 40 ^{o}C, a najmniejszą w 50 i 60 ^{o}C. Największe wartości, dla większości szczepów, uzyskano w temperaturze 30 ^{o}C na podłożu z dodatkiem Tween 40, gdzie najbardziej aktywnym okazał się glebowy *B. mycoides* (0,88 U cm^{-3}). Wyjątek stanowi glebowy *B. cereus*, dla którego wartość aktywności lipolitycznej na podłożu z dodatkiem glukozy wynosiła aż 1,38 U cm^{-3}. Uwzględniając wszystkie analizowane źródła węgla i parametry, najaktywniejszymi były szczepy *B. mycoides.*

Słowa kluczowe: *Bacillus cereus*, *Bacillus mycoides*, lipazy, tributyryna, Tween

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Adam RADKOWSKI[1]

EFFECT OF MICROELEMENT FERTILIZATION ON THE QUALITY AND NUTRITIONAL VALUE OF THE MEADOW SWARD HAY PART II. THE CONTENT OF MACROELEMENTS*

WPŁYW NAWOŻENIA MIKROELEMENTAMI NA JAKOŚĆ I WARTOŚĆ POKARMOWĄ SIANA RUNI ŁĄKOWEJ CZ. II. ZAWARTOŚĆ MAKROELEMENTÓW

Abstract: The one-factor field experiment was designed by means of random block sampling in four replicants (fields of 2.0 × 5.0 m area). The experimental field was located on the acid brown soil (type II B) of the V quality class. The study was conducted in the years 2006–2008 in the individual farm in Pilica administrative district, in Zawiercie county, in the region of Krakow–Czestochowa Jura, at the altitude of 320 m.

The kind of microelement fertilizer was the determining factor in the study. During the experiment foliar fertilizers were applied in the form of single microelements (copper, zinc, manganese) as well as multicomponent preparation – Plonvit P, containing elements in the form of chelates. It was stated that applied fertilization had the most spectacular effect on the sodium content in the meadow sward. As a result of foliar application of multicomponent preparation, copper and manganese significant increase of average content of this element was observed in comparison with the non-fertilized field (2.34; 1.98 and 1.09-time higher values, respectively). Moreover, it was found that foliar treatment with examined microelements caused elevation of calcium and magnesium level by 46.0 % and 45.7 %, respectively. Treatment with the multicomponent fertilizer also resulted in the increased phosphorous content above the standard level. Additionally, applied foliar fertilizers narrowed the proportions between the sum of univalent and divalent cations in the meadow sward.

Keywords: meadow sward, fertilization with microelements, chemical composition

The high level of nitrogen, phosphorus and potassium treatment as well as intensive agricultural production affects the enhancement of microelements uptake by plants, what results in their deficiency both in soil and in plants. The amounts of microelements that are retained by the soil during the application of basic mineral and organic fertilizers usually cannot cover the plant requirements for these components. Therefore,

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there is a need to apply microelement fertilizers [1–3]. Although these elements are absorbed in small quantities they are essential for the proper proceeding of many biochemical and physiological processes in plants [4]. Microelements significantly influence not only the yield level but most of all they positively affect its quality as well as the content of organic and mineral compounds in forage [5, 6].

Therefore, the aim of the three-year long field experiment was an estimation of the effect of foliar fertilization with microelements in the form of chelated multicomponent preparation or single microelements on the content of macroelements in the meadow sward and the proportions between them.

Materials and methods

The study was conducted in the years 2006–2008 on the individual agricultural farm in Solca, in the Pilica administrative disctrict. The experiment was designed by the method of random block sampling, in four replicants, on the brown, acid soil (pH_{KCl} = 5.2) of the V quality class. The soil contained medium levels of assimilable potassium, manganese and zinc and were poor in assimilable phosphorus and copper.

During the vegetation period (April–September) the total rainfall amounted to 338.1 mm; 375.4 mm and 320.3 mm, and the mean temperatures reached the values of 15.2, 14.3 and 14.9 ^{o}C, respectively for the years 2006, 2007 and 2008.

During the experiment plants were treated with foliar preparation of 14 % zinc chelate (chelator – EDTA+DTPA) in a dose of 100 g Zn · ha^{-1}, manganese chelate 14 % Mn (chelator – EDTA+DTPA) in a dose of 100 g Mn · ha^{-1}, copper chelate 12 % Cu (chelator – EDTA+DTPA) in a dose of 60 g Cu · ha^{-1} as well as Plonvit P in a dose of 2 dm^3 · ha^{-1}. The fertilizers were applied for each regrowth.

Zinc, manganese and copper were dosed in such proportions to achieve the equal contents of the relevant microcomponents in the single fertilizers and in the multi-component preparation. Plonvit P is a concentrated, multicomponent, microelement fertilizer containing in a single dose of 2 dm^3: 100 g Zn, 100 g Mn and 60 g Cu in the form of chelates. The spraying solutions were prepared by dissolution of the proper amounts of chelates containing microelements in such water volume to obtain the volume of working liquid corresponding to 300 dm^3 · ha^{-1}. The tap water of the medium hardness degree was used for that purpose.

The first spraying with microelements was done after the beginning of the spring vegetation, the following – after the harvesting at the stage of the initial sward regrowth but not later than 3 weeks before the next mowing. During the investigation period the basic mineral fertilization was also applied: under I regrowth – 80 kg N · ha^{-1} and under II and III regrowths – 60 kg N · ha^{-1} for each regrowth in the form of ammonium saltpetre. Phosphorus was applied once in the spring, in the amount of 120 P_2O_5 · ha^{-1} as a triple superphosphate and potassium – under the first and third regrowths in a dosage of 60 kg K_2O · ha^{-1} (for each regrowth) as 57 % potassium salt. The experimental fields had the area of 10 m^2.

In the collected plant material the chemical composition of forage, the content of dry matter by drying at 105 ^{o}C, phosphorus and magnesium were determined –

colorometrically by the vanadium-molybdenic method, potassium, sodium and calcium – by the flame photometry [7].

On the basis of the obtained results the proportions of the univalent to divalent sum of cations (K + Na) : (Mg + Ca) in the meadow sward were calculated.

All results were subjected to the analysis of variance and verified using the Tukey test at the significance level of $\alpha = 0.05$.

Results and discussion

The conducted study revealed that fertilization with microelements significantly affected the chemical composition of the meadow sward. Foliar application of both the single elements and the multicomponent Plonvit P, which contain the elements in the form of chelate complexes, had the most spectacular influence on the sodium content in the meadow flora (Table 1).

Table 1

The weighted mean of macroelement content and the ionic proportions in the meadow sward as affected by the fertilization with microelements (mean for three years)

Examined parameter	Fertilized objects					Mean	$LSD_{0.05}$
	Control	Multicomponent fertilizer	Cu	Zn	Mn		
P content [g · kg^{-1} d.m.]	2.34	3.27	3.08	3.25	3.04	3.00	0.37
K content [g · kg^{-1} d.m.]	16.31	22.20	16.71	18.99	16.94	18.23	3.97
Ca content [g · kg^{-1} d.m.]	3.51	7.71	4.31	6.07	4.00	5.12	2.59
Mg content [g · kg^{-1} d.m.]	1.03	1.81	1.68	1.64	1.32	1.50	0.31
Na content [g · kg^{-1} d.m.]	0.120	0.402	0.358	0.132	0.251	0.253	0.150
(K + Na) : (Ca + Mg)	1.63	1.10	1.25	1.12	1.44	1.26	0.32

The highest sodium level was observed for the object treated with multicomponent fertilizer. The plants from that field contained 70% higher Na level than the plants collected from the non-fertilized object. Foliar application of multicomponent preparation, zinc, copper and manganese affected also the significant elevation of the mean calcium content when compared with the control object and the differences reached the values of: 55, 42, 19, 12 %, respectively. The great diversification as dependent on the foliar application of microelements was found for magnesium. The highest increase of its content was observed after the treatment with copper and zinc (besides the multicomponent fertilizer). The Mg level was 39 and 37 %, respectively higher than that of the control object. Copper is an element that takes part in the transformational processes of iron compounds in plants and affects the growth and anatomical structure

of many tissues [8]. Zinc is an activator of many enzymes and plant hormones and participates in the synthesis of vitamins B, C and P. Zinc affects the growth and development and enhances the plant good condition [9]. In our investigations we stated also the increase of phosphorus and potassium content after the foliar application of microelements. As a result 26 % higher phosphorus and 13 % higher potassium levels in relation to the control objects were foun under the treatment with microelements.

The quality of plant crops is estimated not only on the basis of optimal concentrations of certain elements but also the proportions between them are of a great importance as the feeding value is taken into consideration [10, 11].

The obtained results indicate that microelement fertilization affected the decreased proportion of univalent to divalent cations. It is worth emphasizing, that the lowest value of the above mentioned ratio was found for the sward foliar fertilized with the solution of multicomponent preparation and zinc.

Conclusions

1. The application of single microelement fertilizers as well as multicomponent Plonvit P preparation highly affected the sodium content in the meadow flora. Foliar application of manganese, copper and Plonvit P caused significant elevation of the Na level by on average 52, 66 and 70 %, respectively in relation to the control object.

2. Fertilization with the examined microelements caused the elevation of calcium and magnesium content by 37 and 36 % on average, in comparison with the control object.

3. The treatment with multicomponent Plonvit P resulted in the highest increase of the examined macroelements. The application of that fertilizer affected 45 % (on average) higher content of the macroelements when compared with the control field.

4. The application of copper, zinc and manganese influenced the elevated content of the examined macroelements by 30, 26 and 23 %, respectively in relation to the control object.

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WPŁYW NAWOŻENIA MIKROELEMENTAMI NA JAKOŚĆ I WARTOŚĆ POKARMOWĄ SIANA RUNI ŁĄKOWEJ CZ. II. ZAWARTOŚĆ MAKROELEMENTÓW

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Abstrakt: Jednoczynnikowe doświadczenie polowe założono metodą losowanych bloków, w czterech powtórzeniach (poletka o wymiarach 2,0 × 5,0 m). Na polu doświadczalnym występowała gleba brunatna kwaśna (rząd II B – gleby brunatne ziemne), zaliczana pod względem bonitacyjnym do klasy V. Doświadczenie prowadzono w latach 2006–2008 w indywidualnym gospodarstwie rolnym położonym w gminie Pilica, powiat zawierciański na Jurze Krakowsko-Częstochowskiej, na wysokości powyżej 320 m n.p.m.

Czynnikiem doświadczenia był rodzaj nawożenia mikroelementowego. W doświadczeniu zastosowano dolistnie nawożenie pojedynczymi mikroelementami (miedź, cynk i mangan) oraz wieloskładnikowy nawóz zawierający pierwiastki w formie schelatowanej – Plonvit P. Wykazano, że zastosowane nawożenie największy wpływ wywierało na zawartość sodu w runi łąkowej. W wyniku dolistnego stosowania wieloskładnikowego nawozu oraz miedzi i manganu stwierdzono znaczny wzrost średniej zawartości tego pierwiastka – w porównaniu z obiektem nienawożonym – odpowiednio: 2,34; 1,98; i 1,09-krotnie. Ponadto stwierdzono, iż dolistne zastosowanie badanych mikroelementów spowodowało wzrost zawartości wapnia i magnezu odpowiednio o 46,0 i 45,7 % w porównaniu z obiektem kontrolnym. Wykazano również, że nawożenie wieloskładnikowym nawozem spowodowało wzrost zawartości fosforu ponad wartość normatywną. Ponadto zastosowane nawozy dolistne zawęziły stosunek sumy kationów jednowartościowych do sumy kationów dwuwartościowych w runi łąkowej.

Słowa kluczowe: ruń łąkowa, nawożenie mikroelementami, skład chemiczny

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Anna KWIECIŃSKA[1] and Krystyna KONIECZNY[1]

RECOVERY OF INDUSTRIAL WATER FROM PIG LIQUID MANURE BY MEANS OF MEMBRANE TECHNIQUES

ODZYSK WODY PRZEMYSŁOWEJ Z GNOJOWICY TRZODY CHLEWNEJ Z WYKORZYSTANIEM TECHNIK MEMBRANOWYCH

Abstract: Liquid manure that is produced during high density livestock farming requires proper utilization methods. Nowadays, it is mainly used as a fertilizer or as a substrate for biogas or compost production. However, these methods are often very limited and do not allow to utilize the total amount of produced liquid manure, thus it is still treated as problematic waste. High water content in liquid manure leads to the assumption that it can be treated as a water source. This assumption is quite realistic if application of low and high pressure membrane techniques is considered. Such a solution would allow not only to recover water that could be further reused on farms, but also to obtain valuable concentrated nutrients solution which can be used as a fertilizer and easily transported to agricultural areas.

The aim of the study was to determine the effectiveness of water recovery from pig liquid manure using integrated system: centrifugation/two step ultrafiltration/nanofiltration. The first step ultrafiltration was performed using PVDF membrane of cut off 100 kDa while the second step using PES membrane of cut off 10 kDa. During the polishing process ie nanofiltration hydrophilic composite membrane of cut off 200 Da was used. The effectiveness of the process was determined basing on the change of values of parameters like: BOD_5 and COD, contents of TOC, IC, TC and N_{tot}, concentrations of NH_4^+, Cl^-, SO_4^{2-}, PO_4^{3-}, Mg^{2+}, Ca^{2+} and K^+, while the capacity of the treatment was determined using volumetric permeate streams. Obtained results allow to conclude that the proposed system can be used to recover water of an industrial quality.

Keywords: liquid manure, water recovery, membrane processes, ultrafiltration, nanofiltration

High density livestock farming results in the production of a significant amount of liquid manure which is a mixture of animal urea, feces and water [1–3]. Nowadays, it is mainly used as a fertilizer or a substrate for biogas or compost production. In Poland, 70 mln m^3 of liquid manure is produced every year, however only ca 21 mln m^3 is applied for fertilizing purposes [4], whereas the rest is treated as a highly load waste and

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requires special treatment methods. However, except for nutrients, liquid manure contains also a significant amount of water (ca 90–97 %) which is usually omitted product during consideration of liquid manure management methods.

The development and improvement of membrane processes create the possibility of water recovery from liquid manure [5–7]. The application of low and high pressure membrane techniques would allow to obtain water of proper quality that could be reused on farms. The recovery of water would be a great advantage in areas which deal with its deficit and the concentrated fertilizing mixtures could be easily transported to agriculture areas [8–11].

Materials and methods

A 50 dm^3 sample of a pig liquid manure was collected from a 13000 m^3 lagoon localized at one of the high density livestock farm in Silesia region. Firstly, the liquid manure was centrifuged for 10 minutes at the rotational speed of 15000 rpm. The obtained supernatant was next introduced to the laboratory membrane cell by Koch (Fig. 1). The device is equipped with the 0.5 dm^3 feed tank and the effective separation area of the installed flat membrane is equal to 28 cm^2. All processes were carried out in the cross-flow mode.

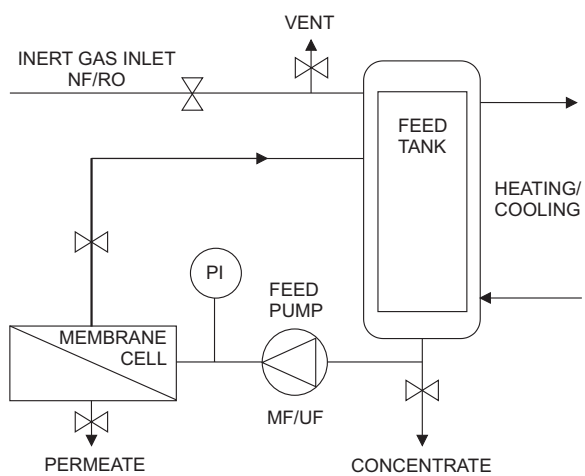

Fig. 1. The scheme of the laboratory installation for membrane filtration by Koch

Firstly, 100 kDa polyvinylidene fluoride (UF-PVDF-100) membrane was used for ultrafiltration of the supernatant and the permeate was introduced to the second treatment step which was carried out with the use of 10 kDa polyethersulfone (UF-PES-10) membrane. The polishing of the second ultrafiltration step permeate was performed using composite nanofiltration membrane of cut off 200 Da (NF-200). All applied membranes were provided by KOCH. The detail scheme of the process is shown in Fig. 2.

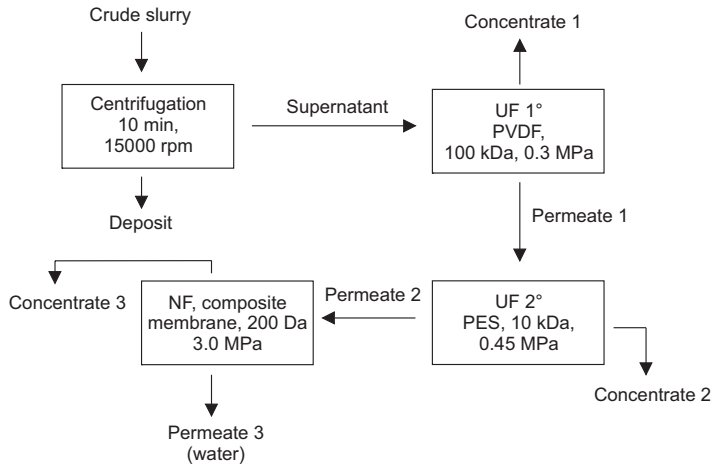

Fig. 2. The scheme of the pig liquid manure treatment process in the integrated system of centrifugation/two step ultrafiltration/nanofiltration

Following parameters were analyzed in the produced process streams: pH, conductivity, COD, BOD_5, contents of TOC, IC, TC, N_{tot}, concentrations of ions NH_4^+, PO_4^{3-}, Cl^-, SO_4^{2-}, Mg^{2+}, Ca^{2+} and K^+ (Na^+ – only in nanofiltration permeate). COD, concentrations of K^+, PO_4^{3-}, $N_{tot.}$ and NH_4^+ were determined according to the methodology by Merck methodology, while BOD_5 by means of the respirometric method with the use of the OXI Top WTW set. Concentrations of Cl^- and SO_4^{2-} ions were obtained during chromatographic analysis using ionic chromatograph DX 120 by Dionex. The content of particular carbon forms was analyzed using Multi N/C analyzer by Jena Analytic. Concentrations of Ca^{2+} and Mg^{2+} were determined by means of the classical titration method while Na^+ content using flame photometer.

The liquid manure membrane filtration was preceded by the determination of the dependence the volumetric deionized water flux on pressure for all membranes applied. After the liquid manure treatment membranes were washed with deionized water and its volumetric flux was again measured.

Results and discussion

The liquid manure treatment was preceded by the characterization of applied membranes ie the determination of dependence of the volumetric deionized water flux on pressure. The results of the experiment are presented in Fig. 3a–c.

The logarithmic dependence of the volumetric deionized water flux on pressure determined for 100 kDa membrane indicated that the increase of the transmembrane pressure above 0.4 MPa would not improve the process capacity. For other membranes ie 10 kDa and 200 Da regular, linear dependences were obtained.

The study of the composition of treated streams indicated that the proposed system of integrated centrifugation/two step ultrafiltration/nanofiltration was sufficient for the

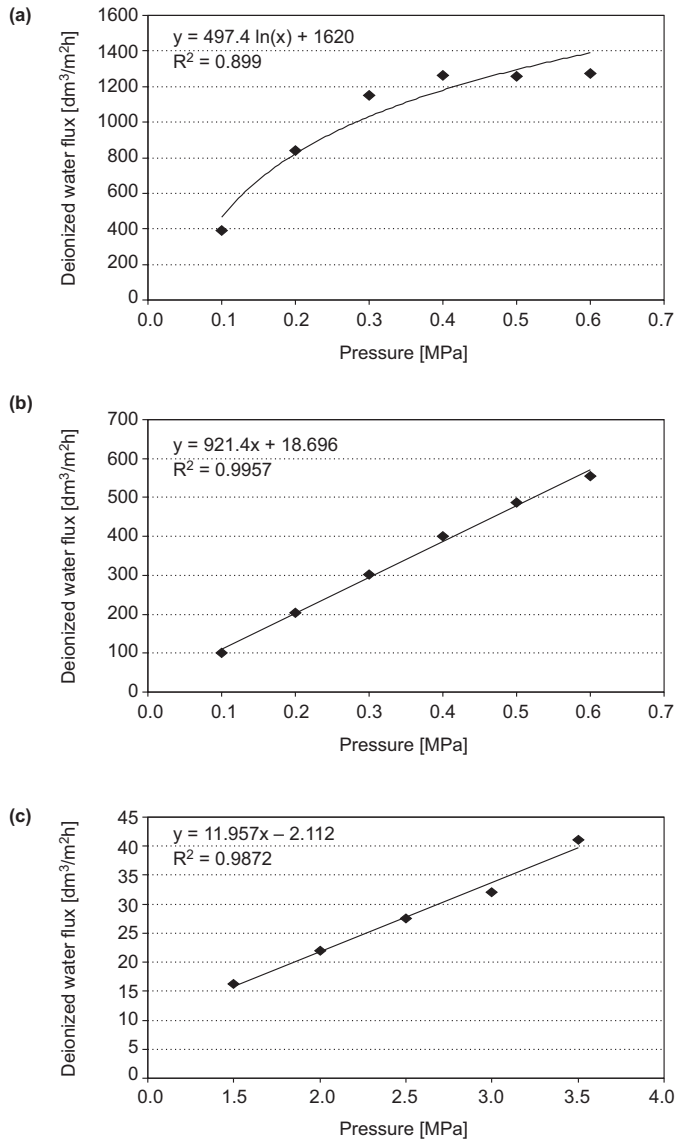

Fig. 3. The dependence of the volumetric deionized water flux on pressure: (a) UF-PVDF – 100 kDa; (b) UF-PES – 10 kDa; (c) NF – 200 Da

liquid manure treatment. The improvement of the quality of treated streams was observed in every step (Table 1). However, finally obtained permeate did not fulfill the regulations for the drinking water quality [12] as concentrations of TOC and NH_4^+ ions, which were exceeded, and Mg^{2+} and Ca^{2+} ions were absent (Table 2). Nevertheless, it can be used as a sanitary safe industrial water for washing of farmhouses and animals as well as for fields watering.

Table 1

The comparison of parameters of treated streams

Parameter	Unit	Crude liquid manure	Supernatant	Permeate 1	Permeate 2	Permeate 3 (water)
pH	—	7.36	7.69	8.1	8.17	8.6
Conductivity	mS/cm	5.96	5.84	5.75	5.6	0.675
TOC	mg/dm^3	1134	671	528	381	9.7
IC	mg/dm^3	466	437	420	370	44
TC	mg/dm^3	1600	1108	948	751	53.7
COD	mgO_2/dm^3	7560	3785	2990	2285	13.9
BOD_5	mgO_2/dm^3	3000	2900	1500	480	n/m*
NH_4^+	mg/dm^3	1350	1200	1478	1414	135
N_{tot}	mg/dm^3	3100	2950	2950	2950	148
SO_4^{2-}	mg/dm^3	199	192	185	49.8	0
PO_4^{3-}	mg/dm^3	63.1	34.6	32.2	24.7	0
Cl^-	mg/dm^3	385	373	371	311	52
K^+	mg/dm^3	820	805	790	790	180
Mg^{2+}	mg/dm^3	19.2	16.8	14	11	0
Ca^{2+}	mg/dm^3	100	100	88	80	0
Na^+	mg/dm^3	—	—	—	—	41

* Non measurable.

Table 2

The comparison of parameters of nanofiltration permeate and drinking water

Parameter	Unit	Permeate 100-10-0.2	Drinking water*, **
pH	—	8.60	6.5–9.5
Conductivity	µS/cm	675	2500
Smell	—	acceptable***	acceptable***
TOC	mg/dm^3	9.7	5.0
Cl^-	mg/dm^3	52	250
SO_4^{2-}	mg/dm^3	0	250
NH_4^+	mg/dm^3	135	0.5
Total hardness	$mgCaCO_3/dm^3$	0	60–500
Na^+	mg/dm^3	41	200

* Permissible values of parameters of drinking water according to the Regulation of Minister of Health on drinking water quality from 20.04.2010; ** There are no regulations considering quality of water for animals watering; *** Slight, hardly sensible, determined during cool study by 4 people.

The evaluation of process capacities indicated that the liquid manure filtration caused fouling of all applied membranes as all permeate fluxes decreased during the treatment

(Fig. 4a–c). Both types of fouling, the reversible and irreversible ones, were observed. However, the second fouling type occurred only in case of ultrafiltration and was most severe during the first step. In comparison with distilled water flux, the membrane capacity during liquid manure treatment decreased by 90 % for 100 kDa membrane, 63 % for 10 kDa membrane and 31 % for 200 Da membrane. However, while comparing initial and final liquid manure fluxes, the capacity of the first step ultrafiltration decreased by 36 %, the second step ultrafiltration by 29 % and the polishing step by 76 %.

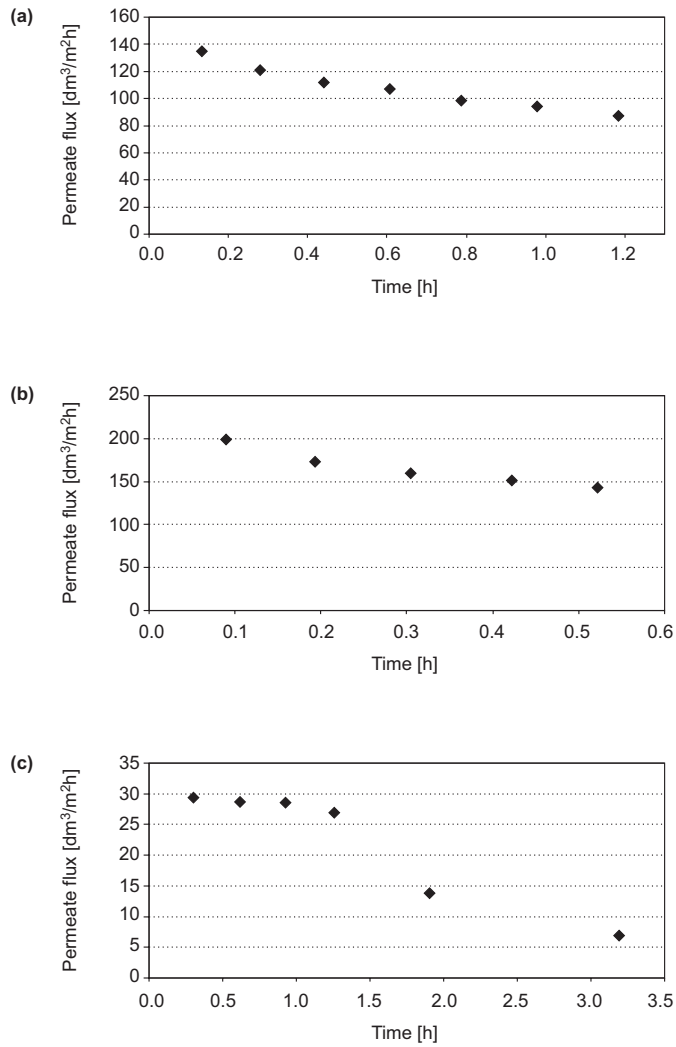

Fig. 4. The decrease of the permeate flux during liquid manure filtration: (a) UF-PVDF – 100 kDa; (b) UF-PES – 10 kDa; (c) NF – 200 Da

The applied washing of membranes with distilled water allowed to partially recover the initial capacity of UF membranes, while in case of the NF membrane the post-process water flux was even greater than the initial one. It was probably due to the modification of the membrane, which could have been caused by the deposition of calcium and magnesium ions and/or organic substances present in the liquid manure on the membrane surface. The washing of membranes with distilled water fluxes enabled the initial capacity recovery at the level of 39 % for 100 kDa membrane, 65 % for 10 kDa membrane and 103 % for 200 Da membrane. The comparison of initial distilled water streams, permeate streams and washing distilled water streams is presented in Fig. 5.

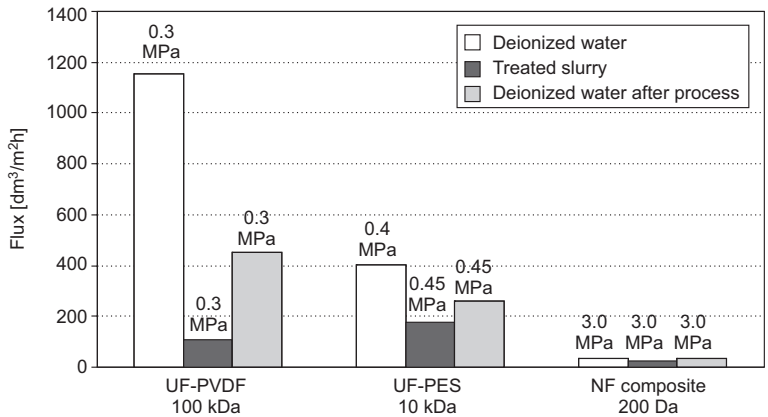

Fig. 5. The comparison of volumetric fluxes of the deionized water (before and after the process) and the treated liquid manure determined for all membranes

Conclusions

The presented study focused on the recovery of the industrial water from the pig liquid manure by means of the integrated system comprised of centrifugation/two step ultrafiltration/nanofiltration.

The obtained results allowed to conclude that the proposed system is suitable for that purpose and the final permeate could be reused on farm eg for animals or farmhouse washing. However, the obtained water did not fullfill the regulations for drinking water quality as concentrations of ammonium ion and TOC were exceeded and it did not contain magnesium and calcium ions.

The appearnce of fouling phenomenon was observed in case of all membranes. Its capacity of greatest influence on the membrane capacity was observed in case of UF membranes. Additionally, for these membranes both forms of fouling, reversible and irreversible, were present. The washing of membranes with distilled water did not allow to recover the initial UF membranes, while for NF membranes the post-process water flux was greater than the initial one. It was explained by the possible modification of the polishing membrane surface, probably by magnesium and calcium ions and/or the organic substances.

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ODZYSK WODY PRZEMYSŁOWEJ Z GNOJOWICY TRZODY CHLEWNEJ Z WYKORZYSTANIEM TECHNIK MEMBRANOWYCH

Wydział Inżynierii Środowiska i Energetyki
Politechnika Śląska, Gliwice

Abstrakt: Gnojowica powstająca podczas wielkoprzemysłowej hodowli zwierząt wymaga stosowania odpowiednich metod utylizacji. Obecnie jest ona wykorzystywana jako nawóz bądź też substrat do produkcji biogazu oraz kompostu. Jednakże metody te są często ograniczone i nie pozwalają na zagospodarowanie całkowitej ilości powstającej gnojowicy, stąd też wciąż jest ona traktowana jako uciążliwy odpad. Duża zawartość wody w gnojowicy pozwala założyć, iż może być ona traktowana jako źródło wody. To założenie jest całkiem realne w przypadku zastosowania nisko i wysokociśnieniowych procesów membranowych. Takie rozwiązanie pozwoliłoby nie tylko na odzysk wody, który mogłaby zostać ponownie wykorzystana na farmie, ale także na otrzymanie wartościowych, stężonych roztworów substancji odżywczych, które mogłyby zostać wykorzystane jako nawóz i łatwo transportowane na tereny rolnicze.

Celem przeprowadzonych badań było określenie efektywności odzysku wody z gnojowicy trzody chlewnej, wykorzystując zintegrowany system: wirowanie/dwustopniowa ultrafiltracja/nanofiltracja. Pierwszy stopień ultrafiltracji prowadzono z użyciem membrany z PVDF o cut off 100 kDa, drugi zaś stopień z wykorzystaniem membrany z PES o cut off 10 kDa. Podczas etapu doczyszczania, tj. nanofiltracji zastosowano kompozytową membranę hydrofilową o cut off 200 Da. Efektywność procesu określono, korzystając ze zmiany wartości parametrów procesu: BZT_5, ChZT, zawartości OWO, WN, OW, $N_{cał}$ stężenia jonów NH_4^+, Cl^-, SO_4^{2-}, PO_4^{3-}, Mg^{2+}, Ca^{2+} i K^+, wydajność zaś wyznaczono na podstawie objętościowych strumieni permeatów. Przeprowadzane badania pokazują, że zaproponowany system może zostać wykorzystany do odzysku wody o jakości przemysłowej.

Słowa kluczowe: gnojowica, odzysk wody, procesy membranowe, ultrafiltracja, nanofiltracja

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APPLICATION OF POLYSULFONE MEMBRANES FOR COKE-MAKING WASTEWATER TREATMENT

MEMBRANY POLISULFONOWE W OCZYSZCZANIU ŚCIEKÓW KOKSOWNICZYCH

Abstract: Considering the complicated and variable type of coke-making sewages their purification strategy is difficult to generalize and it requires an integrated system, which joins biological and physicochemical separated processes. In the paper the researches were presented, which purpose was to define the efficiency of purifying matting pressure membrane techniques – ultrafiltration and reverse osmosis. In the process of low-pressure filtration flat membranes, applied in the laboratory, were different in terms of compactness of structure and porosity.

Keywords: coke-making wastewater treatment, integrated system, membranes pressure techniques, polysulfone ultrafiltration membranes

The aqueous-sewage management environmentally safe is the obligation of each industrial plant. The best solution would be to establish a local sewage treatment plants assuring to neutralize wastewaters.

The negative effect caused by coking plants upon the environment consists in carrying industrial waters cleared in the insufficient rank to plumbing or receivers. The way of cleaning them is of great importance in aspect of nature protection. The coke-making wastewaters industry have changeable compound composition. They contain: polycyclic aromatic hydrocarbons, compounds heterocyclic, oils, tars and substances of inorganic character such as: cyanide, sulfides, sulfates, thiosulfates, ammonia and also heavy metals. One of the ways and the most frequently applied in the wastewater treatment technology and which seems to be environmentally friendly are pressure membrane techniques. It is possible to link them with classical separated processes of purifying wastewaters into so-called hybrid or integrated systems [1–9].

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Apparatus

In the process of membranes cleaning coking industry sewages a system equipped with the plate-frame membrane module of the American Osmonics company of the SEPA CF-NP type, the container of sewers with the radiator was applied as well as cone-and-float meter, the high-pressure pump and manometers and valves. A scheme of the equipment used in investigations is presented below.

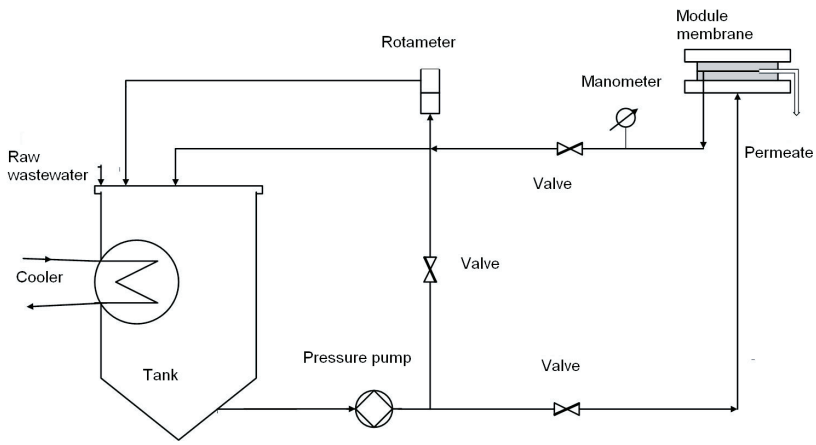

Fig. 1. Scheme of the plate- and frame membrane module applied for cleaning coke-making sewage

Raw wastewater

The process of cleaning was applied in the coke plant effluents coming from Coke Plant ISD Huta Czestochowa "Koksownia" Sp. z o.o., which characteristics are displayed in Table 1.

Table 1

Characteristics of coke-making effluents
from coking plant ISD Huta Czestochowa "Koksownia" Sp. z o.o.

Indicator	Unit	Raw wastewater	Accompanied to receiver set the coefficients of pollutions of sewages*
pH	[-]	8.7–10.9	6.5–9.0
COD	mgO_2/dm^3	4200–3100	125
BOD_5	mgO_2/dm^3	20–80	25
Ammoniacal nitragen	mg NH_4^+/dm^3	25–104	10

* Directive of the Environment Minister from 24th of July 2006 in the matter of conditions one should fulfil which at inserting sewages to waters or soil, and in the matter of substances particularly harmful to the environment aqueous (DzU 2006, nr 137, poz. 984).

As we can observe the values of coefficients describing coke-making wastewater in comparison with standardized values are higher what makes it impossible to pour them directly in to receiver. They are also hard to biodegradation process due to very low value of BOD_5/COD relationship

Analytical methods

During the first stage of researches carried out diversified polysulfone flat membranes were prepared varying from 13 to 17 % mass of the polymer in organic solvent and their appropriate porosity was outlined. In order to form of long-lasting membrane structure they were subjected to the process of conditioning, consisting in pure water filtering with the changeable membrane pressure taking out from 0.5×10^5 Pa to 3.0×10^5 Pa and linear velocity of pure water over membrane surface – 2 m/s. Membranes shaped up to the moment of stabilizing the amount of the flux of pure water.

During the next part of the experiment their transport properties were defined outlining relation between the volumetric flux of pure water from and the trans--membrane pressure changed in the scope of value from 0.5×10^5 Pa to 3.0×10^5 Pa and linear velocity of medium over the membrane surface – 2 m/s.

The last stage of researches consisted in defining the possibilities of applying manufactured ultrafiltrating polysulfone membranes in cleaning coking industry sewages. The usefulness of the membranes depended on: permeability of membranes, relative permeability and the grade of removing the cargo of pollutants from wastewater.

According to interlaboratory studies, purified (with the suitable polysulfone membrane singled out earlier) coke-making industry sewages were characterized by high value of ratio of pollutants which impeded the immediate carrying them to the natural receiver, they were subjected to cleaning with the reverse osmosis method applying of the Osmonics company of the SE type membrane The effectiveness of this process was assessed, as in the case of ultrafiltration taking into consideration the size of the permeate flux and the change in pollutants value of raw and purified sewages characteristics.

Results and discussion

Preparing asymmetric polysulfone membranes and marking their proper porosity

Preparing PSF membranes consisted in pouring the thin film out from membrane solution and gelation into pure water. The membrane solution was prepared by dissolving in period of 24 h polysulfone in *N,N*-dimethylformamide.

The concentration of the polymer changed in the scope from 13 to 17 % weight what resulted in the shift of the porosity and consequently in the density of the membranes structure (Fig. 2).

We can see clearly that the porosity of membranes decrease together with the increase of the concentration of the polymer and their structure became much more

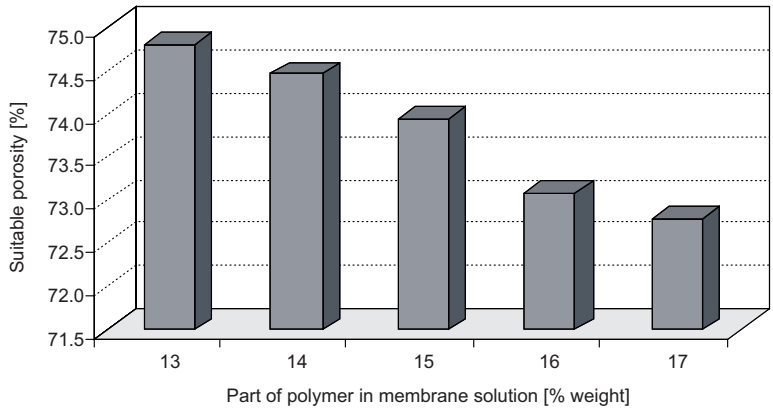

Fig. 2. Dependence of suitable porosity synthesized polysulfone memebranes on content polysulfone in membrane solution

open. So the porosity of the PSF-13 membrane took the 74.8 %, PSF-15 of the 73.94 % and the lowest was the PSF-17 with the 72.8 %.

Transport properties of ultrafiltration polysulfone membranes

Defining the transport property of manufactured membranes consisted in outlining the relation between the temporary volume stream of pure water and the pressure. Measurements made demonstrated that the plumbing membranes productivity dependence on the pressure trans-membrane (Figs. 3, 4).

The biggest volumetric flux of pure water was observed in PSF-13 membrane and the lowest stream was obtained in PSF-17 membrane due to the its clenched structure. For the trans-membrane pressure 3×10^5 Pa it was 97 times smaller in comparison with

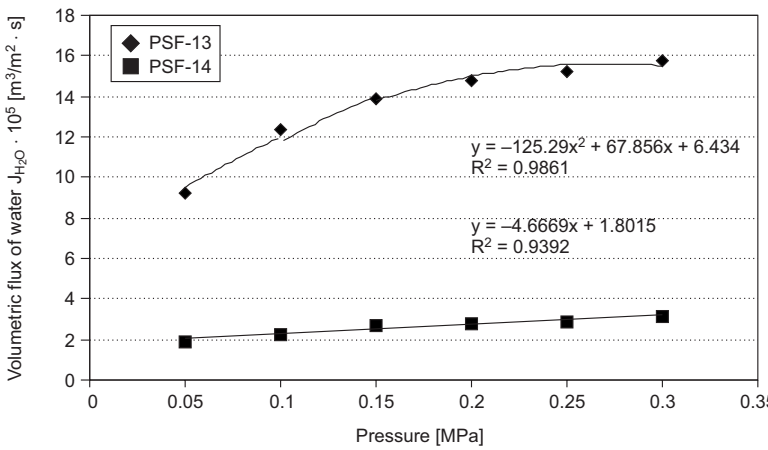

Fig. 3. Dependence of volumetric water flux on use ie pressure for membranes PSF-13 and PSF-14

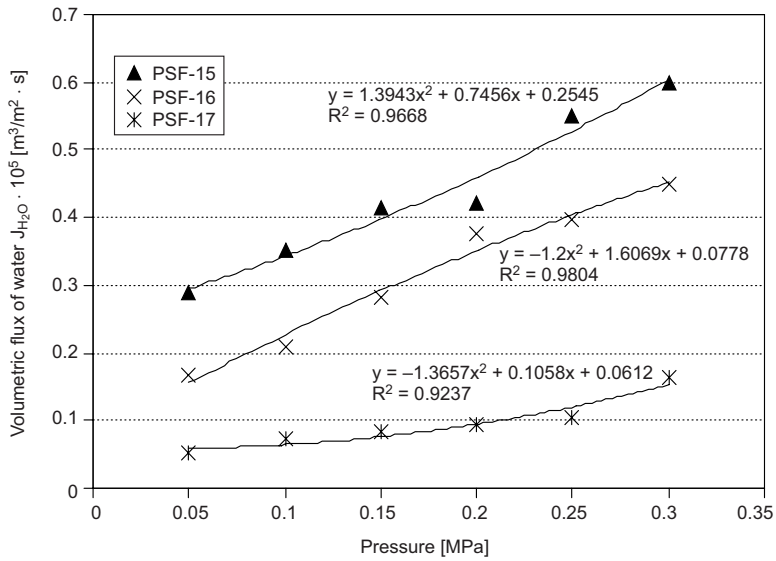

Fig. 4. Dependence of volumetric water flux on use ie pressure for membranes PSF-15, PSF-16 and PSF-17

the flux water for the PSF-13 membrane, in the same conditions of the filtration. In the case of all membranes relations $J_{H_2O} = f(\Delta P)$ described quadratic equations and high value of rates proved the correct selection of the regression line (function).

Coke-making wastewater purification in integrated system of ultrafiltartion-reverse osmosis

Investigations carried out in this stage were supposed to allow to outline a polysulfone ultrafiltrating which would be describe itself with the big efficient and would assure the biggest grade of removing charge of pollutants from cleaning wastewater. Coke-making industry sewages was subjected to cleaning with low pressure filtration outlining for each of membranes the relation between experimental temporary flux and the time filtration (Figs. 5, 6).

The biggest flux post-treat was observed in the process of the low-pressure filtration of the PSF-13 membrane. Its value took 2.73×10^{-5} $m^3/m^2 \cdot s$ out after stabilizing.

The fastest decrease of the stream was observed for this membrane at the same time during what was caused by the biggest intensity of the fouling process. In the 125 first minutes the flux permeate diminished 5 times.

Different post-treat properties were observed in PSF-17 membrane which had the lowest stream and in comparison with PSF-13 47.1 % smaller. Also for this membrane the smallest stream decrease was registered.

Changes estimated as the relationship of the experimental volumetric momentary flux, in their relative permeability were also appointed in the ultrafiltration process of cleaning coking industry sewages for examined membranes permeate (Jv) to the initial

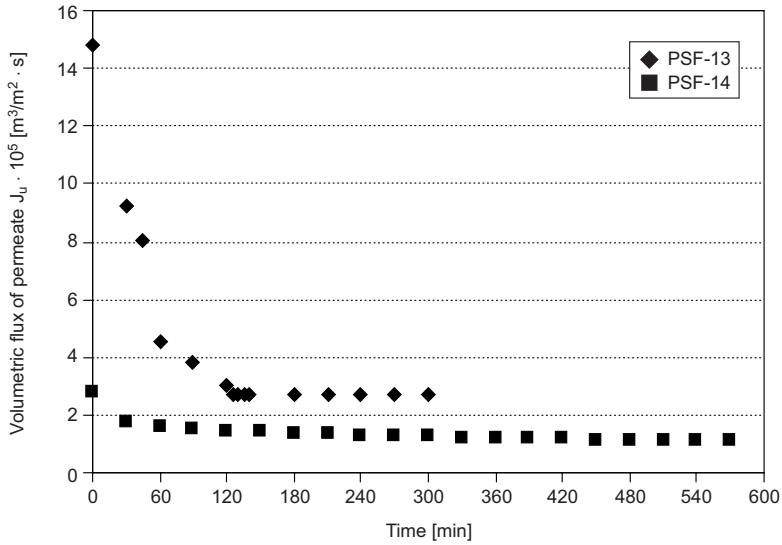

Fig. 5. Dependence of temporary experimental volumetric flux on time treatment coke-making ultrafiltartion process at membranes PSF-13 and PSF-14

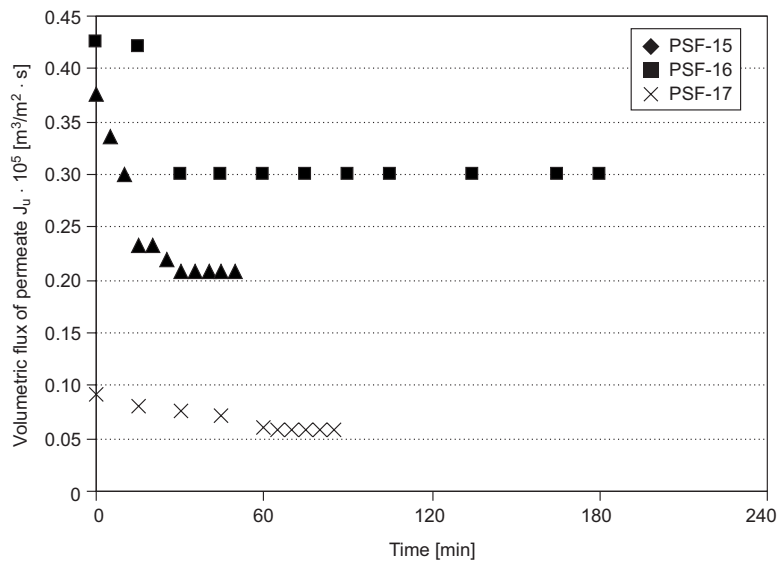

Fig. 6. Dependence of temporary experimental volumetric flux on time treatment coke-making ultrafiltartion process at membranes PSF-15, PSF-16 and PSF-17

flux of pure water (J_0). The change in the permeability of the relative membrane is bound with intensity of the phenomenon setting on its surface and inside pore of the low-pressure process for the membranes fouling separation, into lowering, in the considerable way relative permeability of the membrane. Many factors have effect on to

the step of membrane polluting, and the most important are: affinity of chemical substances presented in sewages in comparison with polymer membrane material and the size and the structure of their particles. In Figs. 7 and 8 changes in the relation for the relative permeability of membranes were compared from the time low-pressure filtration carry out for cleaning coking industry sewages.

It has been proved that the lowest relative permeability had PSF-13 membrane and the highest PSF-16. It was demonstrated that the relative permeability of examined

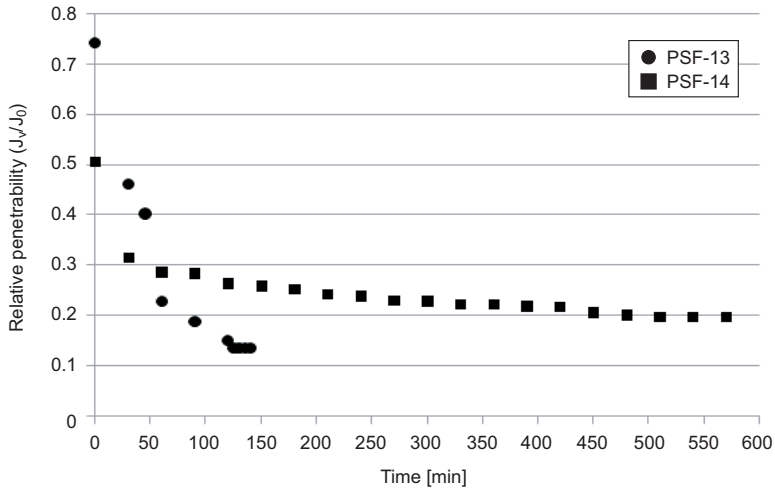

Fig. 7. Compare dependence of changes relative penetrability polysulfone membranes PSF-13 and PSF-14 on time treatment coke-making ultrafiltration process

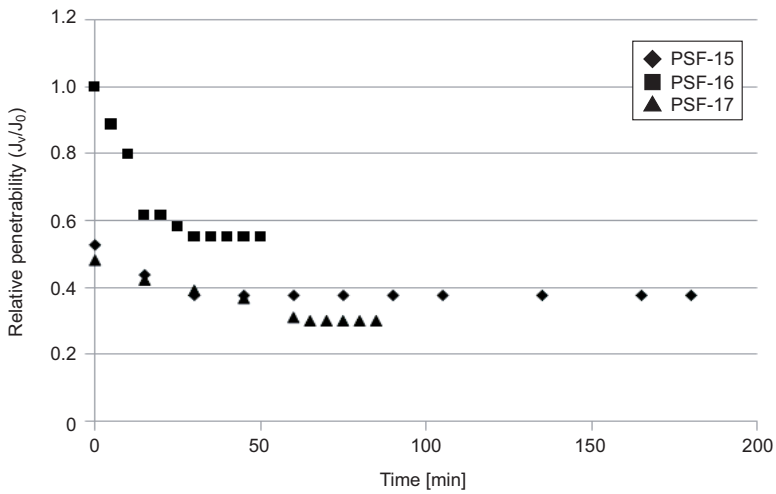

Fig. 8. Compare dependence of changes relative penetrability polysulfone membranes PSF-15, PSF-16 and PSF-17 on time treatment coke-making ultrafiltration process

membranes is a function of the density of their structure. The PSF-13 membrane the most opened structure is relatively easily undergoing the fouling process. The pores are blocked quickly by sewages due to the big diameters. Together with the height membrane polymeric contents their porosity is decreasing and the relative permeability is increasing. Another lowering was observed in the PSF-17 membrane. It is possible to example this fact with the smallest porosity of this membrane that caused layer covering in the form of pollutants as a result in a smaller intensity and blocks the pores of "secondary membrane" which final has the smaller membrane permeability in comparison with membranes with blocked times. The assessment criterion for the effectiveness of low-pressure wastewater filtration was apart from the size of the volumetric flux permeate and relative permeability, step of removing of pollutants. The level of cleaning coke-making wastewater was related with the change of value of the following parameters: COD, TC and TOC, which was typical for "raw" and cleaned sewers. For the PSF-16 membrane additionally a dry substance remains after roasting and conductivity.

From prepared for five polysulfone membranes, as it was expected, none assured the high level of removing charge of pollutants from cleaned sewages appropriately what made it impossible in consequence their immediate carry to the natural environment (Table 2).

Table 2

The stage of removal in process the cargo of pollutions treatment coke-making sewages in ultrafiltration process with use of polysulfone membranes

Membrane	Indicator	Unit	Raw wastewater	Permeate	Degree of removal pollutions [%]
PSF-13	COD	mgO_2/dm^3	3266	2939	10
	TC	mgC/dm^3	720.95	690.7	4.2
	TOC	mgC/dm^3	495.58	481.7	2.8
PSF-14	COD	mgO_2/dm^3	3038.5	2488.5	18.1
	TC	mgC/dm^3	718.2	638.5	11.1
	TOC	mgC/dm^3	483.2	450.8	6.7
PSF-15	COD	mgO_2/dm^3	3808	3027.4	20.5
	TC	mgC/dm^3	707.3	600.5	15.1
	TOC	mgC/dm^3	494.6	448.6	9.3
PSF-16	COD	mgO_2/dm^3	2754	2087.5	24.2
	TC	mgC/dm^3	616.9	515.1	16.5
	TOC	mgC/dm^3	390.7	340.3	12.9
	DS	g/dm^3	6.28	6.09	3
	Compounds of inorganic substances	g/dm^3	0.605	0.35	42
PSF-17	COD	mgO_2/dm^3	2754	1556	43.5
	TC	mgC/dm^3	616.9	70.7	88.6
	TOC	mgC/dm^3	390.7	63.3	83.8

TC – Total carbon; TOC – Total organic carbon; COD – Chemical Oxygen Demand.

As the most suitable was accepted PSF-16 membrane with 16 % weight of the polymer in membrane solution. Its choice was determaid by the the volumetric flux size of permeate flux, which in comparison with the membrane about the most clenched structure (PSF-17) was over three times higher ($\Delta P = 3.0 \times 10^5$ Pa). Besides that this memebrane had the highest relative permeability.

After using this membrane purified sewages were characterized by the following indexes of pollutants: COD – 2087.5 mgO^2/dm^3, the concentration of total carbon TO of 515.1 mgC/dm^3, total organic carbon of 340.3 mgC/dm^3 and ammoniacal nitrogen 98 $mgNH^+_4/dm^3$. As it can be seen all the values exceeded the acceptable standardized levels. Sewages left after the process of ultrafiltration treatment were subjected to in additional cleaning of the reverse osmosis with the method on polymer for SE membrane. Changes in the volumetric flux of pure water and sewages were measured during the investigations and we can concluded, that outlined fluxes during carry out the process (pure water and permeate) moved close. It proves the fact that the applied osmotic membrane is characterized by a big density (Fig. 9).

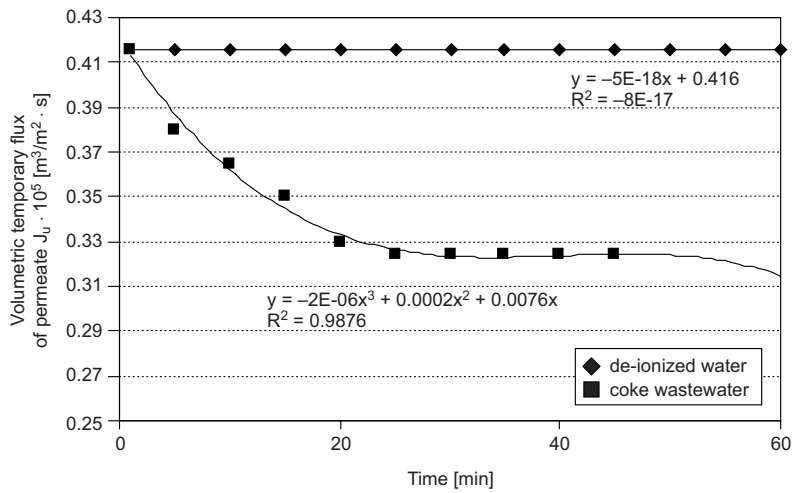

Fig. 9. Dependence of temporary volumetric treatment coke-making wastewater and pure water on time high pressure process

Compared indicators cleaned in the integrated system ultrafiltration-reverse osmosis of pollutants in sewages were described in Table 3.

Unfortunately in this way cleaned effluents did not fulfil qualitative norms presented into the Directive of the Environment Minister from 24th July 2006. Concerning the conditions that should be at inserting sewages into waters or soil, and in the matter of substances particularly harmful to the environment aqueous (Log. Act 2006 number 137 item 984). They stated the exceeding of the acceptable concentration of ammoniacal nitrogen passed 2-ratio (22.4 $mgNH_4^+/dm^3$) that is way the sewages should subjected to the stripping process before carry them to the natural receiver.

Table 3

The efficiency of cleaning in integrated system ultrafiltration-reverse osmosis coke-making sewages

Indicator	Unit	Raw waste-water	Cleaned sewages				Value of coefficients for introduced sewages to land waters
			UF-PSF-16		RO-SE		
			Value	Stage of removal of pollutions [%]	Value	Stage of removal of pollutions [%]	
COD	mgO_2/dm^3	2754	2087.5	24.2	74.3	97.3	125
9TC	mgC/dm^3	616.9	515.1	16.5	29.3	94.3	ns.
TOC	mgC/dm^3	390.7	340.3	12.9	12.1	96.4	ns.
Ammoniacal nitrogen	$mgNH_4^+/dm^3$	131.6	98.0	25.5	22.4	83.0	10

TC – Total carbon; TOC – total organic carbon; ns.– not standardized.

Conclusions

1. Coke-making sewages sequential system of ultrafiltration-reverses osmosis applied in investigations didn't provide properly high degree of treatment.

2. From prepared five polymeric low-pressure filtration membranes the most profitable was a PSF-16 membrane showed itself for 16 % weight contents of the polymer in membrane solution. Since none of membranes assured the high level of coke-making industry wastewater purification the choice was determined by the size of the volumetric flux permeate, which in comparison with the membrane with the most clenched structure (PSF-17) was over three times higher ($\Delta P = 3.0 \times 10^5$ Pa) and had highest relative permeability.

3. Sewages cleaned in the process of the reverse osmosis were characterized by too high concentration on the level, of ammonia 22.4 $mgNH_4^+/dm^3$. They should before carry them to the natural receiver, be subjected to the stripping process.

Acknowledgement

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MEMBRANY POLISULFONOWE W OCZYSZCZANIU ŚCIEKÓW KOKSOWNICZYCH

Instytut Inżynierii Środowiska, Wydział Inżynierii i Ochrony Środowiska
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Abstrakt: Ze względu na złożony i zmienny skład ścieków koksowniczych strategia ich oczyszczania jest trudna do uogólnienia i wymaga prowadzenia tego procesu w układach zintegrowanych, kojarzących biologiczne i fizykochemiczne procesy jednostkowe. W artykule omówiono badania, których celem było określenie efektywności oczyszczania ścieków koksowniczych w układzie kojarzącym ciśnieniowe techniki membranowe, a mianowicie ultrafiltrację i odwróconą osmozę. W procesie niskociśnieniowej filtracji zastosowano wytwarzane w laboratorium płaskie membrany polisulfonowe różniące się zwartością struktury i porowatością.

Słowa kluczowe: oczyszczanie ścieków koksowniczych, układy zintegrowane, ciśnieniowe techniki membranowe, polisulfonowe membrany ultrafiltracyjne

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ECOLOGICAL RISK CLASSIFICATION IN THE REGULATED AND CONSERVED WATERCOURSES

KLASYFIKACJA RYZYKA EKOLOGICZNEGO W CIEKACH REGULOWANYCH I KONSERWOWANYCH

Abstract: The subject of the following study is ecological risk in regulatory and maintenance works conducted in small and medium-sized lowland watercourses. Risk has not been identified well enough. It results from the lack of the data to assess its level objectively. The following research presents a proposal of solving the problem.

The results of the field work conducted between 2007 and 2008 on 10 Lower Silesian lowland watercourses form a basis for this analysis. The research included hydromacrophytes identification and the degree of the bottom coverage by these aquatic plants. The following study showed that in a result of regulatory and maintenance works quality and quantity alternations in aquatic plants communities were observed. The analysis of these alternations enabled assigning measures to the factors of considered risk. It served as a basis for describing the matrix of risk classification.

Risk classification method suggested in the study may be useful in designing plans concerning ecological risk management and at the determination of the safety management rules in case of technical interferences in the watercourses.

Keywords: aquatic vascular plants, ecological risk, maintenance works, watercourses regulation

Introduction

The research on regulatory and conservation works impact on the watercourses biocenosis show it is very variable. The most often effects of the following works are quality and quantity changes in plant and animal communities in the watercourse [1–8]. The level of these changes is dependent on both technical and environmental factors [5–7, 9, 10]. All these should be taken into account when planning regulation or

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conservation. In spite of knowledge base accessible in this matter there is no method allowing for biocenosis changes forecast being a result of technical works occurring in the river bed. The lack of such a tool results in the possibility of the assessment of the project after it has been done. Decisions taken by designers and contractors are assessed after the works completion. Taking into account that the effects of some decisions are irreversible for the environment it is crucial to take fast actions on solving the problem to assure environmental safety.

The following research shows the proposal of using an assessment of ecological risk to cover the problem. The term "risk" defines a degree of exposure to harmful events and their possible consequences [11, 12]. Determination of the level of ecological risk in regulated and conserved watercourses serves a possibility of changes forecast in river beds ecosystems [13–15].

The aim of this study is the determination of the principle to assess and classify ecological risk of regulatory and conservation works basing on one element of watercourse ecosystem – hydromacrophytes. These water plants are good bioindicators of the quality of water environment [16–18]. Therefore, they are one of the basic factors considered in an assessment of the ecological state of flowing waters [19].

Study objects

Field work was performed during vegetation periods in 2007 and 2008 for 10 small and medium Lower Silesian watercourses. These watercourses were divided into 34 experimental sections 100 meters long each. Detailed characteristic of particular sections is showed in the Table 1.

The sections had similar climatic (*moderate, transitional between maritime and continental*), geological (*Foresudetic Monocline* built of Permian and Trias rocks) and soil (*Luvisols* formed from loess and brown soil) conditions [20–22]. Adjacent field was used agriculturally with a domination of arable lands and grasslands. Most of experimental sections was not shadowed. Only some of them were slightly shadowed. Water in experimental sections was contaminated neither with urban or industrial wastes.

Particular sections varied with the degree of antrophogenic transformation – 11 were located in the watercourses where conservatory works were done while other 13 were located in regulated watercourses. Each watercourse had one section where no works were conducted. In the following comparative analyses they served as reference points.

Conservation works in the examined watercourses included: manual scything of scarps and riverside zones, mechanical elutriate of the bottom with removal of the aquatic plants and reparation of the scarps' strengthening. Regulatory works showed deeper interference in the river bed itself. As a result of these works changes in parameters of vertical and cross-sections and the method of the banks protection occurred. In most cases these works were mechanically performed.

Table 1

Study sections

River	Number of study section	Unmodified (U), conserved (C) or regulated (R) section	Bottom width [m]	Water-course depth [m]	Substrate	Inclination of the slope	Slope protection
Czarna Woda	1	C	5	3.5	sand/gravel	1:1.5	fascines
	2	C	3	2	gravel/stones	1:2	fascines
	3	C	3	2	gravel/stones	1:2	fascines
	4	C	3	2	gravel/stones	1:2	fascines
	5	C	3	2	gravel/stones	1:1	fascines
	6	U	7	1.5	sand	1:1	non-protected
Dobra	7	U	3	1.2	sand	1:1.5	non-protected
	8	R	3	0.6	sand	1:3	fascines
	9	R	6.8	2	sand	1:0.2	gabions
	10	R	2	2	sand	1:2	stone coating
Oleszna	11	C	2	3	organic	1:1.5	non-protected
	12	C	2.4	1.36	organic	1:0.8	non-protected
	13	U	1.5	2	organic	1:1.5	non-protected
Olesnica	14	U	7	2.06	sand	1:1.5	non-protected
	15	R	4	2.27	organic	1:1	fascines
Orla	16	U	8	1.8	organic	1:1	non-protected
	17	R	10	1.8	organic	1:0.5	fascines
Potok Sulistro-wicki	18	C	2	3.5	sand/stones	1:2	fascines
	19	U	2	3.5	sand/stones	1:2	non-protected
	20	C	2	3.5	sand/stones	1:2	fascines
Smortawa	21	C	10	2.15	sand	1:2	fascines
	22	U	5	2.15	sand	1:2	non-protected
	23	R	3	1.5	sand	1:2	fascines
	24	R	3	1.5	sand	1:2	fascines
Sleza	25	U	5	3	sand	1:1.5	non-protected
	26	R	5	3	concrete	1:0.2	stone concrete
	27	R	5	1.5	sand/gravel	1:1.5	stone coating
Zalina	28	C	1.5	1.92	sand	1:1.5	non-protected
	29	U	1.5	2.11	sand	1:1	non-protected
	30	R	2	2	organic	1:1	fascines
Zurawka	31	R	3.3	1.4	sand	1:1.5	fascines
	32	U	3	2.2	sand	1:2	non-protected
	33	R	3	2	sand	1:2	fascines
	34	R	3	2.2	sand	1:2	fascines

Methods

In the framework of the following field work macrophytes species was identified on the examined sections and the degree of the bottom coverage with them was de-

termined. All hydromacrophytes rooted in water for at least 90 % of the vegetation period and plants flowing naturally on the water surface or under it were taken into account. Five levels Braun-Blanquet scale was used for the determination of the density degree [23].

In order to assess species variety in the examined sections Shannon-Wiener Index – H [24] was calculated, considering both number of species and their coverage regularity [10].

Statistical analysis of the results was done using Statistica v. 9.1. programme.

Risk level connected with conservation and regulatory works performance was defined according to the following formula [11]:

$$R = P \cdot S$$

In this formula:

P – stands for the probability of change of the hydromacrophyte environment as a result of the performed works,

S – stands for the susceptibility of changes in the aquatic plant species composition.

These factors were ascribed with different measurements. They were indicated by the field work results. In both cases 5 levels scales were used.

Product of P and S parameters formed a basis of the risk level assessment. It was followed by two parametric matrix of the risk assessment [25]. According to accepted scales, matrix was marked due to observed changes in the plants community concerning works conducted in the examined watercourse. Risk classification was based on this analysis.

Results and discussion

In the examined sections 20 species of aquatic macrophytes were determined altogether. This number is small in comparison with the results obtained from other authors in similar studies [5, 26, 27]. The small number of species of aquatic plants may be due to the fact that most watercourses underwent some kind of technical interference in the past as well as due to agricultural settings in the surroundings. Furthermore the small rivers have a lower species richness of aquatic plants than large rivers [26].

The following species were found: *Alisma plantago-aquatica* L., *Berula erecta* (Huds.) Coville., *Butomus umbellatus* L., *Callitriche* sp., *Ceratophyllum demersum* L., *Elodea canadensis* L., *Glyceria Maxima* (Hartm.) Holmb., *Hydrocharis morsus ranae* L., *Myosotis palustris* (L.) L. em. Rchb., *Lemna minor* L., *Nuphar lutea* (L.) Sibth. & Sm., *Phalaris arundinacea* L., *Phragmites communis* Trin., *Potamogeton pectinatus* L., *Potamogeton filiformis* Pers., *Sagittaria sagittifolia* L., *Sparganium emersum* Rehmann, *Sparganium erectum* L. em. Rchb. s.s., *Spirodela polyrrhiza* (L.) Schleid., *Typha angustifolia* L. According to *Method of Macrophytes Rivers Assessment* (MMOR) these species have a wide or medium wide ecological scale and low or medium index value – W measured at 1 or 2 [28].

Figure 1 shows statistic data referring to aquatic plants occurrence in the examined river sections.

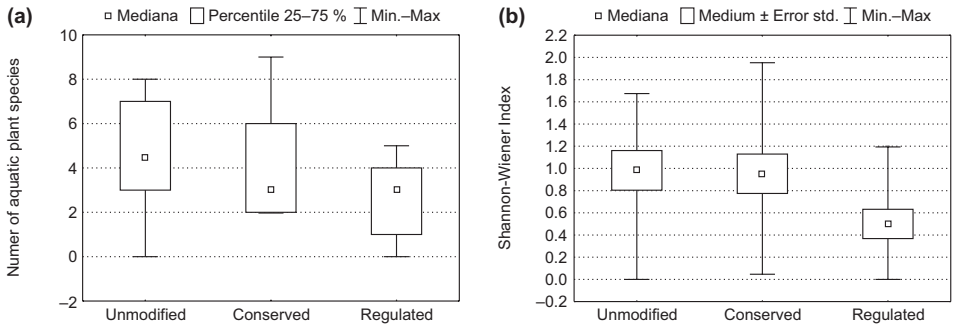

Fig. 1. Diversification of the number of species (a) and species variety Shannon-Wiener Index (b) in the examined sections

The highest mean number of species was observed in natural sections while in modified sections especially those which were regulated a lot less. It is widely believed that any technical interference within a watercourse's channel adversely affects its biocoenosis [5, 29–33]. Values of the diversity Shannon-Wiener Index determined in compared sections were slightly different (Fig. 1b). In unmodified and conserved examined sections they were similar while in regulated watercourses they were considerably lower. The mean index value in unmodified, conserved and regulated river beds was calculated at 0.98; 0.95 and 0.5, respectively.

Ecological risk factors were defined and classified using results of the following research. While assessing P factor, determining the possibility of alternations in the number of aquatic plants species, a strong relation with a range of performed works in the river bed was taken into account. Observations made during the research allowed to acknowledge a five degree scale.

Table 2

Scale of the changes occurrence probability in the aquatic plant communities in the river beds as a result of the watercourses regulation and maintenance works – P

Probability of changes occurence	
Point scale	Description scale
1 Very low	Slope mowing, river bed elutriation with the removal of aquatic plant life
2 Low	Slope mowing, river bed elutriation with the removal of the aquatic plant life, reparation and the strengthening of fascine
3 Medium	Changes in cross-section parameters, modification of the scarps incline to 1:1.5, additional strengthening of a riverbank's foundation with fascine construction
4 High	Changes in cross-section parameters, the strengthening of scarps with stone or stone mattress gabions
5 Very high	Changes in cross-section parameters, modification of watercourse's vertical plane with horizontal scarps, the strengthening of slopes with box gabions or retaining walls

Particular levels in the scale defining risk factor S – changes in the number of species as a result of works conduction were determined on the basis of comparison of species

composition of macrophytes aquatic communities in the sections where technical works were and were not done. In the sections where no human interference was present 15 species (Fig. 2) were found and 10 out of 15 showed medium index value (W = 2) while other species revealed low value (W = 1). None section was defined with stenotopic species of a high index value (W = 3).

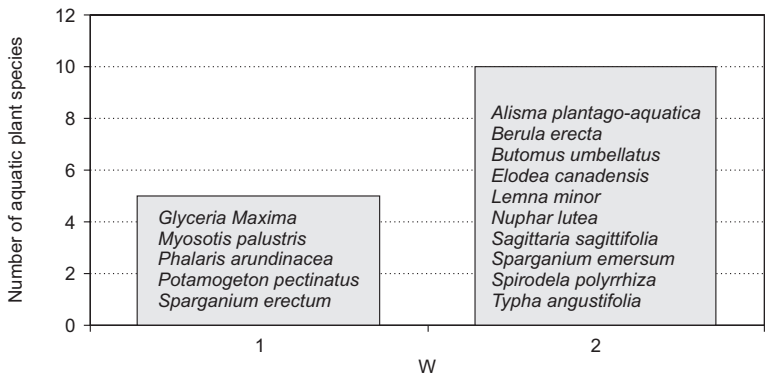

Fig. 2. Index value of the species occurring in unmodified sections

The following research enables to claim that the least severe effects of conducted works occurred in watercourses with wide ecological scale species. The more species with low index value the better they adapt to new, technically changed habitat conditions. On the basis of this relation the effects of conservatory and maintenance works were linked with aquatic macrophytes species composition in particular watercourses (Table 3).

Table 3

The scale of the consequences of the watercourses regulation and maintenance works in the river bed for the aquatic plant communities – S

Susceptibility of changes of aquatic plant species composition	
Point scale	Description scale
1 Lack	No hydromacrophytes in the watercourse
2 Mild	1–3 species of hydromacrophytes with low and medium index values of W = 1 or W = 2 in the watercourse
3 Moderate	More than 4 species of hydromacrophytes dominated with taxons of a low index value of W = 1 in the watercourse
4 Severe	More than 4 species of hydromacrophytes dominated with taxons of a medium index value of W = 2 in the watercourse
5 Very severe	Taxons with the high index value of W = 3 in the watercourse

Classifications of considered risk factors showed in Tables 2 and 3 formed a basis to compile ecological risk matrix in both maintained and conserved watercourses (Fig. 3).

Probability (P)						
	5	5	10	15	20	25
	4	4	8	12	16	20
	3	3	6	9	12	15
	2	2	4	6	8	10
	1	1	2	3	4	5
		1	**2**	**3**	**4**	**5**

Susceptibility of changes (S)

Fig. 3. A matrix of ecological risk in maintained and regulated watercourses

The following matrix shows the level of risk may be placed between 1 and 25 points.

Information obtained during the field work were used for determination of point range for small risk – usually accepted, medium and high – unaccepted. Each section located in modified watercourses were defined with the area of matrix where observed changes in plants communities took place. This data was presented on both matrixes – for watercourses where conducted works comprised of the river bed conservation (Fig. 4) and for regulated watercourses (Fig. 5). Both matrixes were also marked with the direction of the observed changes referring to the number of species and Shannon--Wiener Index.

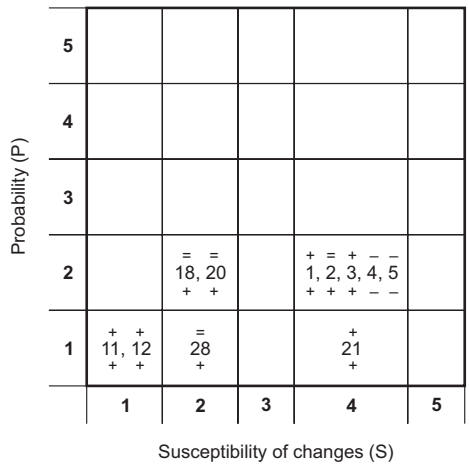

Fig. 4. A matrix of ecological risk for maintained study sections
[ ] – shows a trend of changes in the number of species: "+" – increase, "–" – decrease, "=" – no changes;
28 – the number of the examined section;
[ ] – shows a trend of changes in the Shannon-Wiener Index: "+" – increase, "–" – decrease

Observed alternations in aquatic plants communities concerned enlarging or lowering the number of species and species biodiversity index. Directions of these changes were marked in the Figs. 4 and 5 as upper and lower index given above the number. They show corresponding examined section. In case of conservatory works the most common

situations were those with a growth in both values (Fig. 4). It shows that properly executed maintenance work, including mowing the banks, the removal of plant life from the river bottom and its dredging, in fact does allow the watercourse to function properly, without causing a permanent loss of aquatic plant communities [1, 5, 14, 34–38]. Maintenance works do not cause the disappearance of islands and oxbow lakes, do not change the route of the river bottom and do not restrict the watercourse's capacity during overflowing [4]. Regulated sections in most cases show that the result of river beds regulation is lowering the number of species of aquatic plants and the values of Shannon-Wiener Index. According to Gunkel [30] river regulation is a major controlling factor for the aquatic ecosystems. Conventional river control causes the changes in hydraulic characteristics of the river. The hydrological regime has been widely recognized as an important factor controlling colonization of streambeds by macrophytes [38]. If for these works risk assessment according to proposed method risk level would be contained in the right, upper matrix area (Fig. 5).

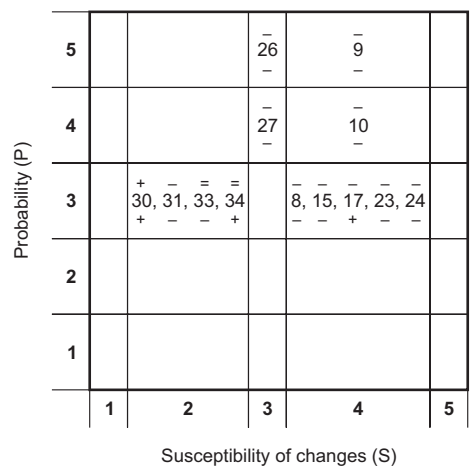

Fig. 5. A matrix of ecological risk for regulated study sections
[ ] – shows a trend of changes in the number of species: "+" – increase, "–" – decrease, "=" – no changes;
34 – the number of the examined section;
[ ] – shows a trend of changes in the Shannon-Wiener Index: "+" – increase, "–" – decrease

Figure 6 shows the matrix where both maintenance and regulatory works were considered. It was a basis for ecological risk classification taking into account its 3 levels – low, medium and high risk (Fig. 7).

The analysis of Fig. 7 reveals that the limits between fixed levels of risk are as follows:

– low risk – ≤ 4 points,
– moderate risk – 6–8 points,
– high risk – ≥ 12 points.

Basing on the research it was impossible to determine the risk level for 5, 9 and 10 points. Therefore, it is necessary to conduct further research, including study objects

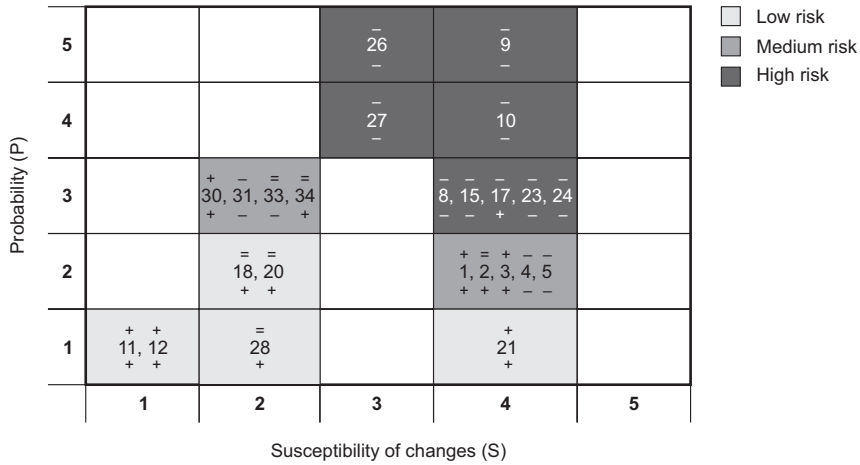

Fig. 6. The ecological risk classification based on the alternations of aquatic plants communities of maintained and regulated watercourses
[ ] – shows a trend of changes in the number of species: "+" – increase, "–" – decrease, "=" – no changes; 28 – the number of the examined section;
[ ] – shows a trend of changes in the Shannon-Wiener Index: "+" – increase, "–" – decrease

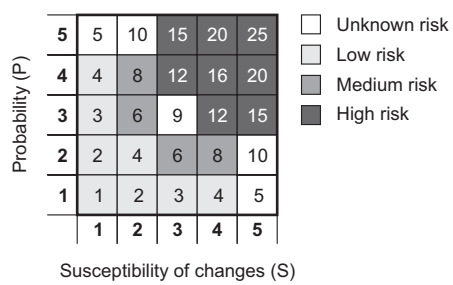

Fig. 7. A matrix showing the three ecological risk levels of maintained and regulated watercourses

with various composition of aquatic plant communities and the different degree of anthropogenic transformation.

Conducted research and analyses should be considered as pilotage for the following project. Better recognition requires further observations performed in the communities of plants and animals in watercourses being a subject to water works.

Conclusions

1. The research conducted on 10 lowland watercourses found in Lower Silesia, where maintenance and regulatory works were done, showed these works resulted in alternations in the number of aquatic plants species and the index of their biodiversity. The range of performed works and ecological tolerance of the plants occurrence in watercourses had a big influence on the size of these changes.

2. The results of the following research allowed for determination and classification of ecological risk factors in five level scales – the possibility of alternations in species in the communities of hydromacrophytes and the level of these changes.

3. Based on the risk matrix, the ecological risk was then classified in three different levels. Low risk was determined for those values ranging from 1 to 4, medium risk was found between 6 and 8, while high risk was determined at values of 12 points or more.

4. The performed analysis found that maintenance works are connected with low or moderate risk, whereas regulatory works comprise of moderate or high risk area.

5. In order to limit adverse alternations of the aquatic plants community, a risk assessment should be performed when planning any maintenance or regulatory works. It requires a detailed environmental assessment of the river bed and a detailed analysis of the range of the planned works.

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KLASYFIKACJA RYZYKA EKOLOGICZNEGO W CIEKACH REGULOWANYCH I KONSERWOWANYCH

Instytut Kształtowania i Ochrony Środowiska
Uniwersytet Przyrodniczy we Wrocławiu

Streszczenie: Przedmiotem pracy jest ryzyko ekologiczne w robotach regulacyjnych i konserwacyjnych wykonywanych na małych i średnich ciekach nizinnych. Ryzyko to nie jest jeszcze dobrze rozpoznane. Wynika to m.in. z braku podstaw do obiektywnej jego oceny. W pracy przedstawiono propozycję rozwiązania tego problemu.

Podstawę analizy stanowią wyniki badań terenowych prowadzonych w latach 2007–2008, na 10 nizinnych ciekach Dolnego Śląska. Badania obejmowały identyfikację występujących w korycie gatunków naczyniowych roślin wodnych oraz określenie stopnia pokrycia przez nie dna. Wykazały one, że w wykonanych prac zachodzą zmiany jakościowe i ilościowe w zbiorowiskach naczyniowych roślin wodnych. Analiza tych zmian pozwoliła na przypisanie miar czynnikom rozpatrywanego ryzyka. Stanowiło to podstawę opracowania macierzy klasyfikacji ryzyka.

Zaproponowana w pracy metoda klasyfikacji ryzyka może być przydatna w opracowywaniu planów zarządzania ryzykiem ekologicznym oraz przy określaniu zasad zarządzania bezpieczeństwem środowiska przyrodniczego w przypadkach ingerencji technicznych w korytach cieków.

Słowa kluczowe: ryzyko ekologiczne, naczyniowe rośliny wodne, roboty konserwacyjne, regulacja cieków

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DNA – kwas dezoksyrybonukleinowy (3) 469, *53*
EPN – nicienie entomopatogenne (5–6) 749, *84*
IAA – kwas indolilo-3-octowy (7) 967, *108*
NAA – neutronowa analiza aktywacyjna(1) 27, *3*
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PM2.5 – (7) 933, *104*
PM10 – (7) 933, *104*
SBR – biologiczny reaktor *s*ekwencyjny (7) 895, *100*
SRP – rozpuszczalny fosfor reaktywny (2) 201, *22*
TDTP – tymczasowe dopuszczalne tygodniowe pobrania (9–10) 1185, *133*
UV – ultrafiolet (3) 469, *53*; (5–6) 827, *95*
VOCs – lotne związki organiczne (7) 991, *111*

Varia

CENTRAL EUROPEAN CONFERENCE ECOpole '11

Short Conference Report

The Conference ECOpole '11 was held in 13–15 X 2011 at the Conference Center "Rzemieslnik" in Zakopane, PL. It was the jubilee – the twentieth ecological conference of the series of meetings organised by the Society of Ecological Chemistry and Engineering, Opole, PL.

178 participants, including delegates representing 10 countries (Bulgaria, Czech Republic, Germany, Latvia, Lithuania, Poland, Romania, Russian Federation, Slovakia and Ukraine) took part in the event and presented 30 oral contributions and 177 posters.

The Abstracts of the Conference contributions were available on the Conference website before and during the Conference.

The Conference issue of the quarterly *Ecological Chemistry and Engineering S* 2011, **18**(3) containing among others keynote speakers papers, was distributed at the Conference Reception desk together with a PenDrive (containing short info on Keynote Speakers, Abstracts of the Conference presentations as well as ECOpole '11 Conference Programme).

On Wednesday (12^{th} October 2011) at 20.30 the participants were invited for Get-Together Party.

The Conference Agenda was divided into 5 sections:

– SI Ecological Chemistry and Engineering
– SII Environment Friendly Production and Use of Energy
– SIII Risk, Crisis and Security Management
– SIV Forum of Young Scientists and Environmental Education
– SV Health, Ecology and Agriculture.

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

Prof. **Paul Jozef CRUTZEN** (*Max-Planck-Institute for Chemistry, Mainz, DE*), the **Nobel Prize Winner**, initiated the First Plenary Session with the invited lecture: "Atmospheric chemistry and climate in the Anthropocene". This contribution was met with great interest of the audience.

During the conference the plenary lectures were also delivered by other invited lecturers: prof. **Marina V. FRONTASYEVA** (*Joint Institute for Nuclear Research,*

Dubna, RU): "NAA for Life Sciences at Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna", prof. **Hartmut FRANK** (*University of Bayreuth, Bayreuth, DE*): "Fresh water pearl mussels, vanishing witnesses of water quality", prof. **Jozef LEHOTAY** (*Slovak Technical University, Bratislava and University of SS Cyril and Methodius in Trnava, SK*): "Trace analysis of some toxic compounds by HPLC" and prof. **Bogdan ZYGMUNT** (*Gdansk University of Technology, Gdańsk, PL*): "Increasingly green approaches to determine selected trace organics in complex matrices".

There were also presented very interesting lectures, *eg*: **S. Ledakowicz and L. Bilinska** (*Lodz University of Technology, Łódź, PL*): "Application of the Fenton reagent in the textile wastewater treatment under industrial conditions", **A. Nowak** (*West Pomeranian University of Technology, Szczecin, PL*): "Are the bacteria necessary in the environment?", **M. Bratychak** (*Lviv Polytechnic National University, Lviv, UA*): "Utilization ways of by-products of hydrocarbon raw material pyrolysis", **S. Bocian and B. Buszewski** (*Nicolaus Copernicus University, Toruń, PL*): "The new approach to the retention mechanism in reversed-phase liquid chromatography", **D. Panasiuk, A. Glodek and J.M. Pacyna** (*NILU Polska Ltd., Norwegian Institute for Air Research Branch Poland, Katowice, PL*): "Scenarios of mercury emission to air, water and soil in Poland to year 2020", **G. Boczkaj, M. Jaszczolt and M. Kaminski** (*Gdansk University of Technology, Gdańsk, PL*): "New methods for process control of the thermal cracking rate of vacuum distillates and products from crude oil vacuum distillation", **E. Kraleva, V. Karamfilov and G. Hiebaum** (*Institute of Biodiversity and Ecosystem Research – BAS, Sofia, BG*): "Determination of polycyclic aromatic hydrocarbons in the Black Sea water by GC/MS following preconcentration with solid-phase extraction", **K. Piotrowska, M. Imbierowicz and A. Chacuk** (*Lodz University of Technology, Łódź, PL*): "Wet oxidation of dairy sewage: the kinetic study of intermediate products decomposition", **M. Siemieniec, H. Kierzkowska-Pawlak and A. Chacuk** (*Lodz University of Technology, Łódź, PL*): "Reaction kinetics of CO_2 in aqueous ethylethanolamine and methyldiethanolamine solutions using the stopped-flow technique", **R. Slavik, M. Julinova, and M. Labudikova** (*Tomas Bata University, Zlin, CR*): "Screening of the spatial distribution of risk metals in topsoil from an industrial complex" and **A.I. Stoica and A.A. Ciucu** (*University of Bucharest, Bucharest, RO*): "Kinetic approach for heavy metals detection by glucose-oxidase inhibition studies".

Thursday, a day of hard work, was finished with the Poster Session of the Section The Quality of Environment and its Monitoring. Many of the discussions started at the posters, lasted until the evening hours.

The next point of Thursday Conference Programme was the 45 minutes-long Musical Soirée. Songs and dances from Rocky Podhale were presented by highlander team "Młode Klimki". The audience applauded the players and claimed for encore.

At 20.00 the Conference participants were invited for a Conference Dinner.

As usually during the ECOpole Conferences, the second day included the Session of the Young Scientists (a forum of young scientists that present and discuss local ecological problems of their countries). During the Young Scientists', Environmental Education and Renewable Energy Poster Session 57 posters were presented.

The Scientific Board (**prof. Stanisław Ledakowicz** (*Lodz University of Technology, Łódź, PL*), **prof. Mikhail Bratychak** (*Lviv Polytechnic National University, Lviv, UA*), **prof. Bohumil Vybiral** (*University of Hradec Kralove, Hradec Králové, CZ*) and **prof. Witold Waclawek** (*Society of Ecological Chemistry and Engineering, Opole, PL*)) granted awards (sponsored by the Society of Ecological Chemistry and Engineering) for the best presentations. The award for oral presentation was given to **Anna Kwiecinska,** MSc (*Silesian University of Technology, Gliwice, PL*) for the oral A. Kwiecinska and K. Konieczny: "Application of membrane processes in treatment of slurry from high-density pig farming". The awards for poster presentations were given to **Lucie Trnkova**, MSc (*University of Hradec Kralove, Hradec Kralove, CZ*) for the poster: L. Trnkova, I. Bousova, F. Altieri and J. Drsata: "Interaction of proteins with low-molecular substances occurring in environment: Structure-activity relationships", to **Agnieszka Baran**, MSc (*University of Agriculture, Kraków, PL*) for the poster: A. Baran and M. Tarnawski: "Content of heavy metals in leachate from bottom sediments as a potential source of contamination of water and ground environment" and to **Aleksander Zaremba**, MSc (*Czestochowa University of Technology, Częstochowa, PL*) for the poster: T. Rodziewicz, J. Nakata, K. Taira, I. Inagawa, A. Zaremba and M. Waclawek: "Performance of flat module made of Sphelar cells in higher latitude areas".

On Saturday morning an excursion was organised to Kuznice. Participants visited Manor-Palace in Kuznice, Memorial Chamber of Count Wladyslaw Zamoyski and an exhibition of works by Antoni Kocjan "Animals of the Tatras".

Closing the conference, prof., prof. Maria and Witold Waclawek made short recapitulation. In general, ECOpole '11 was focused on monitoring of the quality of natural environment, its effects on human life, environmental education as well as application of renewable sources of energy.

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

They asked the participants to publish electronic version of presented during ECOpole '11 Conference contributions (oral presentations as well as posters) at the conference website ecopole.uni.opole.pl – some persons have already took advantage of this call.

They announced, that full texts of the presented papers will be published (after obtaining reviewers' positive opinions) in the successive issues of the journals *Ecological Chemistry and Engineering A* and *S* and they will be distributed to all participants. The Extended Abstracts of the presentations will be published in two subsequent issues of semi-annual *Proceedings of the ECOpole*.

Thanks to the financial support of the Polish Ministry of Scientific Research and Higher Education, it was possible, among others to publish Abstracts and full Conference contributions on the Conference website.

At the end they invited all Colleagues to attend the ECOpole '12 Conference, which will be held at the Conference Center "Rzemieslnik" in Zakopane, PL in the next October.

Maria Wacławek

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Invited Lectures

Jozef Crutzen – Atmospheric chemistry and climate in the Anthropocene

Marina V. Frontasyeva – NAA for Life Sciences at Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna

Hartmut Frank – Fresh water pearl mussels, vanishing witnesses of water quality

Jozef Lehotay – Trace analysis of some toxic compounds by HPLC

Bogdan Zygmunt – Increasingly green approaches to determine selected trace organics in complex matrices

Professor Paul Jozef Crutzen, the Nobel Prize Winner, delivering his lecture (*photo by Andrzej Nowak*)

Professor Marina V. Frontasyeva as a Chairperson (*photo by Tomasz Ciesielczuk*)

Invitation for ECOpole '12 Conference

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 21st annual Central European Conference ECOpole '12, which will be held in **11–13 X 2012** (Thursday–Saturday).

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

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

The Conference language is English.

Contributions to the Conference will be published as:

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

Additional information one could find on the Conference website:

ecopole.uni.opole.pl

The deadline for sending the Abstracts is **15th July 2012** and for the Extended Abstracts: **1st October 2012**. The actualised list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one can find (starting from **31st July 2012**) on the Conference website.

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

At the Reception Desk each participant will obtain abstracts of the Conference contributions as well as the Conference Programme recorded on electronic media (the Programme will be also published on the ECOpole '12 website).

Further information is available from:
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20. 2011 CEC ECOpole '11 Zakopane

Zapraszamy
do udziału w Środkowoeuropejskiej Konferencji ECOpole '12
w dniach 11–13 X 2012

SUBSTANCJE CHEMICZNE W ŚRODOWISKU PRZYRODNICZYM

Będzie to dwudziesta pierwsza z rzędu konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotycząca różnych aspektów ochrony środowiska przyrodniczego.

Doroczne konferencje ECOpole mają charakter międzynarodowy i za takie są uznane przez Ministerstwo Nauki i Szkolnictwa Wyższego.

Obrady konferencji ECOpole '12 będą zgrupowane w pięciu sekcjach:

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

Materiały konferencyjne będą opublikowane w postaci:

– abstraktów (0,5 strony formatu A4) na CD-ROM-ie;
– rozszerzonych streszczeń o objętości 4–6 stron w półroczniku *Proceedings of ECOpole*;
– artykułów: w abstraktowanych czasopismach: *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (*Ecol. Chem. Eng.*) ser. A i S oraz niektórych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*.

Termin nadsyłania angielskiego i polskiego streszczenia o objętości 0,5–1,0 strony (wersja cyfrowa + wydruk) planowanych wystąpień upływa w dniu 15 lipca 2012 r. Lista prac zakwalifikowanych przez Radę Naukową Konferencji do prezentacji będzie sukcesywnie publikowana od **15 lipca 2012 r.** na stronie webowej

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Prof. dr hab. Maria Wacławek
Przewodnicząca Komitetu Organizacyjnego
Konferencji ECOpole ’12

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