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EDITORIAL OFFICE
Opole University
ul. kard. B. Kominka 4, 45–032 OPOLE, PL
phone +48 77 455 91 49
email: waclawek@uni.opole.pl
http://tchie.uni.opole.pl

SECRETARIES
Agnieszka Dołhańczuk-Śródka, phone +48 77 401 60 46, email: agna@.uni.opole.pl
Małgorzata Rajfur, phone +48 77 401 60 42, email: mrajfur@o2.pl

SECRETARIES' OFFICE
phone +48 77 401 60 42
email: mrajfur@o2.pl

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ECOLOGICAL CHEMISTRY AND ENGINEERING A

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Teresa BANASZKIEWICZ[1]

ECOLOGICAL ASPECT OF ADDITION OF DIFFERENT QUANTITY OF ENZYME PREPARATION TO MIXTURE FOR BROILER CHICKENS

EKOLOGICZNY ASPEKT DODANIA RÓŻNEJ ILOŚCI PREPARATU ENZYMATYCZNEGO DO MIESZANKI DLA KURCZĄT BROJLERÓW

Abstract: The aim of the research was to determine the effect of addition of different quantities of enzyme preparation containing xylanase to mixtures for broiler chickens on the excretion and retention of nutrients. The experiment was carried out on 75 one-day old broiler chickens divided into 3 groups each of 25 birds, in five replications, from 1 to 21 days of life. Chickens from group I were fed wheat mixtures with rape cake supplemented with enzyme preparation containing xylanase at the quantity of 0.2 g · kg^{-1}. In mixtures for the remaining groups the quantity of enzyme preparation was increased up to 0.3 g in group II and to 0.4 g in mixture in group III. During the last week of the experiment the excreta were collected. The body weight of broiler chickens in the 21st day of life was not significantly affected, however, together with the increase in enzyme preparation quantity the body weight improved. The increasing in the quantity of enzyme preparation influenced the reduction of nutrient content in excreta. Together with the increasing in the quantity of enzyme preparation the retention of dry matter and crude nitrogen was improved and the most profitable retention of crude fat was found after applying 0.4 g · $kg^{-1}$of enzyme preparation.

Keywords: rape cakes, enzymatic preparation, broiler chickens

Grain, mostly wheat, which provides 55 % of the available energy and 35 % of the protein make up the largest part in the mixtures for broiler chickens. The other part of protein in broiler diets is satisfied by high protein feeds, most often by imported soyabean meal. Kocher et al [1, 2] showed that canola meal could replace soyabean meal in broiler diets without any negative effects on broiler performance, however the addition of commercial feed enzymes to the canola meal diet had no significant effect on feed uptake and feed conversion ratio. Poland is renowned among rapeseed producers. The rapeseed products are characterized by great deal of protein and rape cakes contain a lot of fat. The introduction of rape products can lubricate mixtures

[1] Department of Animal Nutrition, University of Podlasie, ul. B. Prusa 14, 08–110 Siedlce, Poland, phone: +48 25 643 13 42, email: banaszt@ap.siedlce.pl

which facilitates keeping energy in balance, influences the improvement of nutrient utilization, may lead to better sanitary and environmental conditions and to reduction of production risk. The decrease in glucosinolates and elimination of erucic acid from rapeseed 00 give possibilities to replace soyabean meal by rape products [3, 4]. The nutritive value of grains for poultry is reduced by soluble fibre content, which comprise NSP – *non-starch polysaccharides* [5, 6]. These substances are characterized by much viscosity and absorbability, which worsens the digestibility and utilization mainly of fat, amino acids, starch and phosphorus [7]. Rapeseed contains a lot of NSP, whereas the content of available energy and phosphorus is low [8]. The introduction of rape cakes to wheat mixtures increases the content of NSP. The addition of enzymes which decompose the NSP can decrease the antinutritional effect of these substances and can improve the utilization of nutrients from mixtures. The efficiency of enzymatic preparations depends on the kind of enzyme and its quantity [9–13].

The improvement of the nutritive value of mixtures by using enzymatic preparations can influence the quantity of non-use nutrients and nutrients excreted to the environment [14].

The problem regarding excretion of nutrients from mixtures containing rape products as a result of applying enzymes that hydrolyze NSP was analyzed to a small extent.

The aim of the research was to determine the effect of addition of different quantities of enzyme preparation containing xylanase to starter mixtures for broiler chickens on excretion and retention of nutrients.

Materials and methods

The investigations were carried out on 75 one-day-old broiler chickens Ross 308, which were divided into three homogeneous experimental groups, 25 birds in a group (5 × 5 birds), in a period from the 1st to 21st day of chicken life. Broilers were kept in metabolic cages and fed isocaloric and isoprotein experimental starter diets. The diets (in a mashed form) and water were given *ad libitum*. All diets were balanced according to the Nutritional Requirements for Poultry [15]. The broilers from group I were given the experimental starter diet based on wheat (57.85 %), soyabean meal (24 %), rapeseed cake of Lirajet cv. (15 %) supplemented with 0.2 g · kg^{-1} of enzymatic preparation containing xylanase. In the remaining mixtures the quantity of enzymatic preparation increased up to 0.3 g group II and up to 0.4 g · kg^{-1} in mixtures for group III. According to the suppliers' information the enzyme preparation derived from *Aspergilus oryzae* and contained endo-1,4-β xylanase (min. 1000 FXU(W)/g).

In the 1st and 21st day of age, the birds were weighed and their feed consumption was analysed. During the last week of the experiment the balance test of basic nutrients and phosphorus were carried out. During the following 3 days, all excreta were collected and feed consumption was designated. Prior to the analyses the excreta were dried at 60 ^{o}C and ground. In the feeds and excreta the basic nutrient contents were determined by the AOAC procedure [16] and the crude phosphorus content was tested by the colorimetric method (PN-76/R-64781). The excretion of basic nutrients and phosphorus as well as the balance between these feed components were designated. The obtained

data were statistically analyzed by the analysis of variance, and Duncan's multiple range test was used to separate means when the significant effects ($p \leq 0.05$) were detected by the analysis of variance.

Results and discussion

In Table 1 the content of basic nutrients and crude phosphorus in feeds used in experimental mixtures is shown.

Table 1

Content of basic nutrient and crude phosphorus in feeds [$g \cdot kg^{-1}$]

Specification	Nutrient content [$g \cdot kg^{-1}$]					
	Dry matter	Crude ash	Crude nitrogen	Crude fibre	Crude fat	Crude phosphorus
Wheat meal	926.5	17.3	17.3	24.9	13.5	3.2
Soyabean meal	921.3	73.9	79.1	25.6	23.5	6.5
Rape cake of Lirajet cv.	951.7	57.2	41.2	69.9	253.7	9.7

The content of crude nitrogen in wheat was on a slightly lower level than it is given in Nutritional Requirements for Poultry [15], soyabean meal contained similar quantity of crude nitrogen content and rape cake had a slightly smaller amount of the nutrient than in the above-mentioned norms. The basic nutrients and crude phosphorus contents in mixtures were on a similar level (ME – 11.55MJ; crude protein – 21.4 %). The body weight of 21 day-old chickens was similar in all groups and ranged from 603 to 631 g, and it had the highest value when the enzymatic preparation level was the highest, but the obtained results between groups were not significantly different. The contents of basic nutrients and crude phosphorus in broiler excreta from each group were shown in Table 2, whereas in Table 3 the percentage of reductions of basic nutrients and phosphorus in excreta as a result of higher quantity of enzyme preparation in mixtures were showed.

Table 2

Content of basic nutrient and phosphorus in excreta [$g \cdot kg^{-1}$]

Group	Nutrient					
	Dry matter	Crude ash	Crude nitrogen	Crude fibre	Crude fat	Crude phosphorus
I + 0.2 g of preparation	808.3a ± 0.99	101.5 ± 1.26	37.9a ± 0.15	85.7a ± 0.44	42.9 ± 0.40	9.55 ± 0.79
II + 0.3 g of preparation	690.4b ± 5.92	85.9 ± 0.82	33.4b ± 0.35	71.7b ± 1.03	39.2 ± 0.39	8.68 ± 0.53
III + 0.4 g of preparation	695.9b ± 2.47	95.7 ± 1.13	31.4b ± 0.13	74.9b ± 0.58	36.9 ± 0.25	8.91 ± 0.60

a, b – means in columns followed by different letters are significantly different ($p \leq 0.05$); ± – standard deviation.

Table 3

Degree of basic nutrients and phosphorus reduction in excreta due increase quantity enzyme preparation [%]

Group	Reduction of nutrient in excreta [%]					
	Dry matter	Crude ash	Crude nitrogen	Crude fibre	Crude fat	Crude phosphorus
I + 0.2 g of preparation	...	...	...	...	...	...
II + 0.3 g of preparation	14.58	15.36	11.87	16.33	8.62	9.10
III + 0.4 g of preparation	13.90	5.71	17.15	12.60	13.98	6.70

The content of nitrogen in chicken excreta from group III, fed mixture with 0.4 g · kg^{-1} of enzymatic preparation amounted to 31.4 g and from group I in broilers fed mixture with 0.2 g of the preparation – 37.9 g, and the obtained results were significantly proved. As a result of supplementing higher quantity of enzyme preparation, the content of nitrogen in excreta decreased in the range between 11–17 %. Dry matter content in excreta significantly decreased from 80.83 % at the level of 0.2 g of enzyme preparation to 69.04 % when the preparation was at the level of 0.3 and 0.4 g, while crude fibre content decreased from 8.57 to 7.17 %. The level of other nutrients and crude phosphorus in excreta as a result of increasing in the quantity of enzyme preparation decreased by 5.71–15.36 % (Table 3), whereas the content of crude fibre, crude nitrogen and dry matter decreased in the highest degree.

In Table 4 the daily balance of basic nutrients and crude phosphorus as well as coefficients of retention were presented.

Table 4

Balance and retention of basic nutrient and phosphorus

Group	Dry matter	Crude ash	Crude nitrogen	Crude fibre	Crude fat	Crude phosphorus
Quantity of nutrient uptake [g/bird/day]						
I + 0.2 g of preparation	32.2 ± 3.46	2.27 ± 0.24	1.18 ± 0.12	1.39a ± 0.14	1.81 ± 0.20	0.27 ± 0.03
II + 0.3 g of preparation	35.4 ± 2.78	2.57 ± 0.20	1.31 ± 0.10	1.62b ± 0.13	1.98 ± 0.16	0.31 ± 0.02
III + 0.4 g of preparation	34.4 ± 1.41	2.54 ± 0.11	1.28 ± 0.05	1.57b ± 0.06	2.39 ± 0.10	0.30 ± 0.01
Quantity of nutrient excretion [g/bird/day]						
I + 0.2 g of preparation	10.07a ± 0.56	1.26 ± 0.13	0.47a ± 0.03	1.07 ± 0.10	0.53a ± 0.07	0.111 ± 0.01
II + 0.3 g of preparation	11.40b ± 0.93	1.42 ± 0.13	0.55b ± 0.04	1.19 ± 0.20	0.64b ± 0.08	0.138 ± 0.01
III + 0.4 g of preparation	10.95ab ± 0.64	1.50 ± 0.17	0.49a ± 0.03	1.18 ± 0.11	0.58ab ± 0.03	0.136 ± 0.02

Table 4 contd.

Group	Dry matter	Crude ash	Crude nitrogen	Crude fibre	Crude fat	Crude phosphorus
Retention of nutrient [%]						
I + 0.2 g of preparation	68.73 ± 4.22	44.49 ± 9.23	60.17 ± 7.10	23.02 ± 9.98	70.71a ± 2.69	58.88 ± 5.64
II + 0.3 g of preparation	67.80 ± 0.38	44.74 ± 1.49	58.02 ± 0.81	26.54 ± 7.18	67.67b ± 1.70	55.48 ± 1.65
III + 0.4 g of preparation	68.17 ± 0.88	40.47 ± 5.67	61.72 ± 1.49	24.84 ± 5.30	75.73c ± 0.98	54.66 ± 1.76

a, b – means in columns followed by different letters are significantly different ($p \leq 0.05$); ± – standard deviation.

The quantity of dry matter consumption ranged from 32.2 to 35.4 g, nitrogen from 1.18 to 1.31 g daily per bird and phosphorus from 0.27 g in group I to 0.31 g in group II. The quantities of consumed nutrients were not significantly different between groups, except fibre, the uptake of which by chickens from II and III groups was significantly higher. Significant differences between groups in the quantity of crude nitrogen, crude fat and dry matter excretion per bird during balance test were found. The retention of nutrients in the form of dry matter was on a level of 67–69 %, crude nitrogen was kept by chickens on a level of 58–62 %.The highest coefficient of retention for fat (75.73 %) at the level of 0.4 g of enzyme preparation was determined. Retention of phosphorus in groups ranged from 54.66 to 58.88 %, but it was not significantly different.

Mikulski et al [13] reported that the addition of the smallest dose of enzyme preparation containing xylanase (50 cm^3/Mg of mixture) proved the tendency to improve digestibility of protein, fat and NDF, and the improvement of digestibility for the most nutrients at 150 cm^3/Mg of mixture was found. The authors stated the improvement of digestibility of fat and dry matter and that the increasing quantity of enzyme preparation in the range of 50–150 cm^3 profitable influenced rearing results in turkeys. According to Korol et al [14] mixtures, in which the level of fat was increased, decreased in about 10–20 % compared with non-fatted ones and the retention of nitrogen was higher by about 12–14 %. The quantity of phosphorus excretion decreased by 15 % and calcium excretion by 15–20 % per 1 kg of body weight.

Conclusion

An increase in the quantity of enzyme preparation influenced the reduction of nutrient content in excreta. Together with the increasing in the quantity of enzyme preparation the retentions of dry matter, crude nitrogen and crude fat were improved.

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EKOLOGICZNY ASPEKT DODANIA RÓŻNEJ ILOŚCI PREPARATU ENZYMATYCZNEGO DO MIESZANKI DLA KURCZĄT BROJLERÓW

Wydział Przyrodniczy
Uniwersytet Przyrodniczy w Siedlacach

Abstrakt: Celem przeprowadzonych badań było określenie wpływu wzrastającej ilości preparatu enzymatycznego dodanego do mieszanki dla kurcząt brojlerów na wydalanie oraz retencję składników pokarmowych. Badania przeprowadzono na 75 jednodniowych kurczętach brojlerach podzielonych na 3 grupy po 25 ptaków, pięć powtórzeń po 5 sztuk, w okresie od 1. do 21. dnia życia. Kurczęta z grupy I żywiono mieszanką pszenną zawierającą makuch rzepakowy uzupełniony preparatem enzymatycznym zawierającym ksylanazę w ilości 0,2 g · kg^{-1}. W mieszankach skarmianych w dwóch pozostałych grupach zwiększono ilość dodanego preparatu do 0,3 g · kg^{-1} (grupa II) oraz do 0,4 g · kg^{-1} (grupa III). W ostatnim tygodniu doświadczenia przeprowadzono kolekcję odchodów w celu określenia ilości składników pokarmowych wydalanych oraz stopnia ich retencji.

Masa ciała kurcząt w wieku 21 dni nie różniła się znacząco między grupami, jednak wraz ze zwiększaniem ilości preparatu enzymatycznego wzrastała również uzyskana masa ciała. Zwiększenie ilości dodanego preparatu enzymatycznego spowodowało obniżenie zawartości składników pokarmowych w wydalanych odchodach. Wraz ze wzrostem ilości dodawanego preparatu enzymatycznego poprawiała się retencja suchej masy oraz azotu ogólnego, a najkorzystniejszą retencję tłuszczu surowego stwierdzono przy zastosowaniu 0,4 g preparatu.

Słowa kluczowe: makuch rzepakowy, preparat enzymatyczny, kurczęta brojlery

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Joanna DŁUŻNIEWSKA[1] and Ryszard MAZUREK[2]

IMPACT OF THE DISTANCE FROM BLACK LOCUST (*Robinia pseudoacacia* L.) SHELTERBELTS ON SOIL MICROFLORA

WPŁYW ODLEGŁOŚCI OD ZADRZEWIEŃ ROBINII AKACJOWEJ (*Robinia pseudoacacia* L.) NA MIKROFLORĘ GLEBY

Abstract: The paper aimed at determining the quantitative and species composition of soil microorganisms depending on the distance from 30-year-old black locust shelterbelts in Krolewice village situated on the Proszowicki Plateau. Samples of soil, from which bacteria, Actinomycetales and fungi were isolated were collected from three zones at various distances from the shelterbelts: I – 0–2 m; II – 4–6 m and III – 10–12 m.

It was found that bacteria were the most numerous in the tested samples. Also significant increase in bacteria colony number was observed with increasing distance from the shelterbelts. Between 3 and 9 fungi species were isolated from the individual zones. The dominant fungus species in the soils from black locust shelterbelts were *Fusarium solani*, *Trichoderma viride*, *Mucor hiemalis* and *Penicillium* spp.

Keywords: *Robinia pseudoacacia*, soil, microorganisms

Shelterbelts, through increasing diversity of landscape structure, allow to combine agricultural production with environmental protection. Introducing shelterbelts into the agricultural environment protects biodiversity and may also limit spread of pollution. Shelterbelts are greatly involved in regulating important biological processes comprising matter cycling and energy flow between agricultural ecosystem components [1, 2]. One of the plants used for shelterbelts is black locust. The plant influences the increase in soil nitrogen content, whereas its shelterbelts also play humus forming role [3, 4].

Soil environment, including microorganisms is strictly connected with vegetation cover. The effect crops or lack of it and soil abundance in food, condition the differences in microflora communities composition. The number and activities of soil

[1] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 00, email: rrdluzni@cyf-kr.edu.pl

[2] Department of Soil Science and Soil Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 00, email: rrmazure@cyf-kr.edu.pl

microorganisms depend on physical and chemical soil properties, including also mechanical composition, pH, nitrogen and organic matter content [5–7].

The paper aimed at determining the quantitative and species composition of microorganisms present in soils depending on the distance from 30-year-old black locust shelterbelts.

Material and methods

The soil material was collected from the arable field in Krolewice village situated on the Proszowicki Plateau. The arable field adjoined the 8–10 m high and about 30 years old black locust shelterbelt. The soil samples from which the microorganisms were isolated were collected from the zones at the distance of : I – 0–2 m, II – 4–6 m and III – 10–12 m from the shelterbelts. The soil material was classified as typical brown soil developed from loess and revealed granulation of clay silt. Surface soil samples collected in Krolewice had the highest content of organic carbon (19.88 g · kg^{-1}) in the 0–2 m zone and the lowest (13.14 g · kg^{-1}) in the 10–12 m zone (Table 1). Total nitrogen content in the soils was decreasing with the distance from the shelterbelts (from 1.70 g · kg^{-1} in 0–2 m zone to 1.66 g · kg^{-1} in 10–12 m zone). Acid reaction was assessed in all soil samples, but the highest pH values were registered in the zone neighbouring black locust trees (pH 5.75).

Table 1

Selected properties of soils sampled from particular distance zones from black locust

Distance zone from black locust [m]	$C_{org.}$	N_{total}	C/N	pH_{KCl}	pH_{H_2O}
	[g/kg]				
0–2	19.88	1.70	11.71	4.87	5.75
4–6	16.87	1.69	9.99	4.72	5.67
10–12	13.14	1.66	7.93	4.63	5.64

The bacteria and *Actinomycetes* count was assessed using plate method [8].

Soil fungi communities were isolated using sand method after Manka [9]. A soil--sand mixture was prepared from the tested sample, containing 1 g of soil and 149 g of sterile quartz sand. The mixture (28 mg weight) was placed on Petri dishes and poured over with Martin-Johnson medium. After 7-day incubation period at room temperature the growing colonies were counted and inoculated into test tubes with 2 % PDA medium solidified at a slant. The fungi were identified basing on mycological keys.

The following biocenotic indicators were used for mycological characteristics of the tested soils:

– constancy of occurrence coefficient for individual fungi species,
– indicator of fungi colonies and species density,
– proportional share of individual fungi species in the whole community.

Constancy of occurrence coefficient (C) for individual species was computed using the formula [10]:

$$C = 100\ (n/N)$$

where: n – total of samples in which individual species occurred; N – total of all samples.

A percentage scale of the coefficient values was used to assess:
– C4 – absolutely constant species – 76–100 % of samples,
– C3 – constant species – 51–75 % of samples,
– C2 – accompanying species – 26–50 % of samples,
– C1 – accidental species – 0–25 % of samples.

Indicator of fungi colonies density (Ag) was computed according to the formula [10]:

$$Ag = Ng/S$$

where: Ng – number of fungi colonies; S – soil mass [g].

Proportional share of individual species in the whole community was used to define the notions [10] of:
– dominant fungi (the most numerous) – > 5 %,
– influence fungi (moderately numerous) – 1–5 %,
– accessory fungi (not numerous) – < 1 %.

Results and discussion

Significantly highest number of bacteria was found in the tested samples (Fig. 1). Also a marked increase was observed in bacteria colonies with growing distance from the shelterbelts.

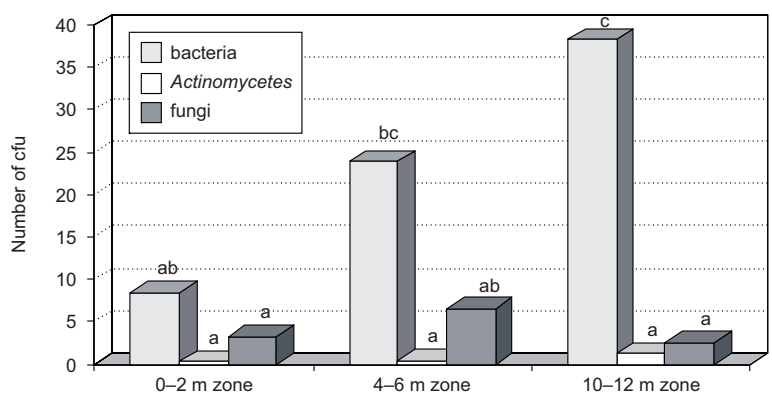

Fig. 1. The effect of distance from Black locust shelterbelts on microorganism number in soil
* Columns marked with the same letter do not differ significantly acc. to Duncan test (p = 0.05)

A total of 129 fungi colonies belonging to 9 species representing 8 genera were isolated from the examined soil samples (Table 2). The number of isolated fungi was diversified in the individual zones and ranged between 23 and 67 isolates, while the colony density indicator fluctuated from 68 to 197. The highest number of fungi colonies were registered in the zone between 4–6 m far from the black locust shelterbelts, whereas the smallest number was noted in soil of the 10–12 m zone. It may be associated with a decline in nutrient content in soil because in the zone most distanced from the trees the quantity of organic carbon and total nitrogen were diminishing. Black locust, which lives in a symbiosis with *Bacillus radicola* nodule bacteria and *Rhizobium* bacteria affects an increase in soil nitrogen content [2]. Also humus forming role of black locust has been observed, which primarily influences the arable field zones close to the shelterbelts. The effect of shelterbelts on humus content in soil is visible in the zones up to 10 m from the trees, which is a distance comparable to their height. Humus content decreases with growing distance from the shelterbelts [3].

Table 2

Species composition of fungi isolated from soils at various distances from black locust shelterbelts and biocenotic indicators

Fungus species	Distance zone from black locust shelterbelts [m]			Species share in the community		Constancy of occurrence coefficient	
	0–2	4–6	10–12	[%]	U	[%]	C
Alternaria alternata (Fr.) Keissl	1		3	3.9	I	11.1	C1
Cladosporium herbarum Link ex Fr.		1		0.8	A	2.7	C1
Fusarium oxysporum (Schlecht.) Snyd. et H.	1	16		3.9	I	8.3	C1
Fusarium solani (Mart.) Sacc.	5	19	7	30.4	D	55.5	C3
Mucor hiemalis Wehmer	8	5	1	13.7	D	38.8	C2
Penicillium spp.	14	17	8	18.3	D	55.5	C3
Rhizoctonia solani Kühn.	2	4	1	5.4	D	8.3	C1
Sclerotinia sclerotiorum (Lib.) de Bary		1		0.8	A	2.7	C1
Trichoderma viride Pers. ex Gray	9	3	1	12.7	D	22.2	C1
Total isolates	39	67	23				
species	7	9	7				
Ag [pcs/g soil]	115	197	68				

On the other hand in the soil of the zone closest to the shelterbelts allelopathic substances secreted by black locust roots were present, such as: robinetin, myricetin and quercetin, which might have inhibitory effect upon some soil microorganism groups [11].

Between 3 and 9 fungi species were isolated from individual zones. The fungi species dominating in the soil from black locust shelterbelts were: *Fusarium solani*, *Mucor hiemalis*, *Penicillium* spp., *Rizoctonia solani* and *Trichoderma viride* (Table 2). *Mucor* fungi are saprophytes active in organic matter decomposition. The greatest

amount of leaves, branches and pods find their way to the area of soil up to 10 m from the shelterbelts. Fallen black locust leaves revealing low C:N ratio are rapidly decomposed and provide an additional source of nitrogen in soil [3].

Fungi of *Fusarium*, *Penicillium* and *Rhizoctonia* genus may cause plant diseases. However, the presence of *T. viride* as a dominant fungus favourably affects plant healthiness [12–14]. *Trichoderma* fungi influence is conditioned by the soil microbial environment resistance to pathogens. The greatest number of potentially pathogenic fungi colonies of *Fusarium*, *Penicillium* or *Rhizoctonia* genus were observed in the 4–6 m zone from the shelterbelts, whereas the highest number of *T. viride* isolates were found in the 0–2 m zone. The number of *T. viride* colonies declined with growing distance from black locust plants. It may be connected with a decrease in C:N ratio in soil. Low C:N ratio in soils causes disappearance of the antagonistic effect between fungi responsible for many diseases and their antagonists revealing considerable fungistaic activity at high C:N ratio [15–17].

The obtained results point to black locust shelterbelt effect on the neighbouring soil environment.

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WPŁYW ODLEGŁOŚCI OD ZADRZEWIEŃ ROBINII AKACJOWEJ (*Robinia pseudoacacia* L.) NA MIKROFLORĘ GLEBY

[1] Katedra Ochrony Środowiska Rolniczego, [2] Katedra Gleboznawstwa i Ochrony Gleb
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem pracy było określenie składu ilościowego i gatunkowego mikroorganizmów glebowych w zależności od odległości od 30-letnich zadrzewień robinii akacjowej w miejscowości Królewice znajdującej

się na terenie Płaskowyżu Proszowickiego. Próbki gleby, z której izolowano bakterie, promieniowce i grzyby, pobierano ze stref oddalonych od zadrzewień: I – 0–2 m; II – 4–6 m; III – 10–12 m.

Stwierdzono, że w badanych próbkach glebowych istonie najwięcej było bakterii. Obserwowano również znaczny wzrost liczby kolonii bakterii wraz ze wzrostem odległości od zadrzewień. Z poszczególnych stref izolowano od 3–9 gatunków grzybów. Dominującymi gatunkami grzybów w glebach z zadrzewień robinii były *Fusarium solani*, *Trichoderma viride* oraz *Mucor* spp. i *Penicillium* spp.

Słowa kluczowe: *Robinia pseudoacacia,* gleba, mikrorganizmy

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Wojciech DMUCHOWSKI[1, 2] and Aneta Helena BACZEWSKA[1]

HEAVY METAL CONTENT IN CITY TREE LEAVES USED FOR COMPOST PRODUCTION

ZAWARTOŚĆ METALI CIĘŻKICH W LIŚCIACH DRZEW MIEJSKICH WYKORZYSTANYCH DO PRODUKCJI KOMPOSTU

Abstract: The object of the study was to determine the levels of heavy metal accumulation (lead, cadmium, zinc, copper, iron, and chromium) in the leaves of eight species of trees. Conducted analyses demonstrated that in Warsaw the emissions caused by motor traffic were decisive in the levels of heavy metal accumulation. They also demonstrated a significant dependence of their content in the leaves on the distance from the road. In an industrial area (steel works in the Bielany district of Warsaw) the highest accumulation of heavy metals was observed, with the exception of copper. In turn, the greatest contamination with copper was found in the street trees growing in the city center. Heavy metal content in the leaves of trees was much lower than the levels considered allowable in compost as specified in the EU Directive and by the Polish law.

Keywords: heavy metals, compost, environmental pollution, trees

Civilization development leads to generating ever larger amounts of various kinds of waste. Legal limitations to apply storage as a method of neutralizing wastes that succumb to biodegradation caused increased interest in their biological processing. Wastes removed from the urban green spaces (branches, shrubs, leaves, grass) constitute over 18 % of all communal wastes and are first of all a huge resource of organic matter. The best method for their utilization is composting, and then using the finished product as a fertilizer [1, 2]. According to the Polish Main Statistical Office (GUS) urban and residential green spaces constitute about 65 000 ha in Poland. Assuming that the annual production of plant mass in the green spaces is 5 tons per ha, we get about 325 000 tons of dry raw material for compost production [2]. Plant wastes originating from upkeep of urban green spaces have been composted for a long time to satisfy internal needs of many large parks, for example Lazienki Park in Warsaw, and in some other cities, for example Lodz. However, because of the possibility of contamina-

[1] Botanical Garden – Centre for Biological Diversity Conservation, Polish Academy of Science, ul. Prawdziwka 2, 02–973 Warszawa, Poland, phone/fax: +48 22 754 14 26, email: dmuchowski@ob.neostrada.pl

[2] Faculty of Agriculture and Biology, Warsaw University of Life Sciences, ul. Nowoursynowska 159, 02–776 Warszawa, Poland, phone: +48 22 593 26 89, fax: +48 22 593 26 82, email: wojciech_dmuchowski@sggw.pl

ting the compost with heavy metals, one needs to study particularly precisely their chemical composition [1]. The objective of the research conducted was to determine the heavy metal content in city tree leaves used for compost production.

Sites and study methods

The study involved eight tree species, growing in sites in Warsaw that were diverse with respect to the degree of pollution. The research areas were localized at an industrial site, near the main communication arteries, residential streets, and in downtown parks. Leaves of the Crimean Linden were collected in the median area between the traffic lanes of Zwirki i Wigury Avenue, and at distances of 10, 50 and 100 m from the road. The control area was set up in The Botanical Garden in Powsin, and in the case of the Crimean Linden, the Bielany Woods, an area with relatively little contamination [3].

From each site leaves of six trees were collected in mid September, from which a collective sample was formed. The spectrophotometric atomic absorption method applied with a spectrophotometer found the heavy metals (cadmium, lead, zinc, chromium, copper, and iron) in the plant material mineralized dry in a muffle oven [4, 5].

Review, results, and discussion

Heavy metal content in the leaves of studied tree species, growing in the Warsaw area varying with respect to the level of contamination, and in the control area, is represented in Table 1. In Figure 1 was illustrated the effect of the distance from the road on heavy metal content in the Crimean Linden leaves.

Research demonstrated that in Warsaw the emissions caused by motor traffic were decisive about the levels of heavy metal accumulation in the leaves of trees. Demonstrated was the fundamental effect of the distance from the road on their content in the leaves. Among the metals studied, the greatest decline was confirmed in the lead accumulation with the increase in the distance from the road, from 7.2 mg/kg in the median area between the traffic lanes to 3.1 mg/kg at a distance of 100m from the road. This clearly points to the road traffic as the main source of lead emissions. In the case of zinc, iron, copper and chromium the decline was smaller, but still distinct. The impact of motor traffic on the contamination of the environment with cadmium was decisively the smallest among all the metals studied, respectively from 0.334 mg/kg in the median area between the traffic lanes to 0.288 mg/kg at a distance of 100 m from the road.

The highest concentrations of heavy metals were found in the leaves of trees growing in the industrial area of Warsaw (steel works in the Bielany district), and second highest were those from the trees growing along the main arteries in the city center. The contamination in the parks and residential streets were significantly smaller. This tendency concerned all species of studied trees. The biggest relative differences between the control area (The Botanical Garden in Powsin), and the polluted area (area of Warsaw) manifested itself in the case of chromium and, to a lesser degree, lead and zinc. These differences in the contents of iron, copper and cadmium were significantly

Table 1

Heavy metal content [mg/kg] in the leaves of trees from the Warsaw area

Localization	Pb	Cd	Zn	Cu	Fe	Cr
Betula pendula Roth						
Steel works ArcelorMittal	10.9	0.568	845	8.3	397	2.34
Sikorskiego St.	5.9	0.231	467	9.8	245	1.35
Wielkopolski Park	3.0	0.167	408	7.6	231	1.23
Control	1.9	0.095	313	7.0	147	0.76
Quercus robur L.						
Marszalkowska St.	7.8	0.295	45	10.6	286	1.40
Wielkopolski Park	4.6	0.283	36	8.8	224	1.09
Control	2.3	0.112	28	7.1	154	0.81
Gleditsia triachantos L.						
Niepodleglosci Av.	4.7	0.197	35	13.3	274	1.22
Krasickiego St.	3.5	0.190	28	6.1	193	1.05
SGGW Park	3.3	0.192	29	8.8	128	1.13
Control	1.7	0.089	17	5.6	89	0.66
Aesculus hippocastanum L.						
Marszalkowska St.	8.1	0.307	44	15.6	303	1.57
Saski Garden	3.1	0.297	35	11.6	275	1.50
Control	2.0	0.123	21	6.0	205	0.81
Acer platanoides L.						
Swietokrzyska St.	7.7	0.283	42	9.0	279	1.46
Saski Garden	3.9	0.277	35	9.3	265	1.40
Control	2.1	0.143	26	5.5	137	0.65
Robinia pseudoacacia 'Umbraculifera'						
Belwederska St.	4.8	0.207	36	8.3	257	1.12
Komorska St.	3.2	0.186	25	6.7	176	1.01
Control	1.8	0.103	18	4.7	132	0.53
Tilia cordata L.						
Marszalkowska St.	7.1	0.289	49	14.6	311	1.49
Saski Garden	3.3	0.274	28	10.2	282	1.33
Control	2.0	0.157	22	6.0	119	0.80
Tilia 'Euchlora'						
Zwirki and Wigury Av.						
Median area between the roads	7.2	0.334	55	9.2	268	1.33
10 m from the road	7.0	0.297	49	9.0	260	1.30
50 m from the road	5.0	0.295	33	8.5	221	1.22
100 m from the road	3.1	0.288	26	7.8	187	1.19
Bielany Woods (control)	2.4	0.232	23	5.4	137	0.66

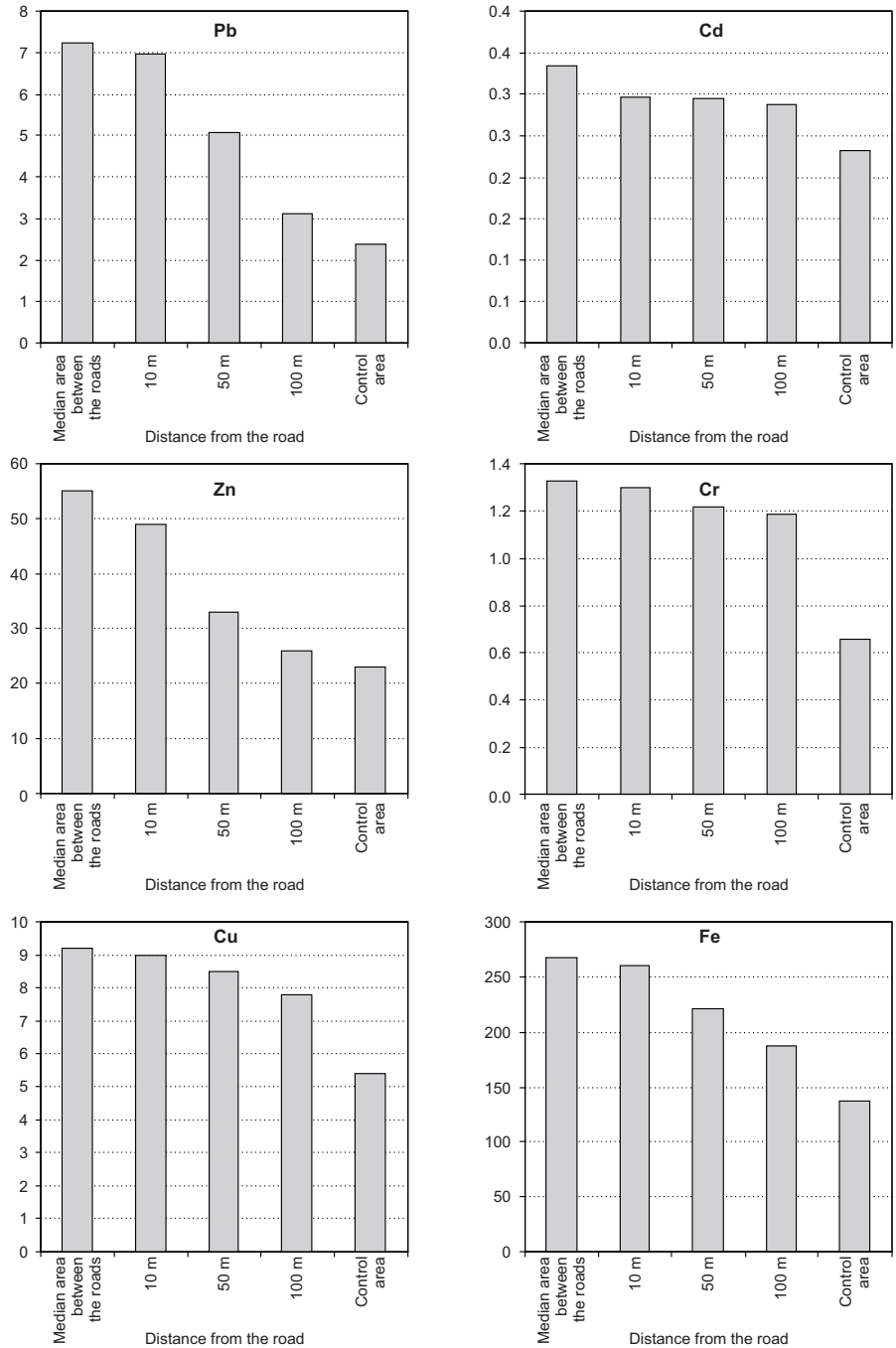

Fig. 1. Heavy metal content [mg/kg] in the Crimean Linden leaves as a function of the distance from the road of Zwirki and Wigury Avenue

smaller. In the industrial area (steel works in the Bielany district of Warsaw) the highest accumulation of heavy metals was recorded (with the exception of copper). In turn, the biggest contamination with copper was concluded for the street trees growing in the city center.

The studied tree species accumulated heavy metals to different degrees. The biggest difference was found in the case of zinc. The leaves of Silver Birch contained the most of this element, whereas with other species the content was smaller by over an order of magnitude. Published data indicate that the leaves of Silver Birch contain naturally higher levels of zinc than those found in other species. This is a species specific characteristic [6, 7].

From the research conducted it follows that the large leaf trees (Chestnut, Maple, Oak and Linden) had accumulated more lead, cadmium, zinc and chromium than the small leaves of trees from the family *Leguminosae* (Legume Family): Honey Locust and Acacia Robinia.

Heavy metal content in the leaves of trees was much lower than those described as the allowable levels specified in the EU Directive and the Polish regulations [8, 9]. Lowering of the plant mass even by 50 % through the process of mineralization will not cause a threat of going over the allowable contamination limits for compost (Table 2).

Table 2

Allowable metal content in compost in the Polish regulations and the EU Directive [8]

		Cd	Cr	Cu	Pb	Zn
Limits of maximum heavy metal content [mg/kg] in the European Union regulations						
Ecological agriculture (2092/91/EEC)		0.7	70	70	45	200
Eco-mark for fertilizers (2001/688 EC)		1.0	100	100	100	300
European Commission Proposals	Class I	0.7	100	100	100	200
	Class II	1.5	150	150	150	400
Poland (Regulation MRiRW, 2004)		3	100	400	100	1500

Despite not going over any legal limits governing contamination with heavy metals of the raw materials used for the production of compost, their application raises doubts. Because the material introduced into the environment is, to some extent, contaminated. However, the existing regulations enforce the production of compost utilizing waste collected from urban green spaces. So their application requires insightful monitoring of the levels of environmental pollution.

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ZAWARTOŚĆ METALI CIĘŻKICH W LIŚCIACH DRZEW MIEJSKICH WYKORZYSTANYCH DO PRODUKCJI KOMPOSTU

[1] Polska Akademia Nauk, Ogród Botaniczny – Centrum Zachowania Różnorodności Biologicznej
[2] Wydział Rolnictwa i Biologii, Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Abstrakt: Przedmiotem badań było określenie poziomu akumulacji metali ciężkich (ołowiu, kadmu, cynku, miedzi, żelaza i chromu) w liściach ośmiu gatunków drzew. Przeprowadzone analizy wykazały, że w Warszawie emisje pochodzenia motoryzacyjnego decydowały o poziomie akumulacji metali ciężkich. Wykazano także istotny wpływ odległości od ulicy na ich zawartość w liściach. Na terenie uprzemysłowionym (huta ArcerolMittal na Bielanach) odnotowano najwyższą akumulację metali ciężkich z wyjątkiem miedzi. Największe skażenie miedzią stwierdzono natomiast w drzewach ulicznych rosnących w centrum miasta. Zawartość metali ciężkich w liściach drzew była o wiele niższa niż przewidują dopuszczalne poziomy w kompostach określone w Dyrektywie UE i polskim prawie.

Słowa kluczowe: metale ciężkie, kompost, zanieczyszczenie środowiska, drzewa

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Katarzyna GLEŃ[1]

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

[1] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 00, email: rrglen@cyf-kr.edu.pl

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°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 (mm) measurements, D – number of days since the experiment outset, b_1, b_x – increment of colony diameter (mm) since the last measurement; d_1, d_2 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	
$NIR_{0.05}$ for fertilizer kind		1.67		2.11		2.31	
$NIR_{0.05}$ 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, respectively 67.88 % and 68.83 % (Table 2). A strongly inhibiting effect on *Fusarium* fungi was also observed after the application of English foliar fertilizer Yeald to the medium, which similarly as Mikrovit Zn contained zinc [21]. Combined application of Mikrovit Zn with urea and magnesium sulphate in the Authors' own

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.

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). 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 56.73–64.03 % 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 %.

Table 3

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

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	

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*

Katedra Ochrony Środowiska Rolniczego, Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Praca dotyczy reakcji 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 koncentracji 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 dolistnej nawozów na zdrowotność roślin.

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

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Joanna JARMUŁ-PIETRASZCZYK[1], Marta KAMIONEK[1]
and Przemysław WILKOWSKI[2]

EFFECT OF LONG FERTILISATION ON SEASONAL VARIABILITY OF OCCURRENCE OF ENTOMOPATHOGENIC NEMATODES AND FUNGI

WPŁYW DŁUGOTERMINOWEGO NAWOŻENIA NA SEZONOWĄ ZMIENNOŚĆ WYSTĘPOWANIA NICIENI I GRZYBÓW ENTOMOPATOGENNYCH

Abstract: This paper presents community structure of entomopathogenic fungi and nematodes in experimental plots fertilised with mineral (NPK) and organic (manure) fertilisers since 1955. Soil samples for experiment were taken from plots of the Warsaw Agricultural University in Chylice, PL. In the laboratory entomopathogenic nematodes and fungi were isolated from these samples using a trap insect *Galleria mellonella.*

Dominating fungal species in studied sites was *Paecilomyces fumosoroseus*. It was isolated from all plots irrespective of the fertiliser applied. *Beauveria bassiana* and *Metarhizium anisopliae* were also common. *Metarhizium flavoviride* was found sporadically. In the analysed soil samples only the entomopathogenic nematode of the genus *Steinernema* sp. was found.

Keywords: fertilise, entomophatogenic fungi and nematode, soil

Contemporary agriculture is largely based, apart from agrotechnical measures, on the application of chemicals including mineral and organic fertilisers. Fertilisation is aimed at improving nutrient availability to plants and, consequently, at increasing plant growth and yielding. Chemical treatment of fields affects not only plants but also accompanying fauna.

Both mineral and organic fertilisation directly affects entomopathogenic nematodes and fungi but it may also have an indirect effect. Fertilisation largely improves soil structure thus affecting habitat conditions of these microorganisms.

[1] Department of Zoology, Chair of Biology of the Animal Enviroment, Warsaw University of Life Sciences SGGW (WULS-SGGW), ul. Ciszewskiego 8, 02–787 Warszawa, Poland, phone: +48 22 593 66 28, email: joanna_jarmul@sggw.pl

[2] Student of Department of Zoology, Chair of Biology of the Animal Enviroment, Warsaw University of Life Sciences SGGW (WULS-SGGW).

Mineral NPK fertilisation may act indirectly on entomopathogenic nematodes. Nitrogen contained in fertilisers increases growth and development of plants making them more attractive to insect pests which are potential hosts for nematodes [1, 2]. Sienkiewicz [3] reported that appropriate mineral fertilisation in combination with manure application enriched soils in nutrient easily available to plants. Moreover, Kozlowska and Domurat [4] demonstrated significant impact of NPK treatment on phytofagous nematodes [5] An increase in plant parasites was found in the soil after its fertilisation with NPK. Lenart [6] showed significant impact of long mineral and organic fertilisation on soil microstructure and provided an evidence for the most favourable role of manure. It might seem that these studies are not relevant to soil microorganisms. However, the effect of fertilisation on soil microstructure means its effect on aggregate soil composition. This in turn is one of the main factors decisive for soil porosity, heat, air, water and nutrient resources and cycling [7–9]. According to Hassink et al [10] aggregate soil structure forms favourable conditions for plant root system and for soil microorganisms, including entomopathogens.

Material and methods

Soil samples collected from 24 experimental plots of the Agronomy Department in Chylice fertilised with organic and/or mineral fertilisers were used for experiment. The study was carried out in the vegetation season of 2007. Samples were taken in May, July and November with the Egner's cane.

Selected plots differed in the type of fertilisation. The plots have been uniformly fertilised since 1955 and in 1979 fertilisation doses were reduced by half.

Four combinations were used to fertilise the plots:

– NPK: 1.25 kg of ammonium saltpetre per 62.5 m^2 (plot + 1 m path), 0.9 kg of triple superphosphate, 2.5 kg of 60 % potassium salt,

– 1/2 NPK + 1/2 manure: 0.62 kg of ammonium saltpetre (34 % N), 0.45 kg of triple superphosphate (46 % P_2O_5), 1.25 kg of potassium salt (60 % K_2O), 125 kg of manure per plot,

– Manure: 250 kg per experimental plot,

– Control: plots not fertilised since 1955.

Caterpillars of the greater wax moth (*Galleria mellonella*) from own culture were used in the experiment to isolate entomopathogenic nematodes and fungi. Three tests were made in every plot. Ten caterpillars of the greater wax moth and the soil from experimental plots were placed in boxes of a volume of 250 cm^3. Boxes were placed in an incubator at a temperature of 20 ^oC. First tests were made after five days. Next examinations were made every second day. Dead insects were transferred onto filtration paper. The experiment was prolonged until the death of the last insect. The reason for insects' death was estimated later.

Table 1

Entomopathogenic fungi and nematodes isolated from soil in relation to fertilisation scheme [%]

Factor of mortality	May				July				November			
	NPK	O	1/2 NPK and 1/2 O	C	NPK	O	1/2 NPK and 1/2 O	C	NPK	O	1/2 NPK and 1/2 O	C
B. bassiana	34	34	28	6	50	0	50	0	0	34	0	67
M. anisopliae	0	63	12.5	25	0	0	0	0	0	0	0	100
M. flavoviride	0	0	34	67	0	100	0	0	100	0	0	0
P. fumosoroseus	27	21	12	38	27	29	20	24	43	8	8	41
Steinernema sp.	20	20	40	20	100	0	0	0	100	0	0	0

NPK – NPK fertilization; O – organic fertilization; C – unfertilized control; values marked with the same letter do not differ significantly (at $p = 0.05$).

Results and discussion

Four species of entomopathogenic fungi: *Paecilomyces fumosoroseus*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Metarhizium flavoviride* were found in studied habitats, the first being the dominating species (Table 1). It was isolated from all plots irrespective of applied fertilisation though it dominated in minerally fertilised plots and in control plots without fertilisation. Representatives of the species were most frequent in samples taken in July and less numerous in May and November. Other entomopathogenic fungi were more frequent in May than in other months.

Nematodes of the genus *Steinernema* dominated in soil samples collected in May but not in samples taken in other months of the study period (Table 1). Moreover, mites pathogenic to insects were isolated from soil samples.

Wasilewska [5] noted that mineral (NPK) fertilisation favourably affected phytopathogenic nematodes by increasing their density. Bednarek [11], however, demonstrated in 1990 that other groups of nematodes of the families *Steinernematidae* and *Heterorhabditidae* were negatively affected by NPK fertilisation which resulted in a decrease in the number of invasive larvae in soil. This author showed that different response of nematodes depended on the time of contact with the fertiliser. The ability of nematodes to infect insects decreased at the longer contact with mineral fertilisers. Short contact, however, stimulated nematodes by increasing their intensity of infection.

The influence of mineral NPK fertilisation on the growth of entomopathogenic fungi was also studied. Particular components of mineral fertilisers were found to affect entomopathogenic fungi in a different way. Bajan and Kmitowa [12] in a long term study found that nitrogen stimulated growth of *B. bassiana* and *P. fumosoroseus*, phosphorus stimulated *B. bassiana*, *P. farinosus* and *P. fumosoroseus* while most intensive growth stimulation by potassium was found in *P. farinosus*.

The next problem is species composition of crop plants which might largely affect soil microorganisms. Mietkiewski and Ignatowicz [13] showed that the soil under beets was dominated by *M. anisopliae*. Fertilisation and other agrotechnical measures affected soil flora and fauna. Rational fertilisation at optimum doses may positively affect microorganisms and stimulate their growth. Too large doses of fertilisers, particularly mineral ones, may bring the reverse effect.

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WPŁYW DŁUGOTERMINOWEGO NAWOŻENIA NA SEZONOWĄ ZMIENNOŚĆ WYSTĘPOWANIA NICIENI I GRZYBÓW ENTOMOPATOGENNYCH

[1] Katedra Biologii Środowiska Zwierząt, Zakład Zoologii
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie
[2] Student Katedry Środowiska Biologii Środowiska Zwierząt, Zakład Zoologii
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Abstrakt: Niniejsza praca ukazuje strukturę gatunkową grzybów i nicieni entomopatogennych na poletkach doświadczalnych nawożonych nawozem mineralnymi (NPK) i organicznym (obornik) od 1955 r. Do doświadczenia pobrano próby glebowe z poletek doświadczalnych SGGW w Chylicach. Z próbek tych w warunkach laboratoryjnych wyizolowano nicienie i grzyby entomopatogenne przy wykorzystaniu owada pułapkowego *Galleria mellonella.*

W badanych siedliskach dominującym gatunkiem grzyba był *Paecilomyces fumosoroseus*. Wyizolowano go ze wszystkich poletek niezależnie od typu nawozów. Występowały także *Beauveria bassiana* i *Metarhizium anisopliae*. Sporadycznie spotykano *Metarhizium flavoviride*.

W badanych próbkach glebowych stwierdzono występowanie tylko nicienia entomopatogenego z rodzaju *Steinernema* sp.

Słowa kluczowe: nicienie i grzyby entomopatogenne, gleba, nawożenie

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Marta KAMIONEK[1], Joanna JARMUŁ-PIETRASZCZYK[1],
Anna M. BADOWSKA-KOZAKIEWICZ[2]
and Elżbieta PEZOWICZ[1]

INFLUENCE OF LEAD AND CADMIUM IONS ON THE ENTOMOPATHOGENIC NEMATODES *Steinernema feltiae* Filipjev

WPŁYW JONÓW OŁOWIU I KADMU NA NICIENIE ENTOMOPATOGENNE *Steinernema feltiae* Filipjev

Abstract: The aim of this study was to determine the influence of lead and cadmium compounds on the entomopathogenic nematodes *Steinernema feltiae* Filipjev. The conducted research allowed to determine the sexual strucure of the nematodes developing inside the body cavity of the host as well as the survival rate of IJs before leaving the insect and after the migration to the external environment. Chemical and biological material as used in the conducted experiments. Chemical material included lead nitrate at the concentration of 40,100,500 ppm and cadmium nitrate at the concentration of 1, 2, 3 ppm. Biological material included the entomopathogenic nematodes *Steinernema feltiae* and test insects – *Galleria mellonella* L. – larvae from own culture.

Keywords: *Steinernema feltiae*, *Galleria mellonella*, lead, cadmium

Ability of bioaccumulation in invertebrates is influenced not only by position in the trophic chain but first of all by their physiology and ability of detoxification and removal of metals from the organism [1]. Invertebrates, which appear in soil environment, constitute approximately 99 % of animal species. There are small animals and they are not able to quickly leave the place of their appearance. These animals constitute a significant source of information about various types of pollutions. Evaluating content of heavy metals in direct way, we gain information about physical amount of a given element. Many studies focused on selection of plant or animal

[1] Faculty of Animal Sciences, Chair of Animal Environment Biology, Department of Zoology, Warsaw University of Life Sciences, ul. Ciszewskiego 8, 02–787 Warszawa, Poland, phone: +48 22 593 66 23, email: joanna_jarmul@sggw.pl

[2] Department of Biophysics and Human Physilogy, Medical University of Warsaw, ul. Chałubińskiego 5, 02–004 Warszawa, Poland, phone: +48 22 628 78 46, email: abadowska@op.pl, abadowska@wum.edu.pl

organisms, which are accumulating toxic substances in their tissues in order to determine toxicity of compounds in the soil [2].

Contamination of the environment with fluoride and metals is the one of the most important ecological problems in modern civilization due to possibility of their toxic action on plants, animals and humans.

Some heavy metals, such as Pb, Zn, and Cu are classified to the group of toxic elements, with very high degree of risk for the environment [3]. Not only invertebrates but also mammals are sensitive indicator of lead in the environment, because especially their bone tissue, reflect environmental pollution [4]. Many investigators have paid attention to ecological features of the nematodes, such as: species and trophic diversity, short time of the development, ability of fast colonization and easy isolation and identification of the respective species, which facilitate their use as bioindicators of the environmental pollution [5]. Species diversity is influenced by soil salinity, fertilization and agricultural intensification, as well as environmental pollution, including pollution with heavy metals. Variety of nematode groups comparing to natural habitats causes increase in number of bacteriophages [6]. In soil, where the limestone or the waste from the lead works was used, higher concentration of bacteriophagic and mycophagic nematodes was established. However, higher concentration of the predatory and omnivorous nematodes was not established. Lead directly and indirectly influences general number of nematode species, their diversity and biomass [7]. Among soil nematodes, we distinguish entomopathogenic nematodes, and there are some of them, which are feeding in the soil, but actively looking for the host and having contact with heavy metal ions. Some ions of heavy metal ions stimulate pathogenicity of the nematodes, but the other heavy metal ions limit it [1, 8]. Many heavy metals are included in coenzymes, active parts of vitamins, and respiratory dyes. They become toxic, if they enter biochemical reactions, which they normally do not take part in, and replace suitable substances [9]. Heavy metals penetrate into mitochondria and cause disturbances in gas exchange; they also influence ability of reproduction. Together with food, an animal organism may be penetrated by the xenobiotic elements (Cd, Pb), which do not take part in metabolism, they are very harmful, but they are consumed and assimilated by the organism, and become toxic when their concentrations exceed the allowable level [3, 10].

Material and methods

Experimental material constituted of entomopathogenic nematodes *Steinernema feltiae* (bio-preparation "Owinema") and test insects – larvae *Galleria mellonella* L. (own culture). Also chemical material was used for the experiments: lead nitrate $Pb(NO_3)_2$ in concentrations of 40, 100, 500 ppm, cadmium nitrate $Cd(NO_3)_2 \cdot 4H_2O$ in concentrations of 1, 2, 3 ppm and the control containing distilled water. Cycle of experiments took place in accordance with items a–c.

a) 10 insects from each container were placed in Petrie dishes and 50 IJs (*S. feltiae*) per one host were introduced. After two days following the death of *G. mellonella*, the dissection was performed in order to specify number of females and males of

the giant generation of the nematodes developing in the host fed with contaminated food,

b) 10 insects of *G. mellonella* from each container were contacted in the Petrie dishes with invasive larvae of *S. feltiae* (in initial dose of 50 IJs/host). After 8 days following death, insect dissection was conducted in order to determine living and dead larvae L3 placed in the body of the host before migration to the external environment,

c) 10 insects of *G. mellonella* from each box were contacted with 50 IJs (*S. feltiae*) insect. Dead insects were placed in the White's traps in order to obtain living larvae of nematodes migrating from the insect into the external environment. Every week for one month, living and dead nematodes were counted in order to determine their survival rate in the external environment. Each experiment comprised three repetitions.

Results and discussion

Together with the increase in the lead ions, the sex structure of the parasitic generation of *S. feltiae* developing in the body cavity of the insect was changing. Global number of the parasitic generation of developing nematodes was significantly lowered. Lead ions more intensively influenced the number of developing males of *S. feltiae* than number of females. However, high concentration of lead also adversely influenced number of females of *S. feltiae*. Together with increase in the lead ions, number of females as well as males of *S. feltiae* was decreasing. In case of the cadmium nitrate concentration at the level of 3 ppm, number of the "giant" males and females of the parasitic generation of *S. feltiae* in the insect, with reference to other concentrations, was the highest, even higher than the value in the control experiment. Based on data, it may be concluded that the cadmium nitrate revealed more significant influence on males than on females. The least development was established at concentration of 2 ppm (Fig. 1).

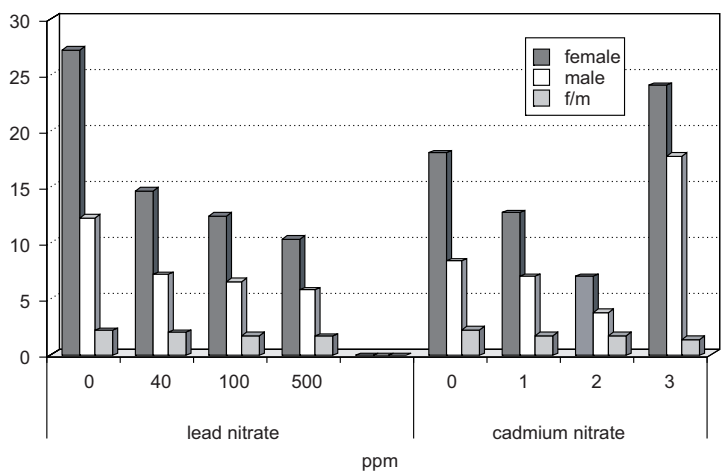

Fig. 1. Influence of lead nitrate and cadmium nitrate included in the food of the insects on the sex structure of the parasitic generation of *S. feltiae*

Influence of the lead nitrate and the cadmium nitrate included in the food of the insects on vitality of saprophagic, mono-phase generation of the (L3) *S. feltiae* larvae before migration from the host.

Increase in concentration of the lead influenced vitality of saprophagic population (L3) of the nematodes. Increase of the lead concentration contributed to reduction of number of living nematodes *S. feltiae* in the insect before migration. Similarly, the cadmium nitrate adversely influenced the nematode numbers. The highest proportion of living larvae of the nematodes to the dead ones was revealed at the cadmium nitrate concentration of 2 ppm (Fig. 2).

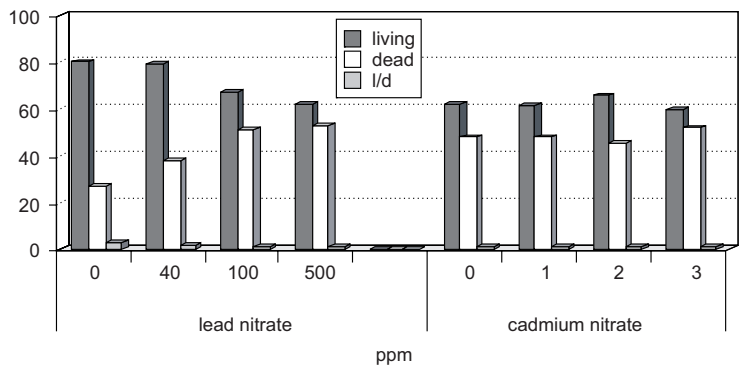

Fig. 2. Influence of the lead and cadmium ions included in the food of insects on vitality of saprophagic, mono-phase generation of the *S. feltiae* larvae before migration from the host

Conducted studies also include evaluation of influence of lead and cadmium ions on the survival rate of (L3) *S. feltiae* larvae in the external environment after migration from the host. Crucial factor influencing number of nematodes in this part of the experiments was not only concentration of the heavy metals, but also time of contact with heavy metal ions. Increase in the mortality of the nematodes inside the insect influenced their ability of migration to the external environment and their vitality. The longer time the larvae stayed in the external environment the higher was their mortality.

In all cases, there was the high correlation between concentration of the lead and cadmium ions, and the number of living and dead invasive larvae, sex structure of *S. feltiae* and the number of individual organisms of the saprophagic generation in the body cavity of the host.

Regression revealed that not only time but also concentration influenced number of males as well as females and there was the obvious influence on mortality rate of the nematodes. Statistical evaluation revealed more significant influence of the heavy metals on the whole development cycle of the nematodes, than on the host insect. Based on conducted evaluation, the conclusion may be drawn that the lead and cadmium ions cause changes in sex structure of the entomopathogenic nematodes *S. feltiae* and in mortality rate of invasive form of these nematodes, as well as in the speed of invasion of the entomopathogenic nematodes to the host. Heavy metal ions influence development of nematodes *S. feltiae* in insects *G. mellonella* and their vitality. Based on conducted

experiments, as well as data published in literature, it may be stated that entomopathogenic nematodes may be used as bioindicators of soil contamination and as biological preparations in battle with the plant pests.

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WPŁYW JONÓW OŁOWIU I KADMU NA NICIENIE ENTOMOPATOGENNE *Steinernema feltiae* Filipjev

[1] Zakład Zoologii, Katedra Biologii Środowiska Zwierząt
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie
[2] Zakład Biofizyki i Fizjologii Człowieka, Warszawski Uniwersytet Medyczny

Abstrakt: Celem pracy było określenie wpływu związków ołowiu i kadmu na nicienie entomopatogenne *Steinernema feltiae* Filipjev. Prowadzone badania umożliwiły określenie struktury płciowej nicieni rozwijających się w jamie ciała żywiciela, jak również przeżywalność IJs przed opuszczeniem owada oraz po migracji do środowiska zewnętrznego. W prowadzonych badaniach użyto materiału biologicznego i chemicznego. Materiał chemiczny stanowił azotan ołowiu w stężeniach 40 100 500 ppm oraz azotan kadmu w stężeniach 1, 2, 3 ppm. Materiał biologiczny stanowiły nicienie entomopatogenne *Steinernema feltiae* i owady testowe – larwy *Galleria mellonella* L. – hodowla własna.

Słowa kluczowe: *Steinernema feltiae*, *Galleria mellonella*, ołów, kadm

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Monika KOWALSKA-GÓRALSKA[1], Piotr ŁAWA[1]
and Magdalena SENZE[1]

IMPACT OF SILVER CONTAINED IN THE NANO SILVER PREPARATION ON THE SURVIVAL OF BRINE SHRIMP (*Artemia salina* LEACH 1819) LARVAE

WPŁYW SREBRA ZAWARTEGO W PREPARACIE NANO SILVER NA PRZEŻYWALNOŚĆ LARW ARTEMII (*Artemia salina* LEACH 1819)

Abstract: At the Department of Limnology and Fisheries at Wroclaw University of Environmental and Life Sciences there was conducted an experiment aimed at examining the impact of silver from the Nano Silver preparation on the survivability of Brine Shrimp (*Artemia salina* Leach 1819).

The experiment lasted for six hours, as Brine Shrimp lives in fresh water for about eight hours. The mean survivability rate in three repetitions for all of the concentrations of Nano Silver amounted to 93 %, with no linear drop in Brine Shrimp's survivability as silver concentrations increased. It was shown that silver nanoparticles were only slightly toxic to aquatic crustaceans. This low toxicity may prove useful to cut losses as regards zooplankton when using the preparation in order to limit large-scale development of plants in ponds.

Keywords: silver, brine shrimp, survivability

Disinfection allows proper hygiene levels to be maintained and prevents illnesses in the breeding of all kinds of animals. Disinfection is achieved by means of various chemicals. These are characterized by a wide spectrum of operation, significant activity, and small toxicity, which ensures that their application is entirely safe. The ideal disinfectant should have an appropriate killing capacity in respect of a broad range of microorganisms and at the same time it should not result in the development of resistance. It should be non-toxic or only slightly toxic so that it can be used in the presence of animals. After a period of effective operation it should undergo fast biodegradation and should not leave any toxic remains in the environment. It should dissolve well in water and produce stable and long-lasting solutions, easy to keep for at

[1] Department of Hydrobiology and Aquaculture, Wrocław University of Environmental and Life Sciences, ul. J. Chełmońskiego 38c, 51–630 Wrocław, Poland, phone: +48 71 320 58 70, fax: +48 71 320 58 76, email: monika.kowalska-goralska@up.wroc.pl

least 24 hours, until they are used. It should not damage the surfaces subjected to disinfection. Unfortunately, so far no ideal preparation has been manufactured, as toxicity seems to grow in direct proportion to the efficiency of the chemical [1]. In the search for the ideal agent attention was drawn to silver, whose beneficial biocidal properties have been known for centuries. Silver nanoparticles offer very good value in terms of biocidal capacity. Nanosilver can be used on a mass scale, as it is ecologically pure and safe to the environment. Furthermore, fragmented nanoparticles have an incommesurately bigger active surface and so have an enormous biocidal potential. Nanosilver kills bacteria both under normal lighting conditions and in the dark. What is more, such properties are permanent – a silver nanoparticle kills bacteria until it is removed [2]. However it is important that nanosilver could be also risky for immune system.

Because of controversial reports regarding silver [3–6], a decision was made to check the influence of the preparation called Nano Silver made by Nanoco on the survivability of Brine Shrimp (*Artemia salina* Leach 1819).

Material and method

The study was carried out on Brine Shrimp (*Artemia salina* Leach 1819).

Kingdom:	Animalia
Phylum:	Arthropoda
Subphylum:	Crustacea
Class:	Branchiopoda
Order:	Anostraca
Family:	Artemidae
Genus:	Artemia
Species:	*Artemia salina*

Silver for the study was sourced from the preparation called Nano Silver, made by Nanoco. The preparation contains 2000 mg Ag · dm^{-3} of silver in 1000 cm^3 of the product. The experiment was conducted at the laboratory of the Department of Limnology and Fisheries of Wroclaw University of Environmental and Life Sciences. The graphic presentation of the results of the study in the form of tables and graphs has been prepared using MS Excel 2002. The results were processed statistically by means of the Statistica 7 package.

Brine Shrimp (*Artemia salina* Leach 1819) is an aquatic crustacean and is the best known feed for fry [7]. Fully developed specimens (obtained after incubation in optimum conditions) were placed in ten Erlenmeyer flasks, ten specimens per flask. The flasks contained ISO standard water [8] and silver. Standard water was used because of its constant chemical composition, as there were reports about the influence of various compounds on the accumulation of silver in organisms [9]. Silver was obtained from Nano Silver made by Nanoco at concentrations of 5–100 mg Ag · dm^{-3} and from silver nitrate at concentrations of 5–100 mg Ag · dm^{-3}. The concentrations were many times higher than those normally present in water [10] or recommended by the WHO [11] and the EPA [12].

Brine Shrimp larvae were kept at 18 ^{o}C. Because under normal conditions Brine Shrimp lives in fresh water for about eight hours, the experiment was set to last six hours. After that time the number of surviving Brine Shrimp specimens were counted and then compared with that obtained in standard ecotoxicological tests, ie tests in which Brine Shrimp are subjected to an experimental factor in the salt water in which they developed [13]. In the study in question Brine Shrimp were tested in fresh water in order to obtain results applicable to pond management.The test was done three times, applying the same parameters.

Results and discussion

The impact of silver on the survivability of Brine Shrimp was tested in three repetitions.

A decision was made to conduct an additional study aimed at verifying whether the preparation Nano Silver has the same damaging effect on Brine Shrimp larvae (Fig. 1) as silver nitrate at similar concentrations (Fig. 2).

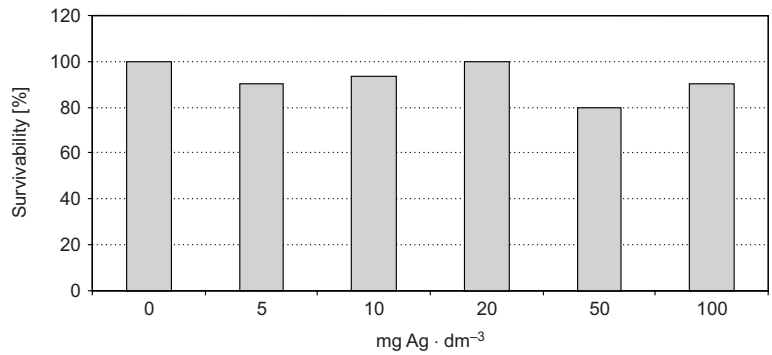

Fig. 1. Average survivability of Brine Shrimp at various concentrations of silver from the Nano Silver preparation (n = 3)

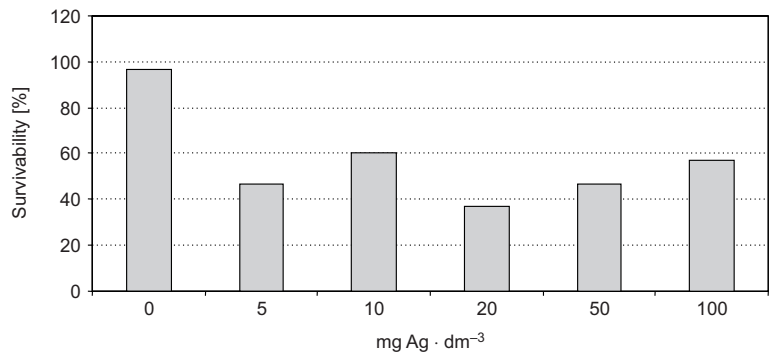

Fig. 2. Average survivability of Brine Shrimp at various concentrations of silver from silver nitrate (n = 3)

In the control sample there were no changes as to the number of surviving specimens. The average survivability, depending on the concentration, was as presented on the graph (Fig. 2). A comparison of the successive concentrations indicates that survivability is not directly proportional to the increase in silver concentration.

The correlation coefficient has been computed for silver nitrate and Brine Shrimp survivability at r = 0.30265. For Nano Silver r = –0.3432.

At the silver concentration of 36 μg · dm^{-3} the copepod *Acartia tonsa* dies in 96 h LC50 [12], whereas the Daphnia (*Daphnia magna*) shows LC50 96 h already at the concentration of 5 μg · dm^{-3} [14]. In the test with Nano Silver the value could not be calculated for Brine Shrimp, as its survivability was higher.

The study regarding Brine Shrimp was carried out using silver nanoparticles and, for comparison purposes, silver from silver nitrate. The study brief provided for checking the effect of Nano Silver only. The results obtained were so surprising that a decision was made to verify them with silver nitrate, which is frequently present in water.

When the preparation was used, Brine Shrimp's survivability was high, as it amounted to 93.33 % for all repetitions. At the highest concentrations Brine Shrimp's survivability increased. Only in one case – at 50 mg Ag · dm^{-3} – the value fell to 60 %, albeit in one repetition only. In the case of the other repetitions with the same concentration the survivability was higher, at 80 %. Only at these two highest concentrations (50 and 100 mg Ag · dm^{-3}) did some specimen die in every repetition. In other cases there was always one concentration at which all specimens survived, which meant that silver had no effect on Brine Shrimp's survivability. It should be pointed out that the silver concentrations used in the experiment may only occur in heavily polluted water reservoirs [15]. At the concentration of 20 m gAg · dm^{-3} the obtained result was similar to that found for the control sample, ie all specimens survived in all repetitions. This is interesting, as such a result was not obtained even at the lowest concentration. The highest mortality rate, observed at 50 mg Ag · dm^{-3}, amounted to 20 %, and the average for all repetitions – only to 7.5 %. The result may indicate low toxicity of silver nanoparticles in respect of this aquatic crustacean. The survivability did not fall linearly with increasing silver concentrations. It seems that silver nanoparticles can be safely used in waters which are home not only to Brine Shrimp but also to other aquatic crustaceans.

The test with silver nitrate was done after Brine Shrimp's very high survivability rate was obtained in the experiment with Nano Silver. The average survivability of Brine Shrimp when silver nitrate was used amounted to as little as 49.33 %, and was much lower than when nanoparticles were applied. This proves that silver may have a different impact on zooplankton, such as Brine Shrimp, depending on its source. At the lowest concentration used, 5 mg Ag · dm^{-3}, 50 % of the specimens died. This was five times more than when Nano Silver was used (10 %). At all of the remaining concentrations a similar result was obtained. In one repetition with 20 mg Ag · dm^{-3} of silver nitrate only 20 % of the specimens survived in one flask. The highest survivability, at 70 %, was determined for 100 mg Ag · dm^{-3}. All of the obtained results point to the advantage of the Nano Silver preparation. However, in one of the control samples one specimen

died. A certain natural mortality rate should always be expected, even in the case of a population not subjected to any toxic substance [16].

In their study concerning *Daphnia magna*, Gloger and Wood observed a very desirable phenomenon – silver was removed by Daphnia after a 24-hour period of its accumulation. Already after 60–70 hours silver concentration was down to the level before the accumulation [17].

The use of the Nano Silver preparation may be very advantageous in pond management.

Use of silver in the form of Nano Silver enables low doses of silver to be used and so limits bioaccumulation and biomagnification of the metal in aquatic environments.

The study indicates that Nano Silver is less toxic to Brine Shrimp than silver nitrate.

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WPŁYW SREBRA ZAWARTEGO W PREPARACIE NANO SILVER NA PRZEŻYWALNOŚĆ LARW ARTEMII (*Artemia salina* LEACH 1819)

Zakład Hydrobiologii i Akwakultury, Instytut Biologii
Uniwersytet Przyrodniczy we Wrocławiu

Abstrakt: W Zakładzie Limnologii i Rybactwa na Uniwersytecie Przyrodniczym we Wrocławiu przeprowadzono doświadczenie mające na celu wykazanie wpływu srebra pochodzącego z preparatu Nano Silver na przeżywalność artemii (*Artemia salina*). Czas trwania doświadczenia wyniósł sześć godzin, ponieważ artemia przeżywalność wodzie słodkiej żyje około ośmiu godzin. Średnia przeżywalność w trzech powtórzeniach dla

wszystkich stężeń dla preparatu Nano Silver wyniosła 93 %, przy czym nie wykazano liniowego spadku żywotności artemii wraz ze wzrastającym stężeniem srebra. Wykazano, że nanocząstki srebra są mało toksyczne dla skorupiaka wodnego. Niska toksyczność może się okazać pomocna przy zmniejszeniu strat w zooplanktonie podczas stosowania preparatu w celu ograniczenia masowego rozwoju roślin w stawach.

Słowa kluczowe: srebro, artemia, przeżywalność

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Katarzyna MALINOWSKA[1]

CONTENT OF ASSIMILATION PIGMENTS IN THE PHOTOSYNTHECTIC APPARATUS OF MAPLE (*Acer platanoides* L.) GROWING IN VARIOUS SITE CONDITIONS OF SZCZECIN

ZAWARTOŚĆ BARWNIKÓW ASYMILACYJNYCH W APARACIE FOTOSYNTETYCZNYM KLONU ZWYCZAJNEGO (*Acer platanoides* L.) ROSNĄCEGO W RÓŻNYCH WARUNKACH SIEDLISKOWYCH SZCZECINA

Abstract: The present study shows the results of the research work on the content of assimilation dyes in the leaves of *Acer platanoides* L., growing in different site conditions in Szczecin. The variance analysis showed a significant influence of the site and the date on the content of the studied physiological parameters. Lower amounts of chlorophyll and carotenoids were obtained in the leaves of trees growing along the street of high volume of traffic. On the basis of the correlation coefficient, a significant negative correlational relationship was recorded between the concentration of Cd, Pb, Fe and Cu in the leaves and the content of chlorophyll *a* and *b* in the leaves of *Acer platanoides.*

Keywords: assimilation pigments, *Acer platanoides* L.

Trees that constitute one of the most important elements of urban green sites are exposed to several stresses concurrently. The main stressful factor are just vegetation conditions in large agglomerations [1, 2]. The degree of plant pollution depends to a large extent on a current emission of pollutants rather than their concentration in soil [1, 3, 4]. The plant dye system is very sensitive to the effect of individual trace metals and gas impurities resulting, among other things, in disorders of the chlorophyll synthesis and their function of capturing photons [5–8].

The results presented in this paper constitute a fragment of multidirectional studies the aim of which is to determine the physiological activity of trees and to prove the

[1] Departament of Plant Physiology, Westpomeranian Technological University in Szczecin, ul. J. Słowackiego 17, 71–434 Szczecin, Poland, phone: +48 91 449 63 82, email: katarzyna.malinowska@zut.edu.pl

usefulness of the examined physiological parameters for the assessment of the degradation degree of urban habitats of Szczecin.

The aim of the study was to determine the amount of assimilation pigments in the leaves of maple growing in the agglomeration of Szczecin and the usefulness of the studied physiological parameters for the assessment of the degradation degree of the environment in Szczecin.

Material and methods

The studies were carried out in the area of Szczecin in two consecutive years (2003–2004) during the period of trees vegetation. Measurement sites were situated along the streets of heavy traffic, in the parks and the control point about 25 km from the city. While selecting research sites, the age of trees and the location of the control points of the Provincial Inspectorate of Environmental Protection (WIOS) were taken into consideration, in which the state of the air pollution in Szczecin is recorded. On each research site, four representative trees were selected. A few one year old shoots from each tree were designated for the studies. The material for the analysis was based on fully formed leaves of the first and the second pair of the year's gain of *Acer platanoides* L. The content of assimilation pigments (chlorophyll *a*, *b* and carotenoids) in the leaves were studied four times during vegetation, according to Lichtenthaler and Wellburm [9].

Table 1 shows the results concerning the level of air pollution in the area of Szczecin, selected from the measurement points of the Provincial Inspectorate of Environmental Protection. The results of average annual measurements of SO_2 in 2003–2004 were differentiated depending on the localization of the control point, but in no control point the allowable value of 20 $\mu g \cdot m^{-3}$ was exceeded. The content of NO_2 in the air was fairly high and both in 2003 and in 2004 the level of pollution with NO_2 exceeded the maximum average annual concentration [10].

Table 1

The air pollution with SO_2 and NO_2 [$\mu g \cdot m^{-3}$] in the area of Szczecin

Pollution measurements site	2003				2004			
	Pollution during the vegetation period		Average annual pollution		Pollution during the vegetation period		Average annual pollution	
	SO_2	NO_2	SO_2	NO_2	SO_2	NO_2	SO_2	NO_2
Św. Łukasza street	5.6	18.3	11.1	21.5	4.2	16.4	7.1	20.6
A. Struga street	5.9	29.9	10.4	32.8	2.4	27.1	6.8	31.0
Wyzwolenia street	b.d.	b.d.	b.d.	b.d.	1.8	16.6	5.3	21.4
Brama Portowa	4.3	43.8	11.6	40.8	2.7	30.8	8.3	36.6

b.d. – lack of data. The allowable value of SO_2 D_a – 20 $\mu g \cdot m^{-3}$ (maximum allowable average annual concentration); of NO_2 D_a – 30 $\mu g \cdot m^{-3}$ (maximum allowable average annual concentration).

Results and discussion

On the basis of the variance analysis it was found that a research site had a significant effect on the content of assimilation pigments in the leaves of maple. The studies showed that the leaves of trees growing along a street of heavy traffic were marked by the significantly smallest content of assimilation pigments. In the assimilation apparatus of trees on these sites an average content of total chlorophyll amounted to 66.2 % in 2003 and in 2004 69.8 % of average value of this dye in the leaves of control trees (Fig. 1).

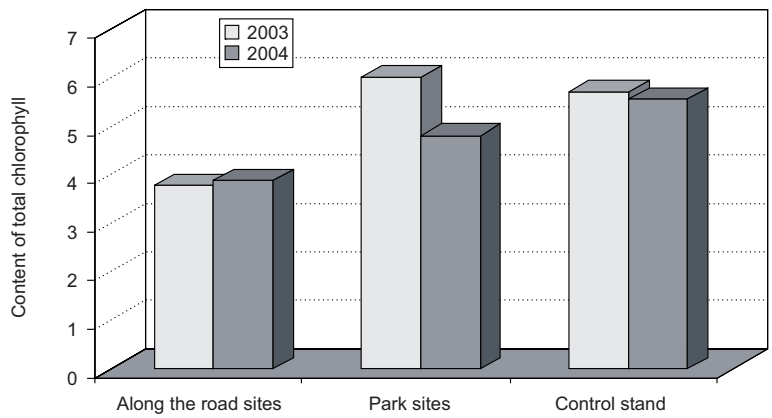

Fig. 1. The average content of total chlorophyll [mg · g^{-1} fresh mass] in the leaves of maple in the area of Szczecin

Significantly less chlorophyll *a* and *b* was observed in September than during a full period of vegetation (Tables 2, 3). The content of chlorophyll *a* in the leaves of trees growing on individual sites was diversified and in 2003 its smallest values were recorded in St. Lukasza street – average: 2.12 mg · g^{-1} of fresh mass, and in 2004 in A. Struga street – average: 2.59 mg · g^{-1} of fresh mass (Table 2). Average values of this dye in these points were lower by 1.92 mg · g^{-1} of fresh matter in 2003 and by 1.45 mg · g^{-1} of fresh mass in 2004 than in the leaves of control trees (Table 2). The average content of chlorophyll in the leaves of trees growing on sites of diverse traffic in 2003–2004 was similar and it amounted to about 1.04 mg · g^{-1} of fresh mass. The content of this dye in the leaves from these research sites was lower by 0.5 mg · g^{-1} of fresh matter than in the control trees (Table 3). Approximate values of the chlorophyll *b* content were observed in the leaves of control trees in both years (on average 1.52 mg · g^{-1} of fresh mass), (Table 3). It was also shown that the content of chlorophyll *b* in the leaves of the examined trees was on average twice as small as that of chlorophyll *a* [11–13]. Lower values of the studied physiological indicators in the leaves growing in the city centre result probably, among other things, from gas and dust impurities of the air. Measurements conducted by the Provincial Inspectorate of Environmental Protection showed that the concentration of SO_2 during the vegetation period was

Table 2

The content of chlorophyll *a* [mg · g^{-1} fresh mass] in the leaves of maple in relation to the site and the date of the studies

Factor I – Stand	Factor II – Date								
	2003				2004				
	VI	VII	IX	The avenage of factor I	VI	VII	VIII	IX	The avenage of factor I
A. Struga street	3.29	3.26	2.70	3.08	2.95	2.63	2.67	2.12	2.59
Wyzwolenia street	3.27	3.49	3.14	3.30	3.44	3.21	3.15	2.96	3.19
Szosa Stargardzka	3.46	2.25	2.06	2.59	3.12	2.65	2.70	2.43	2.72
Św. Łukasza street	2.24	2.14	1.99	2.12	3.36	2.95	2.86	2.12	2.82
E. Orzeszkowej street	3.45	3.34	2.23	3.01	3.03	3.15	3.11	2.56	2.96
S. Dubois street	2.42	2.42	1.86	2.23	3.17	3.28	2.98	2.49	2.98
Park J. Kasprowicza	3.81	4.06	3.23	3.70	3.91	3.51	3.48	3.15	3.51
Park S. Żeromskiego	4.85	5.57	4.30	4.90	3.72	3.46	3.52	3.24	3.48
Control stand	4.20	4.14	3.78	4.04	4.66	3.98	3.96	3.58	4.02
The average of factor II	3.44	3.41	2.81		3.48	3.20	3.16	2.73	
$LSD_{0.05}$ for	factor I – 0.77, factor II – 0.34, Interaction I×II – n.s., Interaction II×I – n.s.				factor I – 0.69, factor II – 0.28, Interaction I×II – n.s., Interaction II×I – n.s.				

n.s. – non-significant.

Table 3

The content of chlorophyll *b* [mg · g^{-1} fresh mass] in the leaves of maple in relation to the site and the date of the studies

Factor I – Stand	Factor II – Date								
	2003				2004				
	VI	VII	IX	The avenage of factor I	VI	VII	VIII	IX	The avenage of factor I
A. Struga street	1.20	1.04	0.75	0.99	0.98	1.08	0.96	0.87	0.97
Wyzwolenia street	1.33	1.00	0.91	1.08	1.24	1.15	1.21	0.89	1.12
Szosa Stargardzka	1.47	1.70	0.96	1.38	1.04	0.91	0.86	0.68	0.87
Św. Łukasza street	0.92	0.87	0.76	0.85	1.12	0.98	1.05	1.03	1.04
E. Orzeszkowej street	1.01	1.29	0.98	1.09	1.25	1.03	1.15	1.10	1.13
S. Dubois street	1.03	0.92	0.82	0.92	0.96	0.92	1.03	0.91	0.95
Park J. Kasprowicza	1.87	1.83	1.26	1.65	1.53	1.23	1.28	1.12	1.29
Park S. Żeromskiego	2.05	1.81	1.46	1.77	1.49	1.31	1.40	1.19	1.35
Control stand	1.70	1.91	0.94	1.52	1.76	1.48	1.56	1.28	1.51
The average of factor II	1.40	1.38	0.98		1.26	1.12	1.17	1.01	
$LSD_{0.05}$ for	factor I – 0.51, factor II – 0.22, Interaction I×II – n.s., Interaction II×I – n.s.				factor I – 0.43, factor II – 0.19, Interaction I×II – n.s., Interaction II×I – n.s.				

n.s. – non-significant.

Table 4

The content of carotenoids [mg · g $^{-1}$ fresh mass] in the leaves of maple in relation to the site and the date of the studies

Factor I – Stand	Factor II – Date								
	2003				2004				
	VI	VII	IX	The avenage of factor I	VI	VII	VIII	IX	The avenage of factor I
A. Struga street	1.29	1.29	0.84	1.14	0.79	0.65	0.65	0.71	0.70
Wyzwolenia street	1.31	1.35	0.97	1.21	0.71	0.69	0.67	0.69	0.69
Szosa Stargardzka	1.42	1.17	0.94	1.18	0.47	0.51	0.52	0.68	0.54
Św. Łukasza street	0.87	0.93	0.89	0.90	0.77	0.78	0.71	0.85	0.78
E. Orzeszkowej street	1.18	1.05	0.85	1.03	0.58	0.62	0.61	0.62	0.61
S. Dubois street	1.00	0.89	0.83	0.90	0.74	0.79	0.72	0.75	0.75
Park J. Kasprowicza	1.88	1.81	1.63	1.77	0.98	0.99	1.02	1.05	1.01
Park S. Żeromskiego	1.42	1.17	0.94	1.18	0.87	1.02	1.00	1.18	1.02
Control stand	1.58	1.65	0.73	1.32	1.12	1.15	1.11	1.21	1.15
The average of factor II	1.35	1.30	0.99		0.78	0.80	0.78	0.86	
$LSD_{0.05}$ for	factor I – 0.27, factor II – 0.12, Interaction I×II – 0.46 Interaction II×I –0.35				factor I – 0.29, factor II – 0.14, Interaction I×II – 0.32, Interaction II×I – 0.28				

highest on sites along St. Łukasza and A. Struga streets (Table 1). The dye system is very sensitive to the effect of SO_2 and that of individual heavy metals. Both SO_2 and heavy metals non-used in metabolism of plants (Cd and Pb) cause disorders in the biosynthesis of chlorophyll and in their function of capturing photons [14, 15]. In the point situated in the street of heavy traffic and near the traffic lights (A. Struga street) a low amount of chlorophyll *a* and *b* was observed in the leaves of maple in both years of the studies (Tables 2, 3). The measurements carried out by he Provincial Inspectorate of Environmental Protection showed that the level of the NO_2 concentration in this point was high and during the vegetation period it amounted to above 90 % of D_a (maximum allowable average annual concentration). Also the average content of carotenoids in the leaves of the street trees was lowest and in 2003 it amounted on average to 1.06 mg · g^{-1} fresh mass and in 2004 to 0.68 mg · g^{-1} of fresh mass and this constitutes, respectively 80 % and 59 % of its average content in the control leaves (Table 4). Lower amounts of carotenoids in the leaves of the trees growing near throughways were obtained by Lorenc-Plucinska [14]. Also lower contents of carotenoids in the leaves of the trees growing in the city centre were observed by Malinowska [11, 12]. On the basis of the correlation coefficient value, a significant correlational relationship was observed between the content of assimilation dyes and the concentration of heavy metals. The concentration of lead, cadmium, iron and copper had a significant negative effect on the content of chlorophyll *a* and *b*, whereas it had a non-significant negative effect on the content of carotenoids (Table 5).

Table 5

Coefficient of correlation between heavy metal concentration and assimilation pigments content in the leaves of maple

Chemical element	Assimilation pigments					
	2003			2004		
	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
Lead	–0.72*	–0.78*	–0.53	–0.87*	–0.79*	–0.66
Cadmium	–0.75*	–0.73*	–0.53	–0.86*	–0.81*	–0.79*
Iron	–0.57	–0.72*	–0.50	–0.80*	–0.76*	–0.57
Copper	–0.79*	–0.75*	–0.71*	–0.81*	–0.75*	–0.70*
Cobalt	–0.40	–0.57	–0.22	–0.69	–0.56	–0.43

* A significant effect of the factor. Significant differences at the level of $\alpha = 0.05$.

Conclusions

1. A significant negative correlational relationship was shown between the concentration of Pb, Cd, Fe and Cu in the leaves and the content of chlorophyll *a* and *b* in the leaves of *Acer platanoides*.

2. The significance of differences in the content of assimilation pigments in the leaves of the trees was shown in relation to the site and the date of the studies.

3. The studied physiological parameters can be useful for the assessment of the environment degradation in Szczecin.

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ZAWARTOŚĆ BARWNIKÓW ASYMILACYJNYCH W APARACIE FOTOSYNTETYCZNYM KLONU ZWYCZAJNEGO (*Acer platanoides* L.) ROSNĄCEGO W RÓŻNYCH WARUNKACH SIEDLISKOWYCH SZCZECINA

Zakład Fizjologii Roślin
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Abstrakt: W pracy przedstawiono wyniki badań zawartości barwników asymilacyjnych w liściach *Acer platanoides* L., rosnącego w różnych warunkach siedliskowych Szczecina. Analiza wariancji wykazała istotny wpływ stanowiska i terminu na zawartość badanych parametrów fizjologicznych. Obniżone zawartości chlorofilu i karotenoidów uzyskano w liściach drzew rosnących przy ulicy o dużym natężeniu ruchu samochodowego. Na podstawie wartości współczynnika korelacji stwierdzono ujemną istotną zależność korelacyjną pomiędzy koncentracją Cd, Pb, Fe i Cu w liściach a zawartością chlorofilu *a* i *b* w liściach *Acer platanoides.*

Słowa kluczowe: barwniki asymilacyjne, *Acer platanoides* L.

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Ryszard MAZUREK[1], Agnieszka JÓZEFOWSKA[1]
and Anna PIOTROWSKA[2]

INFLUENCE OF DISTANCE FROM BLACK LOCUST (*Robinia pseudoacacia*) SHELTERBELTS ON DEHYDROGENASE ACTIVITY IN ARABLE SOILS

WPŁYW ODLEGŁOŚCI OD ZADRZEWIEŃ ROBINII AKACJOWEJ (*Robinia pseudoacacia*) NA AKTYWNOŚĆ DEHYDROGENAZY W GLEBACH UPRAWNYCH

Abstract: Biological activity of soils can be measured on the base of their dehydrogenase activity, mainly influenced by soil organic carbon content. The aim of the paper was to evaluate the effect of distance from shelterbelts plantet with black locust trees on level of dehydrogenase activity in arable soils of Proszowice Plateau derived from loess. Soil samples were taken from area of 20 × 24 m with different soil type (brown soil proper, from layer of 0–25 cm of arable soil. Sixty mixed soil samples were collected from area in growing distance (up to 24 m) from shelterbelt. The highest dehydrogenase activity was measured in samples taken up to 2 m from black locust trees. Dehydrogenase activity amounted 12.48 $cm^3H \cdot kg^{-1} \cdot 20\ h^{-1}$ in these zone. Level of measured activity was the lowest in zone 12–14 m (2.91 $cm^3H \cdot kg^{-1} \cdot 20\ h^{-1}$). It were state statistical differences between dehydrogenase activity in zone 0–2 m and in the rest zones. Level of dehydrogenase activity was strongly influence by organic carbon content and C/N ratio.

Keywords: dehydrogenase activity, black locust, arable soils

The source of enzymes in soil are soil organisms, plant roots and their residue and specific soil flora [1]. Dehydrogenases, which can be regarded the most frequently determined soil enzymes, according to Brzezinska [2] and Wlodarczyk [3] may be also used for assessing total soil biological activity. As results from the investigations, there is a strict relationship between dehydrogenase activity and organic matter content, organisms number and the soil fertility [4].

The aim of the paper was an assessment of the influence of the distance from shelterbelts composed of black locust on dehydrogenase activity in arable soils developed from loess in the Proszowicki Plateau.

[1] Department of Soil Science and Soil Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 6624370, email: rrmazure@cyf-kr.edu.pl

[2] Department of Biochemistry, University of Technology and Life Sciences in Bydgoszcz, ul. Bernardyńska, 85–029 Bydgoszcz, Poland, phone: +48 52 374 95 55, email: ap03@wp.pl

Material and methods

The soil material was collected from an arable field situated in Krolewice village in the Proszowicki Plateau. From the north the arable field adjoins the shelterbelt composed mainly of black locust. The trees are 8–10 m high and about 30 years old. The soil cover of the analyzed field consists of typical brown soils developed from loess.

12 zones, 2 m wide were marked out at the increasing distance from the trees in order to collect soil samples. In each zone 5 plots (2 × 4 m) were designed. 5 soil samples were collected from the surface layer of each plot and a single collective sample was formed of them. There were a total of 60 collective samples taken from the whole tested area, representing the 12 zones.

The soil samples were dried and then sieved through a sieve with 2 mm mesh. In the material prepared in this way basic soil physico-chemical properties were determined by means of standard methods applied in soil science. Dehydrogenase enzymatic activity was determined with Casida et al [5] method, which involves the soil incubation with TTC (2,3,5-triphenyltetrazolium chloride) on DU 640 Beckman spectrophotometer.

The results were elaborated geostatistically using Surfer 8.0 programme. The data were subjected to statistical analysis using the Tukey's RIR test in Statistica 7.0 programme.

Results and discussion

The highest dehydrogenase activity was noted in samples collected within the 0–2 m zone, ie at the distance of 2 m from the shelterbelts (Figs. 1, 2). In this zone mean dehydrogenase activity was 12.48 cm^3 H · kg^{-1} · 24 h^{-1}. In the soil samples taken between 2 and 8 m from the trees, the activity of the analyzed enzyme assumed lower values, however not below 4.95 cm^3 H · kg^{-1} · 24h^{-1}. In the subsequent zones

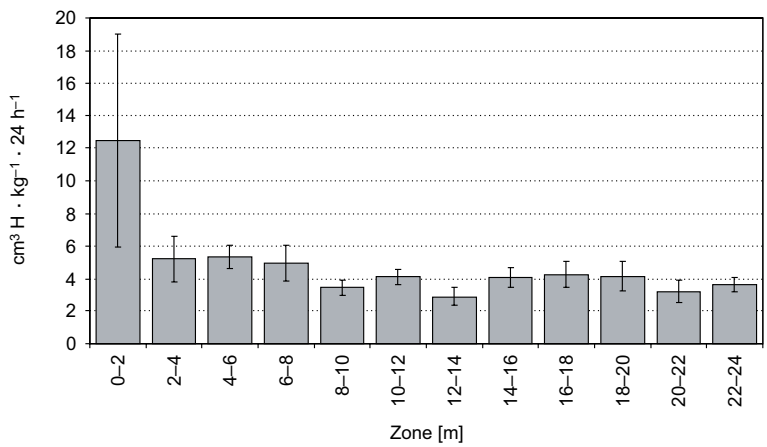

Fig. 1. Dehydrogenase activity in particular zones

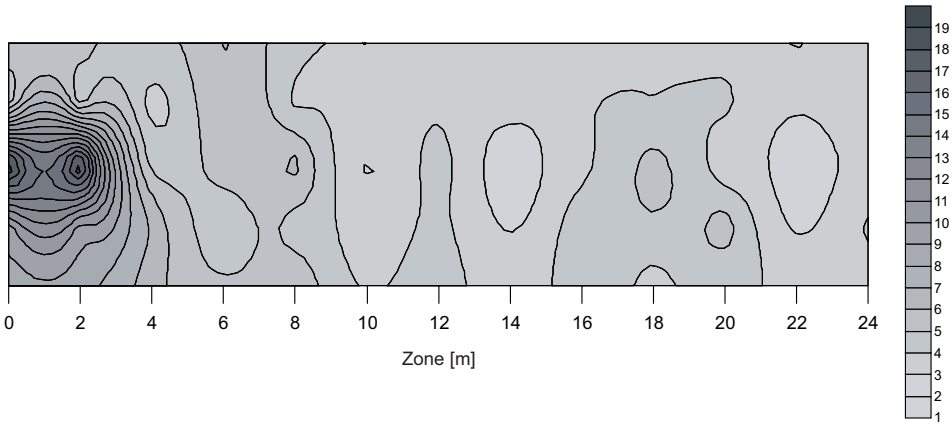

Fig. 2. Spatial distribution of dehydrogenase activity (in cm^3 H · kg^{-1} · 24 h^{-1}) in studied area

dehydrogenase activity was decreasing until 12–14 m where it reached the minimum value of 2.91 cm^3 H · kg^{-1} · 24 h^{-1}. Increased soil enzymatic activity under the influence shelterbelts composed of various species was also registered in former investigations [6, 7]. The research conducted in the Lublin neighbourhood on the effect of 8-year shelterbelts of black locust revealed a similar relationship between dehydrogenase activity and distance from the trees [8]. In the light of these investigations dehydrogenase activity was greater in the soils immediately under the trees as compared with the samples collected 2 m from the black locust, assuming the values respectively 6.67–9.28 and 4.39–5.21 cm^3 H · kg^{-1} · 24 h^{-1}. These slightly lower values of enzymatic activity in the soil close to the trees in comparison with the results presented in this paper are due to the younger tree age. The concentration of soluble carbon in soil and therefore increase in dehydrogenase activity are affected among others by the age and size of plants. It has been evidenced by the activity increasing with the age and size of seedlings in forest nurseries [9].

On the basis of Tukey test it was determined that the difference between the enzyme activity in the 0–2 m zone, as compared with dehydrogenase activity assessed in the other zones, was statistically significant at the level 0.05 (Table 1).

High value of dehydrogenase activity in the "contact" zone with arable fields and shelterbelts evidences a high total activity of soil microorganisms and at the same time is an indicator of the soil fertility and fecundity [10].

A positive influence of black locust shelterbelts on dehydrogenase activity is also connected with increasing content of main nutrients in the zones reached by the inflow of leaves, pods and branches of the black locust. Advantageous effect of black locust on dehydrogenase activity is also connected with the fact that this tree as a plant belonging to legume family enriches the soil in nitrogen. The research aimed at determining the influence of fertilization on dehydrogenase activity demonstrated that it was nitrogen which particularly beneficently affected microorganism activity through its positive influence on their biomass increment [11].

Table 1

Significance level (p) of differences in dehydrogenase activity between particular zones calculated using Tukey test (significant are differences if $p < 0.05$)

Zone [m]	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24
0–2		0.04	0.05	0.02	0.00	0.00	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2–4	0.04		1.00	1.00	0.94	1.00	0.73	1.00	1.00	1.00	0.86	0.97
4–6	0.05	1.00		1.00	0.91	1.00	0.65	1.00	1.00	1.00	0.81	0.95
6–8	0.02	1.00	1.00		0.98	1.00	0.85	1.00	1.00	1.00	0.94	0.99
8–10	<0.01	0.94	0.91	0.98		1.00	1.00	1.00	1.00	1.00	1.00	1.00
10–12	<0.01	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00
12–14	<0.01	0.73	0.65	0.85	1.00	1.00		1.00	0.99	1.00	1.00	1.00
14–16	<0.01	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00
16–18	<0.01	1.00	1.00	1.00	1.00	1.00	0.99	1.00		1.00	1.00	1.00
18–20	<0.01	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00
20–22	<0.01	0.86	0.81	0.94	1.00	1.00	1.00	1.00	1.00	1.00		1.00
22–24	<0.01	0.97	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	

The level of dehydrogenase activity in the analyzed soils was strictly connected with organic carbon content (0.57^{***}) and the degree of organic matter decomposition expressed by C/N ratio (0.51^{***}) and to a lesser extent also with soil sorption complex capacity (0.31^{*}) (Table 2, Fig. 3). Inflow of fresh organic matter stimulates micro-organism activity in the soils situated in the immediate vicinity of black locust. Januszek [12] noted a similar relationship between organic carbon content in the forest soils and their enzymatic activity.

Table 2

Linear correlation coefficients for the relationship between chosen soil properties and dehydrogenase activity

Soil properties	Linear correlation coefficients
pH H_2O	0.13
pH KCl	0.24
C organic [$g \cdot kg^{-1}$]	0.57^{***}
N total [$g \cdot kg^{-1}$]	0.19
C/N	0.51^{***}
TEB [$mmol(+) \cdot kg^{-1}$]	0.22
CEC [$mmol(+) \cdot kg^{-1}$]	0.31^{*}
Hh [$mmol(+) \cdot kg^{-1}$]	0.08
BS [%]	0.10

TEB – *base cations concentration*, CEC – *cation exchange capacity*, Hh – *hydrolytic acidity*, BS – *base saturation*.

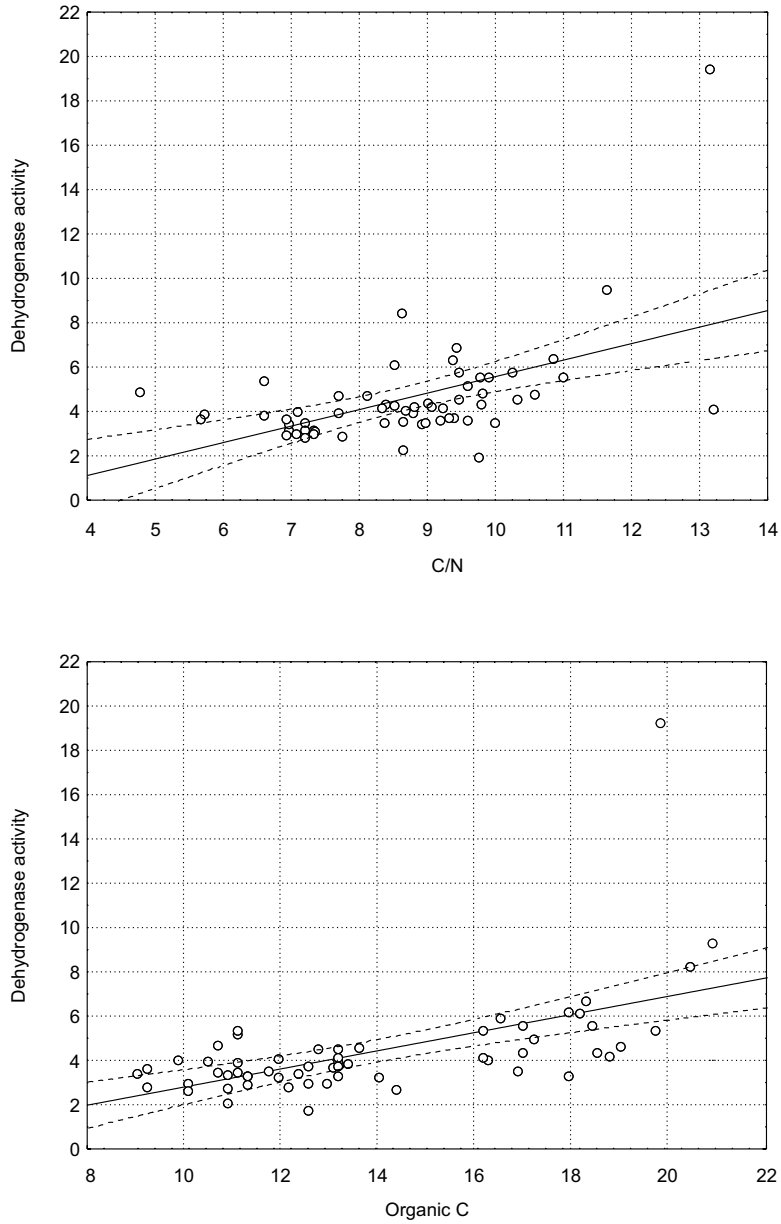

Fig. 3. Dependence between soil carbon content, C/N ratio and dehydrogenase activity

Biomass and microbial soil activity become reduced with increasing temperature and decreasing soil moisture [12]. Shelterbelts which protect the adjoining field against the extreme atmospheric conditions, create more advantageous conditions for soil microflora development and therefore enhance dehydrogenase activity [13].

Conclusions

1. The highest dehydrogenase activity was assessed in the soils collected below 2 m from black locust trees.

2. Dehydrogenase activity in the 0–2 m zone was statistically higher than in all other zones.

3. Organic carbon content, C/N ratio and soil sorption capacity most influenced dehydrogenase activity in the analysed soils.

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WPŁYW ODLEGŁOŚCI OD ZADRZEWIEŃ ROBINII AKACJOWEJ (*Robinia pseudoacacia*) NA AKTYWNOŚĆ DEHYDROGENAZY W GLEBACH UPRAWNYCH

[1] Katedra Gleboznawstwa i Ochrony Gleb
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
[2] Katedra Biochemii
Uniwersytet Technologiczno-Przyrodniczy im. Jana i Jędrzeja Śniadeckich w Bydgoszczy

Abstrakt: Celem pracy było określenie wpływu odległości od zadrzewień złożonych z robinii akacjowej na poziom aktywności dehydrogenazy w glebach uprawnych Płaskowyżu Proszowickiego wytworzonych z lessu. Próbki glebowe zostały pobrane z mikropoletka o wymiarach 20 × 24 m, z warstwy 0–25 cm z gleby brunatnej właściwej użytkowanej jako grunt orny. Ogółem pobrano 60 próbek zbiorczych w rosnącej odległości (do 24 m) od zadrzewień. Najwyższą aktywnością dehydrogenazy charakteryzowały się gleby pobrane w odległości do 2 m od zadrzewień. W strefie tej aktywność dehydrogenazy wynosiła 12,48 cm^3 H · kg^{-1} · 24 h^{-1}. W strefach położonych dalej od zadrzewień aktywność dehydrogenzy stopniowo malała, przyjmując wartości najniższe w strefie 12–14 m od zadrzewień – 2,91 cm^3 H · kg^{-1} · 24 h^{-1}. Stwierdzono statystycznie istotne różnice pomiędzy aktywnością dehydrogenazy w strefie 0–2 m a aktywnością oznaczoną w pozostałych strefach. Poziom aktywności dehydrogenazy był ściśle związany z zawartością węgla organicznego oraz stosunkiem C/N.

Słowa kluczowe: aktywność dehydrogenazy, robinia akacjowa, gleby uprawne

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Małgorzata PACHOLEWSKA[1] and Beata CWALINA[2]

RESISTANCE OF *Acidithiobacillus ferrooxidans* TO As(III) AND Sb(III) IONS

OPORNOŚĆ *Acidithiobacillus ferrooxidans* WOBEC JONÓW As(III) ORAZ Sb(III)

Abstract: The influence of the Sb(III) and As(III) ions on metabolic activity of acidophilic bacteria *Acidithiobacillus ferrooxidans* isolated from the zinc-lead post-flotation tailings have been studied. It may be stated that these bacteria feature high resistance to the As(III) ions and lack of the Sb(III) ions tolerance. Bacteria tolerate the As(III) at concentrations up to 50 mg/dm^3 but do not tolerate Sb(III) even at concentration of 10 mg/dm^3. Thus, the strain being tested cannot be used in processes of the feed electrolyte treatment and cleaning.

Keywords: *Acidithiobacillus ferrooxidans,* Fe(II) oxidation, resistance to As(III) and Sb(III)

The influence of As(III) and Sb(III) ions on metabolic activity of iron-oxidizing bacteria *Acidithiobacillus ferrooxidans* cultured in liquid media stimulating their growth, have been investigated. The bacteria tested were isolated from surface layer of the zinc-lead post-flotation tailings originating from the mining-metallurgy plant ZGH Boleslaw S.A. [1]. Against the popular belief that arsenium and antimonium compounds are toxic to living organisms, it is suggested that these elements-rich natural environments are favourable to many organisms adaptation leading to their resistance to toxic substances [2, 3]. Such effect has been observed in the gold mines where the arsenium-containing minerals are usually present. As contrasted with the arsenium, the antimonium effect on living organisms is not fully recognised as yet [4, 5].

The investigated waste materials acquired from ZGH Boleslaw S.A. are composed mainly of the Ca and Mg minerals (dolomites). They also contain some quantity of heavy metals including Fe, Zn, Pb, Cd, Tl, Cu, Ag, Ni, As, and Sb [6]. Their percentage

[1] Department of Metallurgy, Silesian University of Technology, ul. Z. Krasińskiego 8, 40–019 Katowice, Poland, email: Malgorzata.Pacholewska@polsl.pl

[2] Environmental Biotechnology Department, Silesian University of Technology, ul. Akademicka 2, 44–100 Gliwice, Poland, email: Beata.Cwalina@polsl.pl

in flotation tailings is varying throughout the tailings pond and dependent on storage time (Table 1).

Table 1

Concentrations of As and Sb in flotation tailings from ZGH Boleslaw S.A.

Storage yard	A1 – the oldest part		A2 – the youngest part	
Depth	0.0 m	3.0 m	0.0	3.0 m
As and Sb concentrations [%]				
As	0.098	0.057	0.085	0.075
Sb	0.0063	0.0045	0.0052	0.0041

Total amounts (estimated in 1990s) of some toxic metals present in the tailing ponds are as follows: As-13105.7 Mg, Cd-1967.2 Mg, and Tl-664.9 Mg. Some amounts of As and Sb remain in the zinc-bearing raw materials from which they pass into electrolyte solution. Removal of As and Sb from this solution is carried out due to chemical oxidation of ferrous ions to Fe(III). It is suggested that the iron-oxidizing *A. ferro-oxidans* bacteria indicating resistance to arsenium and antimonium ions could be useful in biological treatment of the feed electrolyte.

Materials and methods

The metabolic activity of acidophilic bacteria *A. ferrooxidans* strain B1 = SB isolated from post-flotation tailings [1] was investigated taking into account these bacteria iron-oxidizing ability. Experiments were carried out in glass bioreactors of 300 cm^3 volume, using synthetic culture medium 9K of Silverman and Lundgren (S/L) [7]. This medium contains [g/dm^3]: $NH_4)_2SO_4$ – 3.0; KCl – 0.1; $MgSO_4 \cdot 7H_2O$ – 0.5; $Ca(NO_3)_2$ – 0.01; K_2HPO_4 – 0.5; Fe(II) – 9.0 ($FeSO_4 \cdot 7H_2O$). The 100 cm^3 samples of 9K medium were inoculated with 2 cm^3 of the culture suspension containing bacteria in exponential growth phase. Solutions were aerated and mechanically stirred. Their temperature was maintained on level 25 ^oC. The Sb(III) and As(III) ions were introduced into 9K solution (pH ca 2.5) as standard water solutions containing respective ions at concentration of 1.0 g/dm^3, to obtain final concentrations (in 9 K solution) of 10, 20 and 50 mg/dm^3 being 10 times higher than those occurring in polluted industrial electrolyte. The standard solutions have been received from the analytical laboratory Boltherm, Bukowno. The standard As(III)-solution has been produced in Regional Verification Office, Lodz whereas the standard Sb(III)-solution has been produced in Tusnovics Instruments Sp. z o.o., Krakow. Solutions with bacteria as well as sterile control solutions without bacteria were investigated. The Fe(II) and Fe(III) concentrations were measured by a complexometric method with sulfosalicylic acid [8].

Results

The influence of Sb(III)

Changes in Fe(II) and Fe(III) concentrations in the 9K solution under the action of *A. ferrooxidans* strain B1 in the presence of Sb(III) ions have been presented in Figs 1–2 and in Table 2.

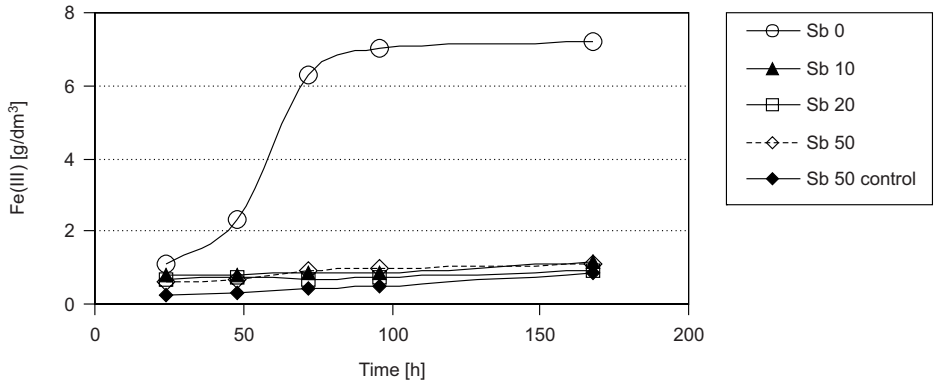

Fig. 1. The influence of Sb(III) mg/dm^3 on metabolic activity of the *Acidithiobacillus ferrooxidans* strain B1, reflected by increase in the Fe(III)-concentration in 9K solution

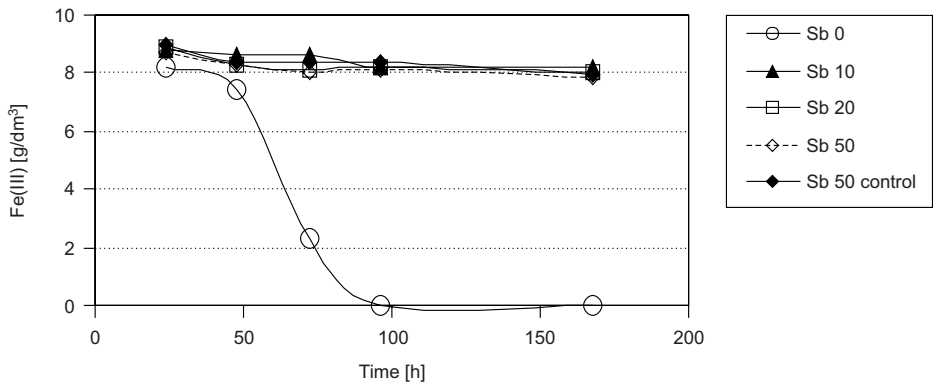

Fig. 2. The influence of Sb(III) mg/dm^3 on metabolic activity of the *Acidithiobacillus ferrooxidans* strain B1, reflected by decrease in the Fe(II)-concentration in 9K solution

From these results it may be concluded that: 1) Sb(III)-presence in 9K culture medium causes an inhibition of the iron-oxidizing activity in *A. ferrooxidans* strain B1; 2) *A. ferrooxidans* strain B1 indicates high susceptibility to Sb(III) ions even at their lowest concentration used (concentration of 10 mg/dm^3 was toxic for B1); 3) average rate of the Fe(III)-concentration increases due to metabolic activity of *A. ferrooxidans*

strain B1 decreases about 6-times (from 42.8 to 7.1 mg/dm^3/d; Table 2) in the presence of Sb(III) ions at the lowest concentration used (10 mg/dm^3).

Table 2

The influence of Sb(III) and As(III) on metabolic activity of the *Acidithiobacillus* ferrooxidans strain B1, reflected by average rates of changes in Fe(II) and Fe(III)-concentrations in 9K solution during 168 hours

Supplement	$\Delta\downarrow$ Fe(II)		$\Delta\uparrow$ Fe(III)	
	mg/dm^3/d	mg/dm^3/h	mg/dm^3/d	mg/dm^3/h
Sb(III) [mg/dm^3]				
0.0	93.7*	3.90*	42.8	1.80
10.0	4.8	0.20	7.1	0.29
20.0	5.6	0.23	5.4	0.22
50.0	6.6	0.27	6.6	0.27
50.0 (control; sterile)	6.2	0.26	5.0	0.21
As(III) [mg/dm^3]				
0.0	93.7*	3.90*	42.8	1.80
10.0	93.7*	3.90*	43.7	1.82
20.0	93.7*	3.90*	45.7	1.90
50.0	93.7*	3.90*	44.1	1.84
0.0 (control; sterile)	7.9	0.33	6.6	0.27
50.0 (control; sterile)	7.0	0.29	4.6	0.19
As(III) 50 + Sb(III) 10 [mg/dm^3]	13.7	0.57	9.5	0.40

$\Delta\downarrow$ Fe(II), $\Delta\uparrow$ Fe(III) – mean rates of Fe(II)-concentration decrease or Fe(III)-concentration increase; * – the rate estimated after 96 hours.

The influence of As(III)

Changes in Fe(II) and Fe(III) concentrations in the 9K solution under the action of *A. ferrooxidans* strain B1 in the presence of As(III) ions have been presented in Figs 3–4 and in Table 2. From these results it may be pointed that: 1) *A. ferrooxidans* strain B1 indicated high tolerance and resistance to As(III) ions; 2) the As(III)-presence at concentration of 50 mg/dm^3 caused inconsiderable increase in the rate of Fe(II) ions bacterial oxidation; 3) simultaneous presence of both Sb(III) and As(III) ions in 9K liquid culture medium caused the decrease in metabolic activity of bacteria tested; average rate of the Fe(III)-concentration increase due to metabolic activity of *A. ferrooxidans* strain B1 decreased almost 5-times (from 44.1 to 9.5 mg/dm^3/d; Table 2) in the presence of Sb(III) and As(III) ions at the lowest concentrations used (10 mg/dm^3).

Presented results indicated that the *A. ferrooxidans* strain B1 possesses increased arsenic resistance. It is accepted that the arsenic resistance and metabolizing systems occur in bacteria in three patterns: the widely-found ars operon that is present in most

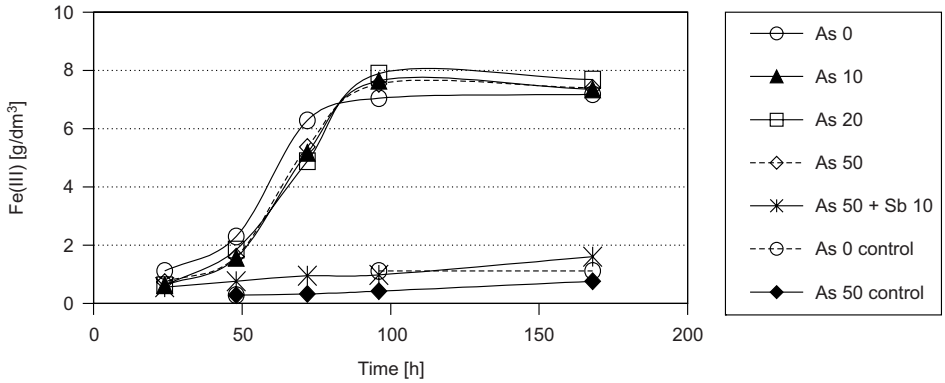

Fig. 3. The influence of As(III) mg/dm^3 on metabolic activity of the *Acidithiobacillus ferrooxidans* strain B1, reflected by increase in the Fe(III)-concentration in 9K solution

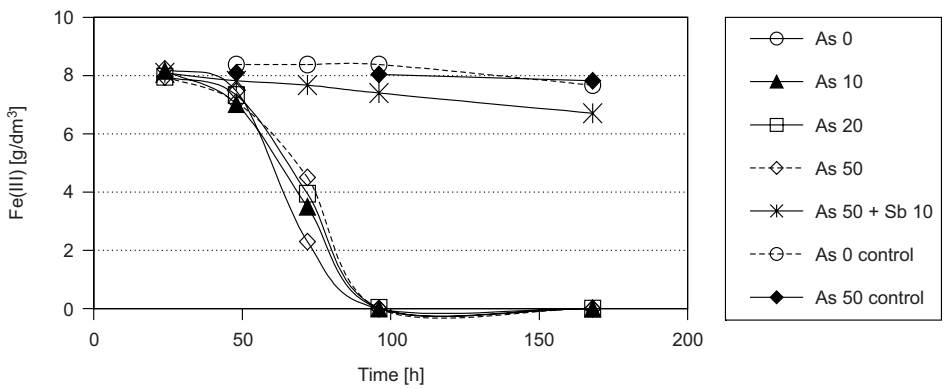

Fig. 4. The influence of As(III) mg/dm^3 on metabolic activity of the *Acidithiobacillus ferrooxidans* strain B1, reflected by decrease in the Fe(III)-concentration in 9K solution

bacterial genomes and many plasmids, the more recently recognized arr genes for the periplasmic arsenate reductase, and the aso genes for the periplasmic arsenite oxidase [10]. It has been shown that after long time (two years) of selection, *A. ferrooxidans* bacteria may become sufficiently resistant to the 13 g/dm^3 total arsenic in solution. The *A. ferrooxidans* strain B1 used in our experiments tolerate the As(III) at concentrations up to 50 mg/dm^3. Such a resistance level would be sufficient for this strain practical using in processes of the feed electrolyte treatment, but these bacteria are not antimony resistant. Taking into account that industrial electrolyte is usually a multi-component solution, in which many other impurities such as Cu(II), Cd(II), and Ag(I) ions (characteristics of the *A. ferrooxidans* strain B1 resistance to these ions have already been investigated and presented [9]), as well as the Sb(III) ions occur simultaneously with As(III) ions, it can be concluded that the *A. ferrooxidans* strain B1 is not useful for

treatment the multi-component feed electrolytes originated in ZGH Boleslaw S.A., due to their high overall toxicity towards bacteria.

Conclusions

Basing on the investigations on the influence of the Sb(III) and As(III) ions on metabolic activity of acidophilic bacteria *A. ferrooxidans* strain B1 = SB, which have been isolated from post-flotation tailings resulting from processes of zinc-lead ores enrichment in the ZGH Boleslaw S.A., it may be stated that these bacteria feature high resistance to the As(III) ions and lack of the Sb(III) ions tolerance (resistance to). It has been demonstrated that bacteria tolerate the As(III) presence at concentrations up to 50 mg/dm^3 but do not tolerate Sb(III) even at concentration of 10 mg/dm^3. Thus, the strain being tested cannot be used in processes of the feed electrolyte treatment and cleaning.

Acknowledgements

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OPORNOŚĆ *Acidithiobacillus ferrooxidans* WOBEC JONÓW As(III) ORAZ Sb(III)

[1] Katedra Metalurgii, [2] Katedra Biotechnologii Środowiskowej
Politechnika Śląska

Abstrakt: Badano wpływ jonów Sb(III) i As(III) na aktywność metaboliczną kwasolubnych bakterii *Acidithiobacillus ferrooxidans*, izolowanych z odpadów poflotacyjnych rud cynku i ołowiu. Ustalono, że bakterie te wykazują dużą oporność na jony As(III) i brak tolerancji Sb(III). Bakterie tolerują As(III) w stężeniach do 50 mg/dm^3, ale nie tolerują Sb(III) nawet w stężeniu 10 mg/dm^3. Tak więc testowany szczep nie może być wykorzystany w procesach przeróbki i oczyszczania nadawy elektrolitu.

Słowa kluczowe: *Acidithiobacillus ferrooxidans*, utlenianie Fe(II), oporność na As(III) i Sb(III)

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Elżbieta PEZOWICZ[1], Dorota TUMIALIS
and Magdalena LEŚNIAK[1]

INVESTIGATIONS ON THE CONTROL OF *Sphaerolecanium prunastri* F. (*Homoptera*, *Coccinea*) BY ENTOMOPATHOGENIC NEMATODES UNDER CONTAMINATED CONDITIONS

BADANIA NAD WPŁYWEM NICIENI ENTOMOPATOGENNYCH NA *Sphaerolecanium prunastri* F. (*Homoptera*, *Coccinea*) W WARUNKACH ZANIECZYSZCZEŃ KOMUNIKACYJNYCH

Abstract: Scale insects (*Coccinea*) are the primary pests of fruit, ornamental and forest trees worldwide. Losses made by scale insects consist in sucking assimilates and causing pathological histological changes. Scale insects are controlled with many methods, most often with chemical insecticides. Biopreparations based on entomopathogenic nematodes may be an alternative way. The effect of nematodes *Heterorhabditidae* and *Steinernematidae* on globose scale fungi was studied under laboratory conditions but they were collected from the combustion gases area near a PKS bus station. Invasive larvae of the nematodes *H. megidis*, *H. bacteripphora*, *S. affinis*, *S. feltiae*, *S. carpocapsae* were used for experiments. *H. megidis* caused 53 % insect mortality and appeared to be most effective. Nematodes of the family *Steinernematidae* were not able to penetrate the body of the globose scale.

Keywords: entomopathogenic nematodes**,** globose scale**,** scale insects, anthropogenic pollution

Scale insects (*Coccinea*) belong to the order *Homoptera*. They are primary pests of fruit as well as ornamental and forest trees worldwide. Losses brought by these insects consist in taking up assimilates and triggering pathological histological changes in plants. They are also the virus disease vectors and large amounts of honeydew excreted by these insects become a medium for fungi [1].

Many methods are used to control scale insects; most popular are the chemical method with the use of insecticides. Zolone, Owadofos, Anthio, Lannante 20L, Ultracid 40 EC are the insecticides now recommended for this purpose [2].

[1] Chair of Animals Environment, Animal Sciences Faculty, Warsaw University of Life Sciences – SGGW, ul. Ciszewskiego 8, 02–786 Warszawa, Poland, phone: +48 22 593 66 24, email: elzbieta_pezowicz@sggw.pl

Insecticides are the means of great efficiency which cause rapid extinction of pests; they are, however, toxic and mutagenic. The substances are easily dispersed in the environment by air and water. Many of them have long waiting periods which means long persistence in soil and a pose threat to the environment. Moreover, chemicals may accumulate in plants and finally reach consumers as dangerous residues tending to accumulate in human blood, fat tissues and milk. There are premises to expect that pesticides are responsible for carcinogenic changes [3]. Common phenomenon of resistance to increasing doses of pesticides observed in many pests is another negative consequence of their application. Moreover, the use of pesticides is harmful to beneficial insects and may deform plant anatomy and physiology [2].

Now, due to a general trend of abandoning chemical pest control, alternative methods are being explored. Such methods include environment friendly biological methods. Pheromone traps with juvenile hormones are used to control scale insects. Their action consists in hampering fertility and growth of insects [4]. The number of scale insects is also controlled by the introduction of predators and parasitoids [1].

In the presented study an attempt was made to check the sensitivity of the globose scale (*Sphaerolecanium prunastri*) collected from the combustion gases contaminated environment, to entomopathogenic nematodes *Steinernematidae* and *Heterorhabditidae*. There is no data on the entomopathogenic nematodes – scale insects system in the world literature so it seems reasonable to check the sensitivity of the latter to selected species of entomopathogenic nematodes.

Material and methods

The globose scale insects were taken for experiments from twigs of a host tree (plum tree) growing on the grounds of a bus station. Trees constantly affected by exhausts from buses were heavily infested by the insects. Twigs were cut into small pieces (ca 9 cm) with several to several dozen insects (larvae of the second growth stage) on each.

Invasive larvae of entomopathogenic nematodes were taken from constant laboratory culture of the Department of Zoology. Larvae are kept in a fridge at a temperature of 4 ^{o}C. Every two months the culture is renewed to maintain uniform pathogenic properties.

Twigs with globose scales were placed in small test tubes with water to maintain viability of the plant and insects. Test tubes were sealed with cotton wool and paraffin. Such prepared twigs with insects were placed in Petri dishes lined with double layer of filter paper. Invasive larvae of a given nematode species at a dose of 4 thousand invasive larvae per 1 cm^3 of water were introduced to Petri dishes. The dishes were then sealed with paraffin and placed in a thermostated chamber at a temperature of 25 ^{o}C.

Five nematode species: *Heterorhabditis megidis*, *Heterorhabditis bacteriophora*, *Steinernema feltiae*, *Steinernema affinis*, *Steinernema carpocapsae* were used in the experiment. Globose scale larvae treated with water devoid of nematodes were the control sample. Mortality of the larvae was checked during 6 days. Dead insects were dissected in order to test whether nematodes were the reason of their death.

Results and discussion

Performed experiments allowed for estimating mortality of the globose scale *Sphaerolecanium prunastri*. Results (Table 1) indicate that the highest mortality of the larvae (95 % as compared with 45 % in the control) was caused by the nematode *H. bacteriophora*. High mortality of the globose scale larvae (Table 2) was a result of the presence of hymenopteran larvae of the family *Chalcidoidae* in their body. As known from the literature [5] most frequent species of *Chalcidoidae* grown from the body of the globose scale were: *Discodes* spp., *Microterys hortulanus*, *Coccophagus lyciminia*. Other literature data [6] indicate a high degree of infection (up to 90 %) in larvae of the II growth stage of the globose scale by *Chalcidoidae*. The latter play a role of superparasites. The larvae of the globose scale are small organisms (1–4 mm) [7]. *Chalcidoidae* present in their bodies make penetration of the second potential parasite impossible.

Table 1

Mortality of the globose scale caused by entomopathogenic nematodes [%]

Nematode species	Mean	SD	Control
H. megidis	88	0.14	86
H. bacteriophora	95	0.05	45
S. affinis	89	0.05	80
S. feltiae	71	0.02	70
S. carpocapsae	69	0.03	70

Table 2

Mortality of the globose scale caused by the presence of *Chalcidoidae* [%]

Nematode species	Mean	SD
H. megidis	19	0.05
H. bacteriophora	73	0.05
S. affinis	60	0.07
S. feltiae	56	0.08
S. carpocapsae	62	0.07

Table 3

The extensity of invasion of the globose scale by entomopathogenic nematodes [%]

Nematode species	Mean	SD
H. megidis	53	0.17
H. bacteriophora	3	0.05
S. affinis	0	0
S. feltiae	0	0
S. carpocapsae	0	0

The extensity of invasion of the globose scale larvae by nematodes is presented in Table 3. The data demonstrate that only the *H. megidis* nematode was able to penetrate and infest the body of the globose scale. The extensity of infection of the insects' larvae was 53 %. The presence of developing nematode population in the insects' body was never found when *Steinernematidae* were used in the experiment. Probably tough body structures did not allow nematodes to penetrate to the insect interior. *Heterorhabditidae* possess a cuticular tooth in the anterior part of their body which enables them to penetrate the globose scale [8].

Conclusions

1. Entomopathogenic nematodes of the family *Steinernematidae* (*S. affinis*, *S. carpocapsae*, *S. feltiae*) do not penetrate the body of the globose scale collected from the combustion gases contaminated environment.

2. The globose scale (*Sphaerolecanium prunastri*) is sensitive to nematodes of the family *Heterorhabditidae*.

3. The globose scale infected by hymenopterans (*Chalcidoidae*) is not attractive to entomopathogenic nematodes.

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BADANIA NAD WPŁYWEM NICIENI ENTOMOPATOGENNYCH NA *Sphaerolecanium prunastri* F. (*Homoptera*, *Coccinea*) W WARUNKACH ZANIECZYSZCZEŃ KOMUNIKACYJNYCH

Zakład Zoologii, Katedra Biologii Środowiska Zwierząt, Wydział Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Abstrakt: Czerwce (*Coccinea*) w skali światowej zajmują pierwsze miejsce jako szkodniki drzew owocowych, ozdobnych, leśnych i innych. Szkody przez nie powodowane polegają na pobieraniu asymilatów i wywoływaniu u roślin patologicznych zmian histologicznych. Czerwce zwalcza się wieloma metodami, z których najczęściej stosowane są środki chemiczne – insektycydy. Alternatywą dla nich mogą być biopreparaty z wykorzystaniem nicieni entomopatogennych. W warunkach laboratoryjnych zbadano wpływ nicieni *Heterorhabditidae i Steinernematidae* na misecznika tarniowego pozyskanego z terenów zanieczyszczonych spalinami komunikacyjnymi. Do doświadczeń użyto larw inwazyjnych nicieni *H. megidis*, *H. bacteriophora*, *S. affinis*, *S. feltiae*, *S. carpocapsae*. Najskuteczniejszy okazał się *H. megidis*, który spowodował 53-procentową śmiertelność owadów. Nicienie *Steinernematidae* nie wykazują zdolności penetracji do ciała miseczników.

Słowa kluczowe: nicienie entomopatogenne, misecznik tarniowy, czerwce, zanieczyszczenia antropogenne

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Anna PODLEŚNA[1]

YIELDING EFFECT OF NITROGEN AND SULFUR AT POT EXPERIMENT CONDITIONS WITH WINTER WHEAT (*Triticum aestivum* L.)

PLONOTWÓRCZE DZIAŁANIE AZOTU I SIARKI W WARUNKACH DOŚWIADCZENIA WAZONOWEGO Z PSZENICĄ OZIMĄ (*Triticum aestivum* L.)

Abstract: The aim of the present research was to proof the yielding role of nitrogen and sulfur in the process of growth and development of winter wheat. The experiment was conducted at a greenhouse, in Mitscherlich pots and soil had a low concentration of sulfur. Three increasing doses of N were applied in this experiment. The N sources were ammonium nitrate (without sulfur) and tetra urea calcium sulfate (with sulfur). No other sulfur fertilizer was used in this experiment. It was found that higher yields of wheat were obtained at objects with use of sulfur containing fertilizer. These plants gave higher yield of grain on all N-S levels in comparison to objects not fertilized with S. Plant supply with N and S produced a larger number of lateral fertile shoots as well as a significantly larger number of grains per ear of main and lateral shoots. Mineral sulfur also caused better plumpness of grain in comparison to parallel objects without sulfur, because in these objects a larger mass of thousand grains was found.

Keywords: pot experiment, winter wheat, nitrogen, sulfur, yield structure

Cereals belong to plants with relatively little requirement for sulfur, however it is known that they uptake up to 20 kg S · ha^{-1} [1, 2]. It is observed in agriculture the considerable sulfur exhaustion from the soil resources by enlarging cereal participation in cropping system and cultivation of cruciferous plants which have the biggest requirement for this nutrient. As an effect of this process as well as permanent decrease of sulfur compounds emitted to the atmosphere, sulfur becomes a deficit nutrient in some regions of Poland. There is an anxiety that in this nutrient shortage conditions, widespread use of NPK fertilization can appear less effective. Balanced fertilization, which is recommended in recent years, is based on the foundation that plants require all

[1] Department of Plant Nutrition and Fertilization, Institute of Soil Science and Plant Cultivation – National Research Institute, ul. Czartoryskich 8, 24–100 Puławy, Poland, phone: +48 81 886 34 21 ext. 251, email: ap@iung.pulawy.pl

nutrients, in adequate amounts, for proper development and production of high and good quality yields.

The aim of the present research was to prove the important role of sulfur in the process of growth, development as well as yield formation of winter wheat.

Material and methods

The experiment was conducted at a greenhouse, in Mitscherlich pots filled with 6,5 kg soil with granulometric composition of heavy loamy sand, which was characterized by following parameters (mg · kg^{-1} soil): 13.6 – P_2O_5, 11.6 K_2O, 3.5 Mg, < 1 mg S-SO_4 and pH_{KCl} 5,6. In each pots were cultivated 12 plants of winter wheat, cv. Mobela. The first experimental factor was the type of nitrogen fertilizer: ammonium nitrate (without sulfur) and tetra urea calcium sulfate – CMSW (with sulfur) [3], and the second factor – N dose (g · pot^{-1}): 0.8, 1.6 and 2.4. At objects fertilized with tetra urea calcium sulfate were applied also the following doses of sulfur (g · pot^{-1}): 0.25, 0.50 and 0.75 for succeeding N doses, respectively. During the establishing of the experiment also 0.8 g P, 1.6 g K and 5.4 g $CaCO_3$ were applied to every pot. Plants were watered with distilled water and soil moisture was kept on the level of 40–60 % of field water capacity in dependence on the developmental stage of wheat. Three plant harvests, at phases evaluated according to the BBCH scale, were performed in the vegetation period: 33 – shooting, 65 – flowering and 92 – full maturity. During all harvests, plants from 3 pots, from each fertilizer object were harvested. The presented data are the mean from the objects and two years of research. The obtained results were calculated statistically with the use of Statgraphics Program v. 5.1.

Results and discussion

From the phase of the second node there were observed some differences in the growth and development of wheat, in dependence on N dose and supply with sulfur. Plants fertilized with ammonium nitrate, and particularly at low doses of this fertilizer, had light yellow pigmentation of leaves as opposed to dark green leaves of wheat fertilized with tetra urea calcium sulfate, which was also observed by Haneklaus et al [4]. Moreover, in the shooting phase retarding the increase of aboveground part dry mass of plants with sulfur deficit was found in spite of nitrogen supply. In this result the lowest dose of N applied with S gave greater yielding effect than the highest dose of nitrogen alone (Fig. 1). This tendency was maintained up to the end of vegetation. Sulfur shortage in the soil influenced change of wheat growth and development because in plants occurs mutual regulation of the NO_3^- and SO_4^{2-} assimilatory pathways [5], as a result of which the deficit of one nutrient decreases assimilation of the other. Karmoker et al [6] found that barley deprivation of sulfate ions led to decrease of nitrogen transport in plant and lower stomatal conductance, transpiration and photosynthesis. Growth reduction as a consequence of a lack of balance between fertilization with N and S was caused probably by protein synthesis inhibition and lower nitrate reductase activity as well as accumulation of low molecular protein compounds in these

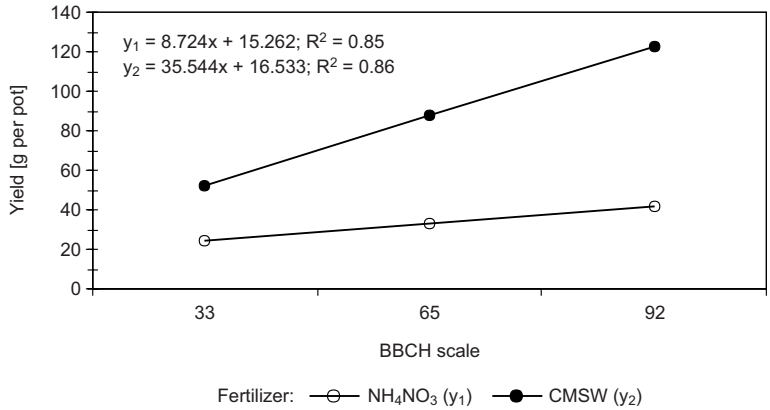

Fig. 1. Aboveground part yield of wheat in dependence on stage of growth and fertilizer

plant vegetative organs [7]. At full maturity it was found, that the used fertilizers and their doses had a significant effect on grain yield, which at objects without sulfur was lower in comparison to the yield found on each N-S level (Fig. 2). Sulfur deficit found at the objects where fertilizers without sulfur were used, caused (mean for N): 75 and 60 % yield reduction of grain and straw, respectively. At a pot experiment conducted by Haneklaus et al [4] and Brodowska [2] wheat from sulfur deficit object gave only 10 and 6 % of seed yield, respectively, in comparison to control. However, Rasmussen et al [8] found in a field experiment a 15 and 20 % seed and straw yield decrease of wheat cultivated in sulfur deficit conditions. It the present author's own research there was observed a significant effect of sulfur and nitrogen on the increase of fertile shoot number, number of grain per ear and number of seeds per plant (Table 1). The obtained results indicated an important role of sulfur in lateral fertile shoot formation as well as their participation in total seeds yield (Fig. 2, Table 1), which was also found in research led by Rasmussen et al [8].

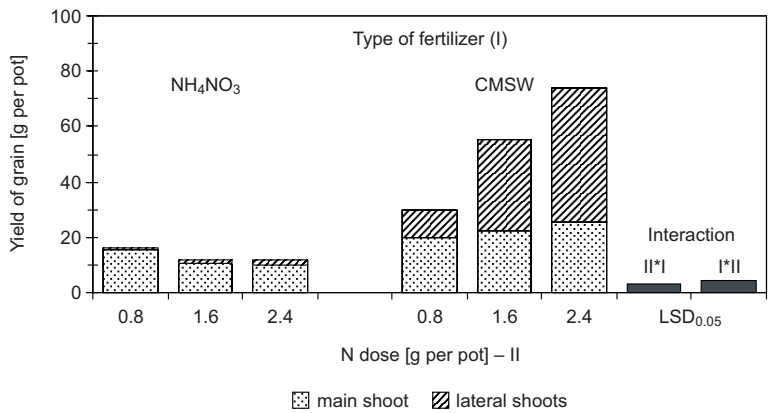

Fig. 2. Yield of wheat grain in dependence on fertilization

Table 1

Yield structure of winter wheat

Fertilizer	N dose [g per pot]	Number of shoots per plant		Number of grain per ear		Number of grain per plant	1000 grain weight [g]
		fertile	sterile	main shoot	lateral shoots		
NH_4NO_3	0.8	0.08	1.5	33.6	1.86	35.2	38.7
	1.6	0.19	1.3	28.8	2.60	31.3	31.2
	2.4	0.39	1.5	28.4	5.10	33.4	29.3
CMSW	0.8	1.00	2.7	35.1	23.8	58.8	42.7
	1.6	2.19	1.7	45.1	70.3	115.3	39.9
	2.4	3.00	1.7	48.7	93.9	142.5	43.3
$LSD_{0.05}$		0.161	0.089	0.470	0.428	0.399	0.631

Haneklaus et al [4] supposes however, that in deficit conditions an intra-plant competition for sulfur takes place and in its effect lateral shoots are kept alive at the cost of ear growth and number of seeds per ear. In the experimental wheat plants fertilized with sulfur also a higher mass of a thousand seeds was noted (Table 1), although it is not confirmed by Karmoker et al [6]. According to Castle and Randall [9], sulfur shortage causes abridgement of time necessary to kernel cell division and acceleration of storage protein synthesis phase, as a result of which the number of cells and possibility of their filling decrease. Moreover, incorporation of nitrogen to seed protein still requires sulfur supply after the end of flowering [10], which probably affected better seed filling in the present experiment.

Conclusions

1. The use of a fertilizer with sulfur caused positive changes in the growth and development of wheat and as a consequence these plants gave significant higher yield in comparison to plants cultivated without sulfur.

2. Efficiency of nitrogen and its usefulness for the yielding potential of wheat was very low in conditions of sulfur deficit.

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PLONOTWÓRCZE DZIAŁANIE AZOTU I SIARKI W WARUNKACH DOŚWIADCZENIA WAZONOWEGO Z PSZENICĄ OZIMĄ (*Triticum aestivum* L.)

Zakład Żywienia Roślin i Nawożenia
Instytut Uprawy, Nawożenia i Gleboznawstwa – Państwowy Instytut Badawczy w Puławach

Abstrakt: Doświadczenie prowadzono w wazonach Mitscherlicha, na glebie o niskiej zawartości siarki. W doświadczeniu zastosowano trzy wzrastające dawki N z użyciem nawozu bezsiarkowego (saletra amonowa) oraz czteromocznikanu siarczanu wapnia jako źródła azotu i siarki. Stwierdzono, że od początku wzrostu i rozwoju pszenicy wyższe plony suchej masy uzyskano na obiektach, w których stosowano dodatkowo nawożenie siarką. Rośliny dojrzałe, które nawożono siarką, dały wyższy plon ziarna na wszystkich poziomach nawożenia w porównaniu do obiektów nawożonych tylko azotem. Uzyskany wzrost plonu wynikał przede wszystkim ze wzrostu liczby pędów bocznych kłosowych oraz liczby ziaren w kłosie pędu głównego i kłosach pędów bocznych. Po zastosowaniu każdej z dawek siarki stwierdzono również wzrost MTZ w porównaniu do równoległych obiektów N bez siarki.

Słowa kluczowe: doświadczenie wazonowe, pszenica ozima, azot, siarka, struktura plonu

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Olga POLESZCZUK[1], Dorota TUMIALIS
and Elżbieta PEZOWICZ

INFLUENCE OF LOW DOSES OF IONIZING RADIATION ON YOUNG AND TWO WEEK OLD INVASIVE LARVAE OF ENTOMOPATHOGENIC NEMATODES (*Heterorhabditidae*, *Steinernematidae*)

WPŁYW NISKICH DAWEK PROMIENIOWANIA JONIZUJĄCEGO NA MŁODE ORAZ DWUTYGODNIOWE LARWY NICIENI ENTOMOPATOGENNYCH (*Heterorhabditidae*, *Steinernematidae*)

Abstract: The performed experiments were aimed at checking the effect of ionising radiation as an abiotic factor on the bionomics of young and two week old larvae of *Steinernema feltiae* and *Heterorhabditis megidis*. Features associated with nematode pathogenicity (intensity of infection) and morphometry (length and width of individuals of the giant generation) were analysed. Three doses of ionising radiation (0.1, 0.05, and 0.01 kGy) were applied. The obtained results indicate the modifying effect of ionising radiation on each of the studied features of entomopathogenic nematodes.

Keywords: ionizing radiation, entomopathogenic nematodes, *Steinernema feltiae*

Together with the advancement of ecological farming and nature protection, plant protection chemicals are being withdrawn from use. Subsidies for farmers and producers associated with ecological farming have opened a new market for biological plant protection measures and consequently for mass production of biopreparations. Rapid growth of the economic importance of entomopathogenic nematodes for agricultural practice creates a constant demand for new strains. These strains should have high potential for reproduction on artificial substrata, resistance to storage and transport and high efficiency of pest insect control competitive with that of chemical

[1] Chair of Animal's Environment, Warsaw University of Life Sciences, ul. Ciszewskiego 8, 02–786 Warszawa, Poland, phone: +48 22 593 66 24, email: elzbieta_pezowicz@sggw.pl

plant protection measures. Biological methods of plant protection were known already in 1900 but their wide application started not earlier than 30 years ago. In orchards, fields, vineyards and greenhouses it is recommended to restrict the use of plant protection chemicals due to environmental protection and for economic reasons [1]. Unfortunately, under specific field conditions the commercial biopreparations are not efficient.

One of the possible solutions is an attempt of genetic improvement through artificial selection, strain hybridisation and genetic engineering. One of abiotic factors affecting nematodes is ionising radiation which interferes in cell processes and leads to biological and chemical changes.

The invasive stage, due to its unique characteristic, plays an exceptional role in the growth cycle of entomopathogenic nematodes and in survival and expansion of their populations. These features are also the basis of the commercial value of nematodes. Even small morphologic modifications may have a significant impact on the activity of invasive larvae of entomopathogenic nematodes.

The aim of the study was to check whether low doses of ionising radiation produce different effects in young and in two weeks old larvae of entomopathogenic nematodes and if the changes in nematode features could be been used in commercial production of biopreparations.

Material and methods

Invasive larvae of *Steinernema feltiae* from Olsztyn and of *Heterorhabditis megidis* in two age groups were used in experiments. The first group consisted of young (2–3 days old) invasive larvae devoid of cuticulum [2]. After irradiation the larvae were left for at least two weeks until they gained infective properties [2]. The second group was composed of invasive larvae at least two weeks old but not older than two months. The larvae had double cuticulum [2].

The Cobalt bomb (RChM-g-20) was the source of radiation used in the experiment. The bomb emits gamma radiation from cobalt isotope ^{60}Co. Radiation doses of 0.1, 0.05 and 0.01 kGy were applied to all nematode species and strains. Selection of these radiation doses was preceded by preliminary studies on nematode reproduction abilities as the main criterion.

The experimental plan was based on using irradiation in the following set-up:

1. Non-irradiated insect + irradiated nematode.

Irradiated nematodes were used to infect the host insect *G. mellonella* (90 caterpillars – 30 in each of the three measurement series). Infected insects were dissected to estimate the invasiveness and to measure hermaphroditic individuals (*Heterorhabditidae*) or giant females (*Steinernematidae*).

2. Non-irradiated insect + non-irradiated nematode (control).

The obtained results were statistically processed with the SPSS v. 12.0 software. Statistical significance of differences was tested with non-parametric ANOVA.

Results and discussion

The intensity of infection is an important parasitic parameter which determines nematode invasiveness towards insects. It was observed during dissection that all caterpillars infected by irradiated nematodes contained giant individuals of *Steinernematidae* or hermaphroditic individuals of *Heterorhabditidae*. It means that gamma irradiation did not hamper the development of invasive larvae. Within-strain comparison between the young and two weeks old invasive larvae showed that two doses of 0.1 and 0.01 kGy applied to *S. feltiae* Olsztyn affected the intensity of infection. The differences were statistically significant (Table 1).

Table 1

Comparison of the mean intensity of infection of *G. mellonella* by *S. feltiae* Olsztyn larvae

Dose	*S. feltiae* Olsztyn two-weeks old L_3	*S. feltiae* Olsztyn young L_3
0.1 kGy	12.4 a	27.3 b
0.05 kGy	18.5 a	15.6 a
0.01 kGy	16.6 a	24.6 b
Control	18.0 a	18.0 a

Different letters denote significant differences at $p \leq 0.05$.

In *H. megidis* only the dose of 0.01 kGy resulted in highly significant difference. Two week old larvae irradiated with the lowest dose showed high intensity of infection but in young nematode larvae this effect was not found (Table 2).

Table 2

Comparison of the mean intensity of infection of *G. mellonella* by *H. megidis* larvae

Dose	*H. megidis* two weeks old L_3	*H. megidis* young L_3
0.1 kGy	8.6 a	9.7 a
0.05 kGy	7.2 a	5.8 a
0.01 kGy	17.3 a	5.3 b
Control	6.4 a	6.4 a

Different letters denote significant differences at $p \leq 0.05$.

The performed measurements of hermaphroditic individuals of *H. megidis* revealed that irradiation of young nematode larvae generated adults of smaller body sizes. The effect was visible at the doses of 0.01 and 0.05 kGy but at the dose of 0.1 kGy the calculated parameters did not differ statistically from the control (Table 3).

Table 3

Comparison of the body size of giant females of *S. feltiae* Olsztyn

Dose	Size [mm]	*S. feltiae* Olsztyn two weeks old L_3	*S. feltiae* Olsztyn young L_3
0.1 kGy	length	10.35 a	11.42 a
	width	0.56 a	0.65 b
0.05 kGy	length	10.61 a	10.25 a
	width	0.52 a	0.60 b
0.01 kGy	length	12.33 a	13.06 a
	width	0.50 a	0.64 b
Control	length	11.64 a	11.64 a
	width	0.51 a	0.51 a

Different letters denote significant differences at $p \leq 0.05$.

In *S. feltiae* Olsztyn the irradiation of young invasive larvae resulted in obtaining adults of body size different from those obtained by the irradiation of two weeks old L_3 larvae. The differences manifested themselves in body widths of the analysed individuals of *S. feltiae* Olsztyn; their body length remained the same (Table 4).

Table 4

Comparison of the body size of hermaphroditic individuals of *H. megidis*

Dose	Size [mm]	*H. megidis* two weeks old L_3	*H. megidis* young L_3
0.1 kGy	length	7.63 a	7.07 a
	width	0.54 a	0.51 a
0.05 kGy	length	8.28 a	7.31 b
	width	0.63 a	0.49 b
0.01 kGy	length	10.35 a	8.27 b
	width	0.69 a	0.53 b
Control	length	11.60 a	11.60 a
	width	0.76 a	0.76 a

Different letters denote significant differences at $p \leq 0.05$.

Conclusions

1. Ionising radiation is the factor affecting the bionomics of entomopathogenic nematodes.
2. The intensity of infection increased when young larvae of *S. feltiae* were irradiated. This effect was not observed in *H. megidis*.
3. Irradiation of young larvae of *S. feltiae* and *H. megidis* increased body sizes of giant nematodes as compared with the irradiation of two week old larvae.

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WPŁYW NISKICH DAWEK PROMIENIOWANIA JONIZUJĄCEGO NA MŁODE ORAZ DWUTYGODNIOWE LARWY NICIENI ENTOMOPATOGENNYCH (*Heterorhabditidae*, *Steinernematidae*)

Katedra Bilogii Środowiska Zwierząt, Wydział Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Abstrakt: Przeprowadzone doświadczenie miało na celu sprawdzenie wpływu promieniowania jonizującego, jako czynnika abiotycznego, na bionomię młodych oraz dwutygodniowych larw inwazyjno-przetrwalnikowych *Steinernema feltiae* oraz *Heterorhabditis megidis*. Badano cechy związane z patogenicznością nicieni (intensywność zarażenia) oraz morfometrią (długość oraz szerokość osobników pokolenia olbrzymiego). Zastosowano trzy dawki promieniowania jonizujacego: 0,1; 0,05; 0,01 kGy.

Otrzymane wyniki wskazują na modyfikujący charakter promieniowania jonizującego u każdej z badanych cech nicieni entomopatogenicznych.

Słowa kluczowe: nicienie entomopatogeniczne, *Steinernema feltiae*, promieniowanie jonizujące

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Adam RADKOWSKI[1] and Iwona RADKOWSKA[2]

ESTIMATION OF THE QUALITY AND NUTRITIONAL VALUE OF HAY FROM THE SELECTED INDIVIDUAL FARMS LOCATED IN THE REGION OF KRAKOW-CZESTOCHOWA JURA PART II. CONTENT OF MACROELEMENTS

OCENA JAKOŚCI I WARTOŚCI POKARMOWEJ SIANA Z WYBRANYCH GOSPODARSTW INDYWIDUALNYCH POŁOŻONYCH NA TERENIE JURY KRAKOWSKO-CZĘSTOCHOWSKIEJ CZ. II. ZAWARTOŚĆ MAKROELEMENTÓW

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

The weighted mean content of macroelements in plants fluctuated in the range of: 1.09–2.59 g P; 14.03–24.06 g K; 3.86–6.10 g Ca; 1.71–3.33 g Mg; 0.41–1.14 g Na · kg^{-1} d.m. All samples collected during the experiment were characterized with low phosphorus, calcium and sodium content. The low level of these elements resulted from the low level of phosphorus fertilization and limited liming of the grasslands located in the area of examined farms. Among all elements only the level of potassium content was optimal. Conducted analyses suggest that grasslands located in the investigated farms are fertilized with liquid manure, what leads to accumulation of potassium, which is a calcium and magnesium antagonist.

Keywords: hay, content of macroelements, antagonism of elements

Hay, which was in recent times the main element of winter feeding for cows, now is more often replaced with silages and haylages. However, complete replacement is considered as a feeding misconception, especially in the case of ruminants, because hay has a positive effect on the fermentation processes in the rumen as well as digesta pH reaction [1]. Moreover, hay of good quality provides many nutrients, mineral com-

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

[2] National Research Institute of Animal Production Balice, ul. Krakowska 1, 32–083 Balice, Poland, phone: +48 666 08 11 49, email: iradkowska@izoo.krakow.pl

pounds and vitamins to animals, which helps, in a natural way, in prevention of metabolic diseases [2]. Especially valuable from a nutritional point of view is the meadow hay composed of valuable grasses, legumes and herbs, which affect the flavor and palatability of the forage, which in turn triggers a higher degree of its consumption [3–5]. Hay should contain at least: 3.0 g P; 17–20 g K; 7.0 g Ca; 2.0 g Mg and 1.5–2.5 g Na · kg^{-1} in dry matter. Providing the proper amounts of mineral compounds is especially important in the case of milk cows, because for the production of 1 liter of milk the balanced forage as regards not only the energy and protein aspect but also containing enough levels of macro- and microelements is required [2].

Thus, the aim of the present study was an estimation of the mineral composition of hay derived from the selected farms specialized in milk production from the region of the Krakow-Czestochowa Jura.

Materials and methods

The investigations were conducted in the years 2005–2007 in 12 farms specialized in milk production located in the region of Krakow-Czestochowa Jura. The investigated farms were located in the Pilica administrative districts (Zawiercie county, Silesia province) at the altitude of 320 m. The experimental grasslands were located on the brown, acid soils and on the podsolic soils. The soils were classified from IVb to VI bonitation class. The soil was characterized with the pH_{KCl} acidity of 4.5–5.5 (acid and very acid), medium content of assimilable potassium and a low level of assimilable phosphorus and magnesium.

During the vegetation period (April–September) the following average rainfall amounts were determined for the years 2005, 2006 and 2007: 356.8 mm; 338.1 mm; and 375.4. The mean temperatures for the following years amounted to: 14.8, 15.2 and 14.3 ^{o}C.

The experimental hay was derived mostly from the first and second regrowth of grass flora and in a minor amount from the third regrowth.

The plants were collected at the turn of the heading and flowering stage – the first regrowth and during the heading stage – the second and the third regrowths. Before the feeding the 4 samples of hay were collected from each farm and subjected to the chemical analysis, which comprised: the estimation of phosphorus and magnesium content – by the colorimetric vanadium-molybdenic method, potassium, sodium and calcium – by the flame photometry method.

The results presented in this paper were limited to the mean values for the following years. The obtained results were subjected to the analysis of variance and the significance of differences was estimated using the Duncan test at the significance level of $\alpha = 0.05$.

Results and discussion

The content of mineral components in the investigated hay samples was diversified. The weighted mean content of macroelements fluctuated in the range of: 1.09–2.59 g P;

Table 1

The weighted mean content of macroelements and the ionic proportions in hay

Item	Investigated farm											
	1	2	3	4	5	6	7	8	9	10	11	12
	$g \cdot kg^{-1}$ d.m.											
P content [g kg^{-1} d.m.]	1.53ab*	2.27b	2.46c	2.57c	1.91b	2.44c	2.45c	2.59c	1.09a	1.83b	1.74b	1.62ab
K content [g kg^{-1} d.m.]	23.44c	18.31b	19.65b	17.93b	24.06c	18.01b	15.85a	16.83ab	14.88a	14.03a	17.24ab	15.45a
Ca content [g kg^{-1} d.m.]	5.82bc	5.28b	4.85ab	4.42ab	5.94c	6.10c	3.91a	4.22a	3.88a	3.86a	4.33a	4.03a
Mg content [g kg^{-1} d.m.]	1.71a	2.26b	2.38b	1.82a	2.27b	3.33c	2.10ab	2.44b	2.42b	1.93a	3.08c	2.63cb
Na content [g kg^{-1} d.m.]	0.46a	0.60ab	0.71b	0.59ab	0.49a	1.14c	0.41a	0.52a	0.62ab	0.48a	0.60ab	0.56ab
Ca:Mg	3.39c	2.34bc	2.04b	2.43bc	2.62bc	1.83ab	1.86ab	1.73ab	1.60a	2.00b	1.41a	1.53a
K:Mg	4.28d	2.54b	2.59b	3.08c	3.32c	1.69a	2.36b	2.16ab	1.92a	2.27ab	1.75a	1.84a
K:Ca	2.06b	1.78a	2.08b	2.08b	2.08b	1.51a	2.08b	2.04b	1.97ab	1.86ab	2.04b	1.97ab
K:(Ca + Mg)	1.39c	1.04ab	1.15b	1.24b	1.27b	0.80a	1.10ab	1.05ab	0.97a	1.02ab	0.94a	0.95a
K:Na	51.33d	30.30b	27.68b	30.31b	49.57d	15.87a	38.84c	32.42b	24.14a	28.93ab	28.81ab	27.57ab

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

14.03–24.06 g K; 3.86–6.10 g Ca; 1.71–3.33 g Mg; 0.41–1.14 g Na · kg^{-1} d.m. (Table 1). According to the feeding requirements the good quality forage should contain at least 3.0 g P; 17–20 g K; 7.0 g Ca; 2.0 g Mg and 1.5–2.5 g Na · kg^{-1} in dry matter [5–7]. In our study we noticed that respectively 75 % and 58 % of samples were characterized with optimal magnesium and potassium content. On the contrary, phosphorus, calcium and sodium concentrations in all samples were below the optimal levels.

For evaluation of the forage quality the quantitative or ionic proportions between elements are important [6–8]. The Ca:Mg weight ratio, which should be equal to 2–3:1, was optimal only in 42 % of samples, whereas none of them was characterized with the proper K:Mg weight proportion, which should be close to 6–8:1 [8, 9]. The 2:1 K:Ca ratio assumed as optimal was found in 75 % of the trials. The forage of good quality derived from grasslands should exhibit the 1.6–2.2:1 K:(Ca + Mg) ionic ratio [7, 9]. Regarding this level none of the samples met the requirements. The range of 5–7:1 is assumed as the most optimal for the K:Na weight proportion [10, 11]. In the case of our investigations the K:Na proportion was too wide and exceeded the optimal level from 3 to 10 times. The bad forage quality resulted mainly from the high deficiency of sodium in the hay samples.

The fact of a low level of mineral components in the examined hay samples can be explained by the wrong fertilization of grasslands in the examined farms. The low level of phosphorus and calcium fertilization indicates that high amounts of liquid manure, abundant on such farms, were utilized. Under the intensive fertilization with liquid manure the higher yield increase is achieved but the effect of component dilution also appears. It results also in the changes of the nutrients availability as a consequence of the strong soil acidification visible as the decreased level of basic cations, especially Ca in plants [12]. The majority of hay samples were characterized with unfavourable weight and ionic proportions.

The supplementation of the deficient macroelements, especially P, Ca and Na in the fertilization of grasslands as well as in ruminants feeding are recommended for the examined agricultural farms [13].

Conclusions

1. Hay samples collected from the all examined farms were not characterized with optimal phosphorus, calcium and sodium content. The low level of these elements resulted from the low level of phosphorus fertilization and limited liming of the grasslands located in the area of examined farms.

2. Too high concentration of potassium indicates that the grasslands were fertilized with liquid manure. This leads to accumulation of potassium, which is calcium antagonist.

3. The Ca:Mg and K:Mg weight proportions reached the optimal values only in the case of 42 and 75 %, respectively. Other ratios between elements were characterized with unfavourable values.

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OCENA JAKOŚCI I WARTOŚCI POKARMOWEJ SIANA Z WYBRANYCH GOSPODARSTW INDYWIDUALNYCH POŁOŻONYCH NA TERENIE JURY KRAKOWSKO-CZĘSTOCHOWSKIEJ CZ. II. ZAWARTOŚĆ MAKROELEMENTÓW

[1] Katedra Łąkarstwa, Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
[2] Instytut Zootechniki – Państwowy Instytut Badawczy w Balicach

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

Średnia ważona zawartości makroelementów w roślinach wahała się w zakresie: 1,09–2,59 g P; 14,03–24,06 g K; 3,86–6,10 g Ca; 1,71–3,33 g Mg; 0,41–1,14 g Na · kg^{-1} s.m.

W przeprowadzonych badaniach stwierdzono we wszystkich próbkach siana małą zawartość fosforu, wapnia i sodu. Niska zawartość tych pierwiastków w roślinach wskazuje na małe nawożenie fosforem i ograniczenie wapnowania użytków zielonych w badanych gospodarstwach. Jedynie zawartość potasu kształtowała się w granicach optymalnej zawartości. Przeprowadzone analizy wskazują na to, iż w badanych gospodarstwach użytki zielone nawożone są gnojowicą, w wyniku czego następuje kumulacja potasu, który jest antagonistą wapnia i magnezu.

Słowa kluczowe: siano, zawartość makroelementów, antagonizm pierwiastków

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Monika RAJKOWSKA[1], Kamila POKORSKA
and Magdalena HOLAK

RELATIONSHIPS BETWEEN MACRO- AND MICROELEMENTS AND HEAVY METALS IN SELECTED ORGANS OF RUDD (*Scardinius erythrophthalmus* L.) FROM LAKES MIEDWIE AND ZELEWKO

ZALEŻNOŚCI POMIĘDZY ZAWARTOŚCIĄ MAKRO- I MIKROELEMENTÓW ORAZ METALI CIĘŻKICH W WYBRANYCH NARZĄDACH WZDRĘGI (*Scardinius erythrophthalmus* L.) Z JEZIOR MIEDWIE I ŻELEWKO

Abstract: The aim of this study was to determine the levels of selected metals (Al, Cu, Fe, Mn, Zn, Ca, K, Mg, Na, Pb, Cd) in gills, gonads, kidneys, livers, skin with scales and muscles of rudd from lakes Miedwie and Zelewko. The relationships between fish sex and metal concentrations in their tissues were also investigated. Na, Zn, Mn, Pb had the highest affinity to the gills, Fe to kidney, Cu to liver, while Al and Ca were accumulated mostly in the skin. Some significant ($p \leq 0.05$) variations in metal concentrations in selected tissues were dependent on fish sex. The data indicate that the rudds from both examined lakes were not polluted with metals. The average content of toxic metals (Cd, Pb) in the muscle tissue of the examined fish were within the limits for fish and fishery products specified by EU Legislation, so the fish were safe for human consumption.

Keywords: macro- and microelements, heavy metals, *Scardinius erythrophthalmus*

Heavy metals from natural and anthropogenic sources are constantly released into aquatic ecosystems. They pose a serious threat to organisms because of their toxicity, long persistence, bioaccumulation and biomagnification in food chains [1]. Trace elements may exert beneficial or harmful effects on plants, animals and humans. These elements get their way into aquatic systems (rivers, lakes or oceans) through atmospheric fallout, dumping wastes, accidental leaks, runoff or terrestrial systems (industrial and domestic effluents) and geological weathering. Fish are located at the

[1] Department of Toxicology, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology in Szczecin, ul. Papieża Pawła VI 3, 71–459 Szczecin, Poland, phone: +48 91 449 65 56, email: Monika.Rajkowska@zut.edu.pl

end of the aquatic food chain, and many accumulate metals and pass them to human beings through food, causing chronic or acute diseases [2]. Rudd (*Scardinius erythrophthalmus,* L.) is a medium-sized cyprinid fish native to European freshwaters. In Poland, usually up to 25–30 cm in length and 300–400 g in weight [3, 4], it is a very popular fish species in Pomeranian lakes. The aim of this study was to compare metal concentrations in rudd from two West Pomeranian lakes: Miedwie and Zelewko, which are linked by the river Plonia. Lake Miedwie is the fifth largest lake in Poland and the second largest in the Province of Western Pomerania. It is a reservoir of drinking water for Szczecin, with its 34.9 km^2 area, 19.3 m mean depth, and 43.8 m maximum depth. The largest tributary to the lake is the river Plonia, inflowing from the south and leaving the lake on its western side to feed Lake Zelewko. Zelewko is a small lake with its 7.5 km^2 area, 3.7 m mean depth, and 6.5 m maximum depth [5].

Material and methods

Samples of rudd of both genders were collected from lakes Miedwie and Zelewko, during autumn 2006. A total of 30 individuals were collected, and kept in a freezer (–20 ^oC) until dissected. To dissect organs, the fish were thawed, their length and weight were measured, and then dissection of organs: gills, gonads, kidneys, livers, muscles and skin was carried out. Gender was determined by inspection of gonads. The tissue samples were placed into clean dry polyethylene bags and frozen at –20 ^oC until analysed. Fish tissue samples of approximately 1 g (except for muscle 2 g) were digested with 3 cm^3 HNO_3 (65 %) in Teflon bombs in a microwave digestion system (MDS 2000). The samples were quantitatively transferred into polypropylene bottles, made up to 20 cm^3 with deionised water. All tissue samples and reagent blanks were prepared in triplicate. Metal concentrations were measured by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) (Cd and Pb) and by Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) (Al, Cu Fe, Mn, Zn, Ca, K, Mg and Na). The accuracy of the methods applied was checked with a certified reference material (Fish paste 2). The results were processed using statistical methods (ANOVA). The analysis involved the Duncan's test at the significance level ($p \leq 0.05$).

Results and discussion

Heavy metals (Pb, Cd, Fe, Mn, Zn, Cu) studied in the present investigations are all regarded as potential hazards to both animal and human health [5]. Pathways of metal uptake by fish may be direct (water via gills) or indirect (diet via the alimentary tract) [6]. The mean concentrations of elements are expressed as μg/g wet weight (Table 1). Metal concentrations in fish organs were dependent on the fishing area. Aluminum, zinc and calcium levels in fish tissues showed no significant differences ($p > 0.05$) amongst lakes. Liver concentrations of the other metals such as: Fe (124.3), Cu (4.20), Na (914.0) and K (1941.0) were higher in Lake Miedwie than in Lake Zelewko (Fe – 47.7; Cu – 1.88; Na – 408.0; K – 902.0) [μg/g w.w.]. Among fish organs, the liver is most often recommended as an environmental indicator of water pollution. This is probably

Table 1

Mean concentrations [μg/g w.w.] and standard deviations of metal in selected organs of rudd (*Scardinius erytrophtalmus*) from Miedwie and Zelewko lakes

Lake	Organs	Al		Zn		Fe		Mn		Cu		Pb		Cd		Ca		Mg		Na		K	
		$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD
Miedwie	gonads	2.8	2.1	62.7	11.6	11.3	1.9	1.4	1.3	1.46	0.87	ns	ns	0.008	0.006	452	568	203	26	629	74	2 254	265
	kidneys	0.6	0.3	251.8	54.0	126.8	11.5	0.5	0.1	0.76	0.22	ns	ns	0.039	0.017	69	89	113	84	701	485	1 396	955
	liver	1.4	0.3	53.3	25.3	124.3	45.3	0.9	0.2	4.20	1.02	ns	ns	0.012	0.009	133	48	209	29	914	42	1 941	121
	muscle	1.5	0.6	10.8	5.3	2.7	0.8	0.1	0.1	0.25	0.11	ns	ns	0.018	0.014	244	120	345	11	482	53	2 281	22
	skin	101.0	29.5	56.0	15.5	4.4	1.8	4.9	2.3	0.52	0.12	ns	ns	0.034	0.026	59 257	28 740	1 528	352	2 021	115	2 258	41
	gill	58.3	3.9	233.0	93.9	39.4	4.1	8.5	2.6	0.65	0.20	0.06	0.07	0.051	0.028	17 308	4 493	999	294	1 249	166	1 773	308
Zeliwo	gonads	0.8	0.6	41.7	17.2	12.6	4.0	1.7	1.7	0.62	0.50	0.01	0.01	0.002	0.000	74	67	95	7	259	22	1 477	276
	kidneys	1.2	0.6	94.2	30.2	119.6	12.8	0.3	0.0	0.44	0.05	0.01	0.01	0.035	0.013	43	8	69	4	357	24	1 151	72
	liver	3.2	1.3	35.1	7.0	47.7	10.8	1.4	0.5	1.88	0.64	0.03	0.03	0.009	0.004	136	162	103	10	408	26	902	90
	muscle	1.3	0.8	4.3	0.9	1.8	1.1	0.1	0.0	0.06	0.05	ns	ns	0.001	0.001	126	38	157	6	201	25	938	32
	skin	110.4	17.3	29.7	11.9	7.4	5.4	9.5	5.5	0.01	0.18	0.03	0.02	0.027	0.023	46 957	31 689	929	419	1 532	783	1 629	779
	gill	45.0	3.6	248.1	44.0	37.7	5.5	16.2	1.6	0.28	0.17	0.26	0.12	0.020	0.009	15 470	2 111	936	58	1 722	71	2 482	110

nd – below in detection limit.

attributed to the tendency of liver to accumulate high levels of various kinds of pollutants from the environment, as previously reported by Galindo et al [7]. In our study, the content of Mn, Pb, Na, K proved to be much higher in the gills of rudd from Lake Zelewko than from Lake Miedwie, while Cd content in gills was higher in rudds from Lake Miedwie (Table 2).

Table 2

Comparison of metal concentrations in organs of rudd (*Scardinius erytrophtalmus*) between Miedwie and Zelewko lakes

Organs	Al	Z	Fe	Mn	Cu	Pb	Cd	Ca	Mg	Na	K
Gonads											
Kidneys											
Liver			M		M					M	M
Muscle											M
Skin				Z					M	M	M
Gill				Z		Z	M			Z	Z

M – higher content of metal in rudd from Miedwie lake; Z – higher content of metal in rudd from Zelewko lake (Duncan's test $p < 0.5$).

In both lakes Na, Zn, Mn and Pb were accumulated mostly in gills, Fe in kidney, Cu in liver, Al and Ca in skin, while K and Cd showed various tendencies, dependent on lakes (Table 1). The highest zinc accumulation in gills was observed also by Amundsen et al [8]. Zinc is an essential element in most metabolic pathways in humans and its deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities [9]. The distribution pattern of Mn in both lakes followed the order: gills > skin > gonads >liver > kidneys > muscle; while Fe followed the sequence: kidneys > liver > gills > gonads > skin > muscle. In the other cases tendencies in metal accumulation were not the same. Most elements occurred in the lowest concentrations in the muscle. Similar observations were reported by Amundsen et al [8]. The differences in metal concentrations in the tissues might have resulted from their different capability to induce metal-binding proteins such as metallothioneins [10]. Metal levels differed ($p < 0.05$) between male and female tissues of rudd from both lakes, indicating that rudd gender influenced metal accumulation (Table 3).

Table 3

Significant differences between metal concentrations in organs of female and male of rudd (*Scardinius erytrophtalmus*) (Duncan's test $p < 0.05$)

Lake	Organs	Al	Z	Fe	Mn	Cu	Pb	Cd	Ca	Mg	Na	K
Miedwie	gonads											
	kidneys										m	
	liver					f						
	muscle											
	skin	m			m				m	m		
	gill		m		m		m			f		

Table 3 contd.

Lake	Organs	Al	Z	Fe	Mn	Cu	Pb	Cd	Ca	Mg	Na	K
Zelewko	gonads					f						m
	kidneys							m				
	liver					m						
	muscle											
	skin	m			m			m	m	m	m	m
	gill						f				m	

f – higher concentrations in female; m – higher concentrations in male.

The average concentrations of Al, Mn, Mg, Ca in the skin of fish from both lakes were found to be significantly higher ($p < 0.05$) in males compared to female fish. Gender dependant differences in metal concentrations might have been influenced by a combination of factors, such as dietary preferences, physiological metabolism in relation to the reproductive cycle stage or foraging behaviour [11]. The highest Cu concentrations were found in the liver tissue – similarly as in the study of Papagiannis et al [12]. Aluminium is not considered to be an essential element in humans. In the Polish Standards there is no information about maximum aluminium levels in fish samples. The average content of toxic elements (Pb, Cd) were relatively low. Especially lead was below the detection limit in most organs of the rudd from Lake Miedwie (Table 1). In the muscle tissue, lead and cadmium were within the limits for fish and fishery products specified by EU Legislation [13, 14], so the fish were safe for human consumption.

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ZALEŻNOŚCI POMIĘDZY ZAWARTOŚCIĄ MAKRO- I MIKROELEMENTÓW ORAZ METALI CIĘŻKICH W WYBRANYCH NARZĄDACH WZDRĘGI (*Scardinius erythrophthalmus* L.) Z JEZIOR MIEDWIE I ŻELEWKO

Katedra Toksykologii, Wydział Nauk o Żywności i Rybactwa
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Abstrakt: Celem pracy było oznaczenie poziomu wybranych metali (Al, Cu, Fe, Mn, Zn, Ca, K, Mg, Na, Pb, Cd) w skrzelach, gonadach, nerce, wątrobie, skórze z łuskami oraz w tkance mięśniowej wzdręg obu płci

z jezior Miedwie i Żelewko (Żelewo). Określono również zależności pomiędzy płcią ryb a zawartością metali w ich tkankach. Największe powinowactwo do nerki wykazywały Na, Zn, Fe, Pb, podczas gdy Al i Ca akumulowały się głównie w skórze. Zaobserwowano istotne ($p < 0,05$) zależności pomiędzy zawartością metali w danym narządzie a płcią ryb. Przeprowadzone badania wskazują, że wzdręgi z obu jezior nie były skażone pod względem zawartości metali, a udział pierwiastków toksycznych (Pb, Cd) w mięśniach był poniżej dopuszczalnej wartości w rybach i rybnych produktach konsumpcyjnych określonych przez prawodawstwo Unii Europejskiej.

Słowa kluczowe: makro- i mikroelementy, metale ciężkie, *Scardinius erythrophthalmus*

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Dariusz ROPEK[1] and Krzysztof FRĄCZEK[2]

EFFECT OF THE SOLID WASTE LANDFILL IN TARNOW ON THE OCCURRENCE OF BENEFICIAL ENTOMOFAUNA ON HORSE BEAN

WPŁYW SKŁADOWISKA ODPADÓW STAŁYCH W TARNOWIE NA WYSTĘPOWANIE POŻYTECZNEJ ENTOMOFAUNY BOBIKU

Abstract: The research on the occurrence of beneficial entomofauna on horse bean was conducted in 2006 and 2007 on plots located in the immediate vicinity of the municipal landfill site in Tarnów. The structure of horse bean entomofauna dominance differed depending on plot localisation with respect to the active landfill sector. A larger proportion of harmful entomofauna was observed on the plots located closest to the active sector, whereas the share of beneficial insects on these plots was smaller.

Keywords: municipal landfill sites, beneficial insects, pests

Landfill sites pose an important economic and social problem. Moreover, the areas adjoining landfills are exposed to various hazards, such as microbiological or chemical pollution leading to degradation of surrounding soils, surface and underground waters or vegetation. Dispersal of gaseous, dust and microbial pollutants in the atmosphere may pose a grave hazard to human life and health [1–3].

Landfill sites may disturb the balance in the environment eg through change of habitat conditions. A large accumulation of various types of waste, including organic one may favour the occurrence of one organism but eliminate the others [4]. It refers to both animal and plant organisms. Landfill sites are a place of habitation of organisms which are troublesome or dangerous for humans, eg rats. Numerous occurrences of *Muscidae*, *Calliphoridae* and *Sarcophagidae* dipterans, which may carry many pathogenic organisms, are also often observed in the vicinity of municipal landfill sites.

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

[2] Department of Microbiology, University of Agriculture, al. A. Mickiewicza 24/28, 30–058 Kraków, Poland, phone: +48 12 662 041 81, email: rrfracze@cyf-kr.edu.pl

Agricultural activity is conducted in the neighbourhood of municipal landfill sites. Due to the character of field crops the balance is frequently disturbed which is visible as a sudden increase in population numbers feeding on cultivated crops. Numerous appearances of pest are often prevented among others by their natural enemies, which are often called beneficial insects. Changes of insect numbers in arable crops may be caused by eg application of chemical plant protection [5–7]. As an effect of pesticide application the number of beneficial insects usually declines, however, not only pesticide application poses a hazard for balance in agrocenoses. Also other factors, such as fertilization, industrial or traffic pollution bring about changes in the numbers of entomofauna. Municipal landfill sites also emit numerous pollutants which may affect organisms settling arable crops but this effect has not been fully investigated yet. Maintaining biodiversity is one of the most important challenges which humans face in the nearest future.

The present research aimed to investigate the occurrence of beneficial entomofauna on horse bean plants in the area immediately adjoining a municipal landfill site.

Material and methods

The research was conducted in 2006 and 2007 in Tarnow. The solid waste landfill site in Tarnow, around which the studies were carried out, is located in the northern city quarter called Krzyz. The landfill site area is surrounded by arable lands, wasteland and forest. Observations were conducted on experimental plots located in the immediate vicinity of the landfill. The experimental points were set up on each side of the landfill in two zones: below 250 m and 250–500 m from its boundaries. Labelling of experimental plots is presented in Table 1. Spring wheat, potatoes and horse bean were cultivated on each 20 m^2 plot. The experiment was set up in four replications. Identical cultivation measures were applied on all plots.

Table 1

Soil sampling sites in the vicinity of the municipal landfill site in Tarnow

Point	Localization of points with respect to landfill site	
	Direction	Zone [m]
W I	West	below 250
W II	West	250–500
N I	North	below 250
N II	North	250–500
E I	East	below 250
E II	East	250–500
S I	South	below 250
S II	South	250–500

Wind from the west dominate in the area of Tarnow city, the mean wind speed is 2.2 m/s, which points to light wind. Detailed wind distribution data are as follows: north

winds – 6 %, north-east winds – 7.1 %, east winds – 16.7 %, south-east winds – 4.8 %, south winds – 14.8 %, south-west winds – 7.4 %, west winds – 22.6 %, north-west winds – 8.8 % and calm air – 11.8 %

Measurements of emission and composition of biogas were conducted in the immediate vicinity of active municipal landfill site in Tarnow in places where plants where cultivated. The measurements were carried out from April 2006 to October 2007 using a device for measuring landfill gas composition – Polytector II G 750 (Germany). The contents of biogas components in the air surrounding the landfill are presented in Table 2. The highest methane concentrations occurred in zone I on the eastern, northern and southern side of the landfill.

Table 2

Mean value of biogas components in the air surrounding the municipal landfill site in Tarnow (from April 2006 to October 2007)

Indicator	Unit	Measuring point							
		S I	S II	E I	E II	N I	N II	W I	W II
Methane (CH_4)	ppm	0.9	0.4	0.7	0.5	0.5	0.0	0.0	0.0
Hydrogen sulfide (H_2S)	ppm	Not registered							
Carbon dioxide (CO_2)	%	0.04	0.04	0.03	0.04	0.04	0.04	0.04	0.04
Oxygen (O_2)	%	20.9	20.9	20.9	20.9	20.9	20.9	20.9	20.9

Immediate observations of entomofauna presence on horse bean plants were conducted every week during vegetation period. Insects were captured on each plot using bucket traps making 10 strokes diagonally on each plot. The insects were captured at the same time on subsequent dates. The collected insects were brought to the laboratory and identified. The Peus scale was used for an assessment of dominance structure of individual insect groups: D-dominants > 5 %, S-subdominants 2.1–5.0 % and A-accessory < 2.1 %.

The results were verified statistically using Statistica programme. Three factor ANOVA was conducted, Newman-Keuls critical intervals were computed and the value of the final step was used for means differentiation at significance level $p < 0.05$.

Results and discussion

Numerous entomofauna occurred in the surrounding of the solid waste landfill site in Tarnow-Krzyz (Table 3). Several thousand specimens were captured on individual plots during the observation period. The most numerous entomofauna was observed on the plots located on the eastern and northern landfill side. The most numerous group of trapped insects consisted of neutral ones, ie species which do not directly affect horse bean plant healthiness. Horse bean pest were also numerous, whereas the least numerous were the so-called beneficial insects, ie these which are the natural enemies of horse bean pests.

Table 3

Occurrence of entomofauna on horse bean plants depending on plot localization with respect to the landfill site

Insects captured on horse bean plants	Year	Number of captured plants [pcs]								$LSD_{p<0.05}$
		Points								
		S		E		N		W		
		I	II	I	II	I	II	I	II	
Captured insects total	2006	4521	4680	4629	4592	4724	4579	4329	4555	
	2007	4469	4576	5116	4948	5222	4927	5041	4811	
	Mean	4495	4628	4872	4770	4973	4753	4685	4683	*324.4*
Neutral insects	2006	2470	2504	2122	2073	2200	2130	2174	2354	
	2007	2337	2367	2319	2365	2449	2335	2505	2445	
	Mean	2403	2435	2220	2219	2324	2232	2339	2399	*272.6*
Harmful insects	2006	1405	1537	2025	1987	2022	1935	1579	1571	
	2007	1526	1582	2193	1946	2179	1971	1878	1684	
	Mean	1465	1559	2109	1966	2100	1953	1728	1627	*137.7*
Beneficial insects	2006	646	639	482	532	502	514	576	630	
	2007	606	627	604	637	594	621	658	682	
	Mean	626	633	543	584	548	567	617	656	*54.4*

Among the beneficial entomofauna the most numerous were the representatives of *Heteroptera* and *Coccinelidae* beetles (Table 4). The representatives of *Chrysomelidae* and *Syrphidae* family were less numerous. Also single specimens from *Cantharidae* family were trapped. The greatest number of *Heteroptera* was captured on the plots on the western side of the landfill in zone II and the smallest on plots localized in zone I on the eastern side in the immediate vicinity of the active landfill sector.

Neutral insects prevailed on all plots, irrespective of their location with respect to the landfill (Table 5). They had the greatest share in the dominance structure on horse bean plots located on the southern and western side of the landfill. Harmful insects were almost as numerous as the neutral ones on the horse bean plots on the eastern and northern side of the landfill. Obviously a lower share of harmful insects in the dominance structure was observed on horse bean plots situated at a long distance from the active landfill sector. Beneficial insects had the smallest share in the dominance structure, usually between 10 and 14 %. They had the greatest share on the plots on the southern and western side of the landfill.

The analysis of the dominance structure of beneficial entomofauna groups (Table 6) points to a prevalence of two groups, ie *Coccinelidae* beetles and *Heteroptera*. The latter dominated in horse bean cultivation on the northern side of the landfill in the first zone. A considerable proportion of *Coccinelidae* beetles was undoubtedly connected with numerous aphids settling horse bean plants. Also for *Chrysomelidae* or *Syrphidae* aphids are the basic source of food [8]. The *Cantharidae* and *Chrysomelidae* population numbers did not differ significantly between the analyzed plots. On the other hand, the numbers of *Coccinelidae* beetles, *Syrphidae* or *Heteroptera* were significantly smaller on the plots situated closest to the active landfill sector. This evidences a stronger sensitivity of this group of beneficial entomofauna to pollution emitted by the municipal landfill site.

Municipal landfills are not only the places of waste accumulation but also, as indicated by Chan et al [4] they may provide comfortable living conditions for many insect species. Gaseous and microbial pollutants which are emitted from the landfill may affect the adjoining area, as in [3]. Because of the localisation, the plots situated on the eastern and northern side of the landfill are the most exposed to the emission effect. It is connected both with east wind prevailing in this area and with the fact that the active sector is located in the north-eastern part of the landfill. The highest methane concentrations were registered on these plots. The gas originates on municipal waste landfill sites and moves to the adjoining terrains with air currents. A greater proportion of harmful entomofauna was registered on the plots located in these places. Also other authors point to a negative effect of air pollution on the composition of entomofauna of cultivated crops [8–11]. On the other hand the share of beneficial insects was smaller despite the fact that the number of beneficial insects captured on all plots was similar. This evidences a lower efficacy of pest natural enemies in reducing their population numbers.

Table 4

Occurrence of entomofauna on horse bean plants depending on plot localization with respect to the landfill site

Taxon	Year	Number of captured plants [pcs]								$LSD_{p<0.05}$
		Points								
		S		E		N		W		
		I	II	I	II	I	II	I	II	
Heteroptera	2006	169	151	135	147	153	144	156	190	
	2007	175	169	175	176	186	172	192	206	
	Mean	172	160	155	161.5	169.5	158	174	198	*23.2*
Coccinellidae	2006	190	196	146	155	133	154	183	171	
	2007	167	174	174	171	150	176	181	173	
	Mean	178.5	185	160	163	141.5	165	182	172	*20.1*
Cantharidae	2006	21	27	15	17	16	16	17	20	
	2007	23	23	21	23	22	20	24	25	
	Mean	22	25	18	20	19	18	20.5	22.5	*n.s.*
Chrysomelidae	2006	32	36	17	29	25	25	29	31	
	2007	38	38	36	42	34	37	41	36	
	Mean	35	37	26.5	35.5	29.5	31	35	33.5	*n.s.*
Syrphidae	2006	59	62	34	44	31	39	38	48	
	2007	57	56	41	46	37	54	46	55	
	Mean	58	59	37.5	45	34	46.5	42	51.5	*13.2*
Other	2006	175	167	135	140	144	136	153	170	
	2007	146	167	157	179	165	162	174	187	
	Mean	160.5	167	146	159.5	154.5	149	163.5	178.5	*n.s.*

n.s. – non-significant differences.

Table 5

Structure of entomofauna dominance [%]

Insects captured on horse bean plants	Year	Proportions of individual insect groups								$LSD_{p<0.05}$
		Points								
		S		E		N		W		
		I	II	I	II	I	II	I	II	
Neutral insects	2006	54.6	53.5	45.8	45.1	46.5	46.5	50.2	51.6	
	2007	52.2	51.7	45.3	47.7	46.8	47.3	49.6	50.8	
	Mean	53.4	52.6	45.6	46.4	46.7	46.9	49.9	51.2	*3.33*
Harmful insects	2006	31.0	32.8	43.7	43.2	42.8	42.2	36.4	34.4	
	2007	34.1	34.5	42.8	39.3	41.7	40.0	37.2	35.0	
	Mean	32.6	33.7	43.3	41.3	42.3	41.1	36.8	34.7	*2.60*
Beneficial insects	2006	14.2	13.6	10.4	11.5	10.6	11.2	13.3	13.8	
	2007	13.5	13.7	11.8	12.8	11.3	12.6	13.0	14.1	
	Mean	13.9	13.7	11.1	12.2	11.0	11.9	13.2	14.0	*1.22*

Table 6

Structure of beneficial entomofauna group dominance [%]

Taxon	Year	Proportion of insects from individual taxons								$LSD_{p<0.05}$
		Points								
		S		E		N		W		
		I	II	I	II	I	II	I	II	
Heteroptera	2006	26.2	23.6	28.0	27.6	30.5	28.0	27.1	30.2	
	2007	28.9	27.0	29.0	27.6	31.3	27.7	29.2	30.2	
	Mean	27.5	25.3	28.5	27.6	30.9	27.9	28.1	30.2	*3.66*
Coccinelidae	2006	29.4	30.7	30.3	29.1	26.5	30.0	31.8	27.1	
	2007	27.6	27.8	28.8	26.8	25.3	28.3	27.5	25.4	
	Mean	28.5	29.2	29.5	28.0	25.9	29.2	29.6	26.3	*3.01*
Cantharidae	2006	3.3	4.2	3.1	3.2	3.2	3.1	3.0	3.2	
	2007	3.8	3.7	3.5	3.6	3.7	3.2	3.6	3.7	
	Mean	3.5	3.9	3.3	3.4	3.4	3.2	3.3	3.4	*n.s.*
Chrysomelidae	2006	5.0	5.6	3.5	5.5	5.0	4.9	5.0	4.9	
	2007	6.3	6.1	6.0	6.6	5.7	6.0	6.2	5.3	
	Mean	5.6	5.8	4.7	6.0	5.4	5.4	5.6	5.1	*n.s.*
Syrhpidae	2006	9.1	9.7	7.1	8.3	6.2	7.6	6.6	7.6	
	2007	9.4	8.9	6.8	7.2	6.2	8.7	8.1	4.6	
	Mean	9.3	9.3	6.9	7.7	6.2	8.1	7.3	6.1	*2.20*
Other	2006	27.1	26.1	28.0	26.3	28.7	26.5	26.6	27.0	
	2007	24.1	26.6	26.0	28.0	27.8	26.1	26.4	27.4	
	Mean	25.6	26.4	27.0	27.2	28.2	26.3	26.5	27.2	*n.s.*

n.s. – non-significant differences.

Conclusions

1. Horse bean plants growing in the immediate vicinity of the active landfill sector are settled to a greater degree by harmful entomofauna.

2. Numbers of beneficial insect populations on plots were similar irrespective of their localisation with respect to the landfill.

3. In the area most exposed to the effect of active landfill sector the share of beneficial and negative insects was declining.

4. Beneficial representatives of *Coccinelidae, Heteroptera* and *Syrphidae* were more sensitive to the negative effect of pollutants originating from the landfill.

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WPŁYW SKŁADOWISKA ODPADÓW STAŁYCH W TARNOWIE NA WYSTĘPOWANIE POŻYTECZNEJ ENTOMOFAUNY BOBIKU

[1] Katedra Ochrony Środowiska Rolniczego
[2] Katedra Mikrobiologii
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstract: Badania nad występowaniem entomofauny pożytecznej bobiku przeprowadzono na poletkach zlokalizowanych w bezpośrednim sąsiedztwie składowiska odpadów komunalnych w Tarnowie w 2006 i 2007

roku. Struktura dominacji entomofany bobiku różniła się w zależności od lokalizacji poletek względem czynnego sektora składowiska. Na poletkach zlokalizowanych najbliżej czynnego sektora zaobserwowano większy udział szkodliwej entomofauny. Natomiast udział owadów pożytecznych w tych lokalizacjach był mniejszy.

Słowa kluczowe: składowiska odpadów komunalnych, owady pożyteczne, szkodniki

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Paweł SKONIECZEK[1, 2] and Józef KOC[2]

ROLE OF RETENTION RESERVOIR IN SODIUM MIGRATION FROM AGRICULTURAL AND AFFORESTED CATCHMENT AREAS

ROLA ZBIORNIKA RETENCYJNEGO W MIGRACJI SODU ZE ZLEWNI ROLNICZO-LEŚNYCH

Abstract: The analisis of the role of retention reservoir in sodium migration from agricultural and afforested catchment areas and wastewater treatment plants was presented. The study was conducted on a stream with a pond at its outlet. For technical purposes, water was partially directed via a band ditch. The investigated site is situated in north-eastern Poland, in the Olsztyn Lakeland mesoregion. Detailed investigations of surface waters were carried out over a period of 3 years.

The highest sodium concentrations were noted in treated effluents (84.19 and 96.64 mg Na · dm^{-3} on average), lower levels were found in the outflows from agricultural catchments (7.54 to mg Na · dm^{-3} to 11.55 mg Na · dm^{-3}), while the lowest – in the outflows from afforested catchments (3.77 to 4.52 mg Na · dm^{-3}). In the outflow from the retention reservoir, sodium concentrations reached 7.87 mg Na · dm^{-3}, and in the outflow from the band ditch – 8.90 mg Na · dm^{-3}. Total sodium concentrations in water flowing from the catchments to Lake Wulpinskie was 8.09 mg Na · dm^{-3}. A total of 4706 kg sodium was discharged into the lake each year, which means that flow per ha of the catchment area was 3.4 kg Na. The retention reservoir significantly reduced the total sodium load flowing from the catchments into the lake. The main role of the reservoir was to equalize sodium concentrations. The sodium load was reduced by approximately 50 %.

Keywords: load reduction, sodium, stream, pond

Sodium is one of the most abundant elements on earth and it comprises 2.63 % of the earth's crust. Sodium is found in water in the form of various compounds, mostly chlorides (NaCl), and less frequently, sulfates (Na_2SO_4), carbonates ($NaHCO_3$ and Na_2CO_3) and nitrates ($NaNO_3$). The above compounds are highly water-soluble. The sodium content of natural water ranges from several grams to 30 g · m^{-3}. Underground

[1] Plant Breeding and Acclimatization Institute – National Research Institute, Division in Bydgoszcz, al. Powstańców Wielkopolskich 10, 85–090 Bydgoszcz, Poland, phone: +48 52 581 69 20, fax: +48 52 322 44 54, email: p.skonieczek@ihar.bydgoszcz.pl

[2] Department of Land Improvement and Environmental Management, University of Warmia and Mazury in Olsztyn, pl. Łódzki 2, 10–718 Olsztyn, Poland, phone: +48 89 523 38 97, fax: +48 523 39 92, katemel@uwm.edu.pl

water may contain up to 100 g · m^{-3} sodium, while sea and ocean water – up to 10 kg · m^{-3}. In sea water, sodium ions account for around 48 % of total cation mass [1, 2].

The presence of sodium bicarbonate is responsible for the sodium alkalinity of water. Water containing high levels of this compound is not fit for use in the pharmaceutical industry, the brewing industry or in the production of plant tannins. Sodium compounds found in fresh water are insignificant from the technical and industrial perspective, and they do not pose a health hazard. In certain circumstances, sodium salts occurring in the form of sodium nitrate(III) and (V) may be a measure of water pollution if supplied from municipal effluents or fertilized soil.

In naturally occurring water, sodium originates from the hydrolytic decomposition of minerals (plagioclase, nephelite, albite and others), magmatic rock weathering and leaching of sedimentary rocks containing sodium salts. Pollution is an additional source of sodium in surface waters. Sodium is used in households in the form of kitchen salt and it has numerous applications in various industries, including paper, glass, chemical, food processing industries, as well as medicine. The largest quantities of sodium are evacuated with industrial effluents from soda and salt plants, mineral salt processing plants and dye factories. Sodium chloride is used in de-icing roads in the winter. Every day, man excretes 5 g of sodium which reaches surface water deposits via municipal effluents. Sodium is difficult to remove because it does not form insoluble compounds with substances used in the treatment of water, while it participates in sorptive processes. Therefore, in most part, sodium is discharged with treated effluent water.

The chemical composition of substances dissolved in the waters of agricultural and afforested areas is determined by geochemical conditions in the catchment, biological processes and human activity. In afforested areas where the above pollution sources are negligent, the composition of water-borne compounds is determined by geochemical conditions and phytocenosis. In agricultural areas, geochemical conditions are difficult to separate from farming and household sources of pollution. The volume of sodium outflows from the catchment is determined mainly by land relief, vegetation cover, soil cohesion, farming intensity and treated effluents. Water flow through a preliminary reservoir on a stream reduces point-source pollution (effluents from sewage treatment plants) as well as surface washings from agricultural and afforested areas [3, 4].

The objective of this study was to determine the sodium load discharged with water from the agricultural and afforested catchment, and to investigate the role of retention reservoir in reducing sodium concentrations and loads in surface waters contaminated with treated effluents.

Materials and methods

The study was carried out in the Olsztyn Lakeland, and it covered three hydrological years. The investigated site was the Szabruk stream with a catchment area of 13.2 km^2, occupied by forests (33 %) and farmland (67 %). The catchment features two residential estates (population of 607), each with a wastewater treatment plant (no. 1 and no. 2), as well as scattered buildings with approximately 40 residents with no wastewater treatment option.

For the needs of the study, water evacuated via the Szabruk stream from afforested areas, water evacuated via drains from agricultural areas and wastewater were sampled for analyses. Water flow measurements were performed with the use of an electromagnetic flow meter. Samples were collected at 13 test sites (Table 1, Fig. 1).

Table 1

Water sampling sites

Site no.	Location (Fig. 1)
1 and 2	Inflow from the afforested catchment
3	Stream above the inflow from the wastewater treatment plant in Unieszewo
4	Inflow from the wastewater treatment plant in Unieszewo
5	Inflow from the agricultural catchment with housing
6	Szabruk stream above the inflow from the wastewater treatment plant in Szabruk
7	Inflow from the wastewater treatment plant in Szabruk
8	Stream below the wastewater treatment plant in Szabruk during the effluent discharge period
9	Drain flow from the agricultural catchment to the band ditch
10	Band ditch at stream inlet below the pond
11	Drain flow from the agricultural catchment to the pond
12	Inflow from the pond to the stream
13	Outflow to the lake

Water flow at test sites on the Szabruk stream was characterized by high variability. In general, water flow was intensified in the spring thaw period. The pond can act as a flow regulator because its structure minimizes rapid flow fluctuations. It is also capable of accepting large quantities of water and evacuating it to the stream with a delay.

Water runoffs at each flow measurement site differed throughout the period of the study (Fig. 2).

In the group of agricultural catchments, the most intensive flow per ha of catchment area was noted in the smallest catchment (82.4 ha, test site no. 11) feeding into the pond at 3.1 $dm^{-3} \cdot s^{-1} \cdot km^{-2}$. Size is not the only characteristic feature of this catchment. It features a deep and drained hollow from which water is evacuated via a drain pipeline directly to the pond.

Runoff per area unit was most variable in drained agricultural catchments (no. 9 and 11), while the highest average runoff was observed in the afforested catchment (no. 1) at 3.9 $dm^{-3} \cdot s^{-1} \cdot km^{-2}$, ie slightly below the characteristic values for the Masurian Lakeland [5].

The outflows from the wastewater treatment plant in Unieszewo supplied 7.8 m^3 of pre-treated effluents daily at a rate of 0.09 $dm^{-3} \cdot s^{-1}$. The inflow of treated sewage did not significantly affect the flow rate. The treatment plant in Szabruk discharged an average of 25 m^3 of treated sewage per session at a rate of 0.29 $dm^{-3} \cdot s^{-1}$. There were two discharge sessions per day, each lasting 30 minutes. Treated wastewater was evacuated to an indirect ditch which significantly delayed the time of reaching the

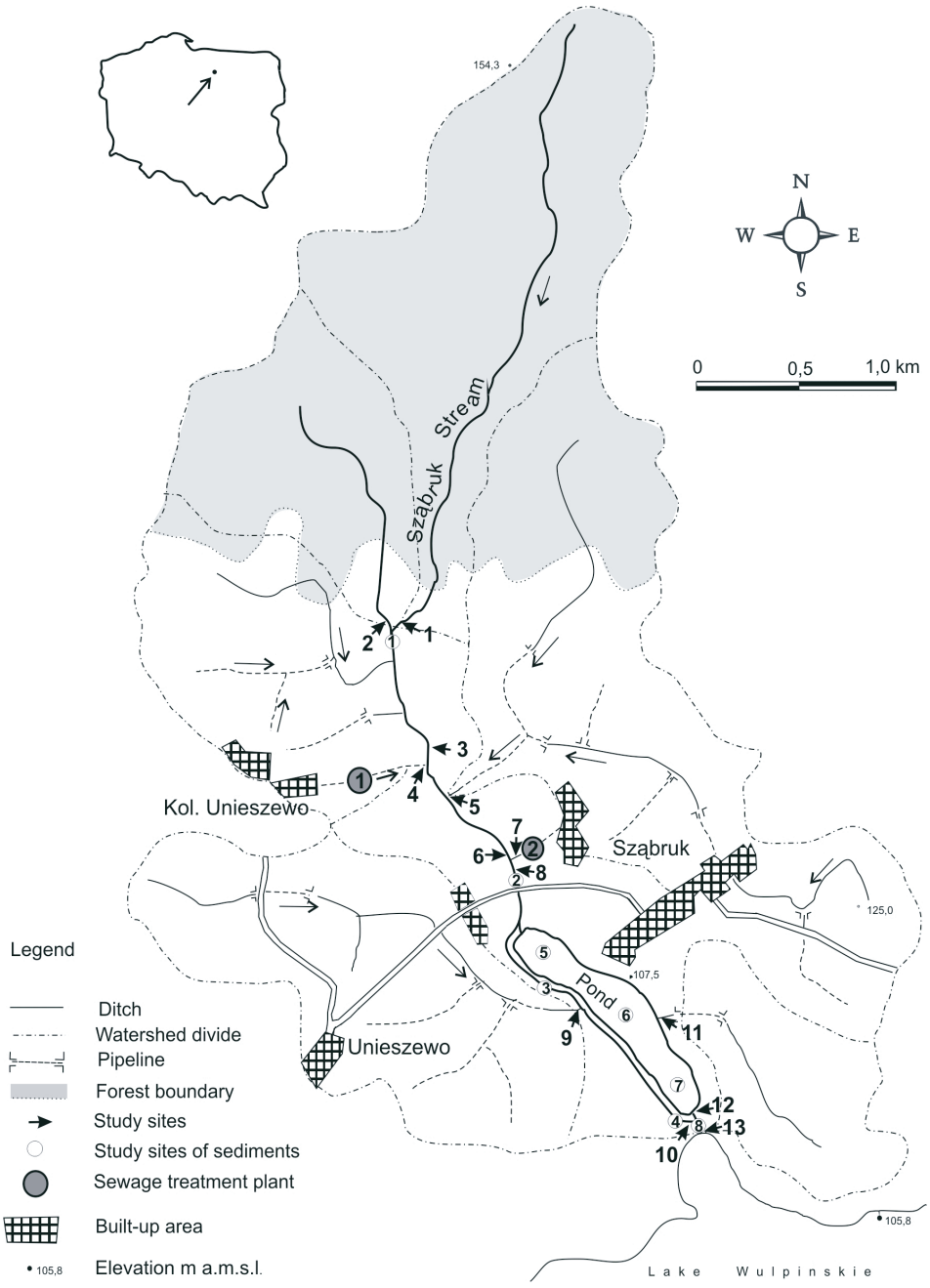

Fig. 1. Location of test sites in the catchment of the Szabruk stream

Szabruk stream. As a result, the waste was diluted and it did not visibly increase the flow in the stream, thus supporting the self-purification process. The total volume of treated wastewater fed by both treatment plants into the Szabruk stream was estimated at 58 $m^3 \cdot day^{-1}$, ie 0.38 $dm^{-3} \cdot s^{-1}$, and it did not have a significant impact on the volume of water flowing in the stream. The main inflow of treated wastewater to the stream was observed in the area of test site no. 8. In view of the flow rate determined at the above site at 26.85 $dm^{-3} \cdot s^{-1}$, the duration of the discharge session and the volume of discharged waste, the treated wastewater had a 2.5 % share of instantaneous flow and accounted for 33 % during discharge.

Water samples were collected once a month. Flow measurements were performed during sample collection. Sodium (Na^+) concentrations were determined by atomic emission spectrophotometry in water samples, and by colorimetry in vegetation and deposit samples. Analyses were conducted in accordance with the universally accepted methodology [6].

Results

The process of sodium flow through water bodies under natural conditions is highly complex, it varies over time and, therefore, it is difficult to observe.

Table 2

Sodium concentrations in the investigated catchments

Type of water	Site no.	Na^+ concentrations [$mg \cdot dm^{-3}$]			Na^+ load [$mg \cdot s^{-1}$]
		Average	Range	±SD	
Inflow from the afforested catchment	1	3.77	2.50–4.80	0.50	36.7
Inflow from the afforested catchment	2	4.52	2.00–5.60	0.71	20.4
Stream above the inflow from the wastewater treatment plant in Unieszewo	3	4.22	2.10–5.60	0.83	69.1
Inflow from the wastewater treatment plant in Unieszewo	4	84.19	23.30–158.0	37	7.6
Inflow from the agricultural catchment with housing	5	8.23	4.40–53.20	9.7	54.3
Stream above the inflow from the wastewater treatment plant in Szabruk	6	7.14	4.40–23.50	3.38	191.8
Inflow from the wastewater treatment plant in Szabruk	7	96.63	12.60–172.7	40	29.0
Stream below the wastewater treatment plant in Szabruk during the sewage discharge period	8	39.19	4.80–99.00	27	1052.3
Stream below the wastewater treatment plant in Szabruk (daily average)	8’	8.48	5.22–27.92	8.9	227.7
Drain flow from the agricultural catchment to the band ditch	9	11.55	4.00–24.8	6.0	66.7
Band ditch at stream inlet below the pond	10	8.90	2.90–12.90	1.94	136.6
Drain flow from the agricultural catchment to the pond	11	7.54	1.80–15.80	3.0	26.8
Inflow from the pond to the stream	12	7.87	4.80–12.40	1.81	24.4
Outflow to the lake	13	8.09	2.50–12.00	1.75	148.9

±SD – standard deviation.

The dynamics of those processes is determined by fluctuations in the factors determining the volume of external load as well as changes taking place inside the water bodies. The concentrations of sodium evacuated to surface waters in afforested and agricultural areas, and sodium levels in pre-treated sewage discharged from two residential sewage treatment plants were determined (Table 2).

Sodium concentrations in the inflows feeding into the Szabruk stream varied subject to weather conditions and, above all, the supply source. Inflows from afforested catchments were characterized by very low sodium levels. Despite the lowest average sodium concentrations (3.77 and 4.52 $Na^+ \cdot dm^{-3}$), inflows from afforested catchments were also marked by the lowest fluctuations in sodium levels throughout the year (Fig. 2).

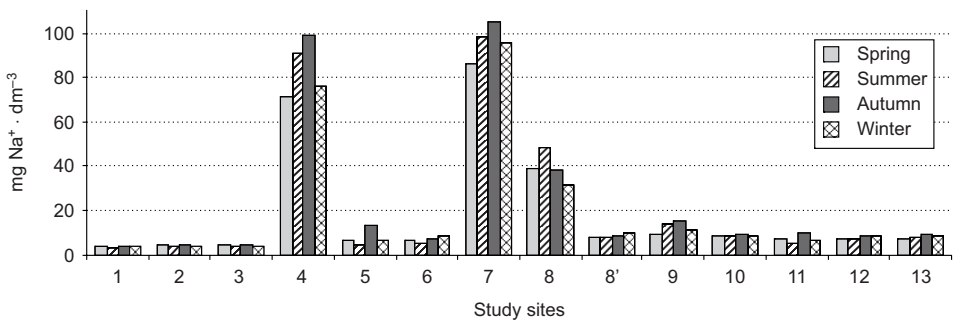

Fig. 2. Seasonal variability in sodium concentrations at each test site in mg · dm^{-3}. Test sites are described in Table 1

Inflows from agricultural catchments supplied the highest sodium load in the autumn, ranging from 10.52 $Na^+ \cdot dm^{-3}$ (directly to the pond) to 15.10 $Na^+ \cdot dm^{-3}$ (to the band ditch). Outflows from agricultural catchments feeding into the stream and into the pond carried nearly twice more sodium, and inflows to the band ditch – more than twice the sodium load supplied by afforested catchments in every season of the year. Sodium levels in surface waters in the investigated catchment were lower than in areas marked by intensive farming production [7, 8].

The effluents discharged by two residential treatment plants showed high sodium concentrations. At both treatment plants, the lowest sodium ion levels were noted in the spring: 70.87 $Na^+ \cdot dm^{-3}$ in the Unieszewo plant and 86.31 $Na^+ \cdot dm^{-3}$ in the Szabruk plant. Similarly to agricultural catchments, the highest sodium concentrations were observed in the autumn at 99.09 and 105.04 mg $Na^+ \cdot dm^{-3}$, respectively (Fig. 2). Sodium concentrations were lower in the effluents from the treatment plant in Unieszewo than in the sewage discharged from the Szabruk facility due to the applied sewage treatment process. The Unieszewo plant relies on three filter beds and two reservoirs. Sewage from the treatment plant continuously feeds into the stream by overflow and via an underground pipeline. Wastewater treated at the Szabruk plant is aerated by Kessener brushes. The plant operates periodically with two pauses during which wastewater is clarified and discharged to the stream. Both treatment plants process domestic sewage.

Inflows from the treatment plants were significantly diluted in the stream. Effluents from the treatment plant in Unieszewo and inflows from the agricultural catchment with residential housing decreased water quality and increased sodium levels by 32 %. Effluents from the Szabruk plant raised sodium concentrations 4.5-fold during the sewage discharge session and by 19 % on a daily basis on average. Treated sewage from Szabruk dramatically decreased water quality, in particular as regards biogenic element supplies, leading to oxygen deficit [9–11].

A rapid increase in sodium concentrations was observed in the Szabruk stream mostly during sewage discharge sessions from the Szabruk treatment plant. The sodium content of the pond was reduced by more than 7 % on average per year. Sodium concentrations in the ditch band increased by nearly 5 % over the same period, mainly due to inflows from the agricultural catchment which supplied an average of 11.55 $Na^{+} \cdot dm^{-3}$.

An analysis of the investigated parameters at each test site showed that despite an increase in catchment area and the inflow of pollutants, sodium concentrations at stream outlet to the lake were lower than at the point of wastewater inflow from the Szabruk treatment plant (Table 2). The load discharged from the treatment plant and evacuated from the catchments was reduced in the preliminary reservoir. It can be concluded that during passage through the pond with an area of 24.8 ha, the sodium load of water polluted with pre-treated sewage became accumulated in bottom deposits and littoral vegetation [12].

In the overall sodium balance (water flow times Na concentrations), treated effluents had a 15 % share, afforested catchments – a 24 % share, while agricultural catchments – the highest 61 % share of sodium load fed into the stream. In the course of three experimental years, the catchment fed an average of 6355 kg sodium into the pond per year. Sodium outflow from the pond accounted for 12 % of its inflow, therefore, vast quantities of this element were retained in the water body. The sodium load passing through the pond was reduced by 88 %. Such a significant reduction of sodium load probably resulted from intense percolation of water from the pond to the band ditch due to an altitude difference of around 140 cm between the pond outflow and the terminal part of the band ditch. For this reason, the sodium load balance was determined collectively for the pond and the band ditch. In the overall balance, the total reduction in sodium concentrations passing through the pond and the band ditch reached 54 %, despite the inflow from agricultural catchments.

Sodium levels in the biomass of littoral vegetation were generally in a range of 0.03–0.49 % DMA (dry matter accumulation, air-dry basis). Significant variations were reported between different vegetation sampling sites, and a 16-fold difference between the lowest and the highest sodium concentrations was noted. Vegetation samples collected in the direct proximity of the inflow were marked by the highest sodium levels, and the sodium content of water decreased with distance from the main supply source. A repeated increase in sodium concentrations in littoral plants was observed in the terminal part of the pond due to inflows from the agricultural catchment. Pond vegetation accumulated 318 Na on average, ie 31 kg Na per ha.

The thickness of bottom deposits in the stream did not exceed 10 cm, and in the pond deposits were formed in the course of 25 years and their thickness was determined by pond depth. The thickest deposits of up to 20 cm were observed in the deepest point of the pond. Their thickness gradually decreased towards shallower parts of the pond, and layers of 0–5 cm occupied the largest pond area of 11.9 ha. For this reason, this layer contained the highest sodium load. An increased sodium content of bottom deposits was observed below points of intensified sodium supply, ie the treatment plant in Szabruk, at 475 mg · kg^{-1} DMA, and the inflow from the agricultural catchment to the band ditch where the highest sodium content was noted at 653 mg · kg^{-1} DMA (Table 3). The investigated water body accumulated 2515 tons of bottom deposits with 1097 kg Na, ie 101 Mg of deposits with 44.3 kg Na per one hectare of pond area.

Table 3

Sodium concentrations in bottom deposits [mg · kg^{-1} DMA]

No.	Sampling site	Na^+ in layers of 0–10 cm	Na^+ in layers of 10–20 cm
1	At point of stream connection below the forest	134	—
2	Below the wastewater treatment plant in Szabruk	475	—
3	Band ditch	230	—
4	Terminal part of the band ditch	653	—
5	Pond-beginning	341	223
6	Pond-middle	549	549
7	Pond-end	564	564
8	At point of connection with pond outflow	490	—

— deposit thickness did not exceed 10 cm.

Conclusions

1. Effluents discharged by small rural treatment plants supply higher sodium concentrations than agricultural and afforested areas, but agricultural catchments are responsible for the highest sodium load introduced to water bodies.

2. Water flow through the retention reservoir reduces the concentrations and load of sodium which becomes accumulated in littoral vegetation and bottom deposits.

3. Pond vegetation accumulated 31 kg Na/ha. Reed canary grass (*Phalaris arundinacea* L.) was the predominant species of littoral vegetation in the studied pond.

4. Bottom deposits with a thickness of 5 to 20 cm, 7.7 cm on average, were formed in the course of the pond's life of 25 years. On average, 101 Mg of deposits containing 44.3 kg Na were accumulated per ha of pond area.

5. The construction of a pond on the watercourse fed with pre-treated effluents improved water quality. Sodium concentrations were reduced by 7 % on annual average. The reduction of sodium load passing through the pond and the band ditch reached 54 %.

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ROLA ZBIORNIKA RETENCYJNEGO W MIGRACJI SODU ZE ZLEWNI ROLNICZO-LEŚNYCH

[1] Instytut Hodowli i Aklimatyzacji Roślin – Państwowy Instytut Badawczy
Oddział w Bydgoszczy
[2] Katedra Melioracji i Kształtowania Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

Abstrakt: Praca zawiera wyniki badań dotyczące znaczenia zbiornika retencyjnego w migracji sodu dopływającego ze zlewni leśnych, rolniczych oraz oczyszczalni ścieków. Obiektem badań był ciek, na ujściu którego zbudowano staw. Ze względów technicznych część wód kierowano rowem opaskowym. Obiekt badań położony jest w północno-wschodniej Polsce, w mezoregionie Pojezierza Olsztyńskiego. Badania szczegółowe wód powierzchniowych prowadzono 3 lata.

W wyniku przeprowadzonych badań stwierdzono, iż największe stężenie sodu występuje w odpływach z oczyszczalni ścieków średnio 84,19 i 96,64 mg Na · dm^{-3}, na odpływie ze zlewni rolniczych średnio od 7,54 mg Na · dm^{-3} do 11,55 mg Na · dm^{-3}, najmniejsze zaś ze zlewni leśnych 3,77 i 4,52 mg Na · dm^{-3}. Na odpływie ze zbiornika retencyjnego stężenie sodu wynosiło 7,87 mg Na · dm^{-3}, natomiast z rowu opaskowego 8,90 mg Na · dm^{-3}. Całkowite stężenie sodu w wodzie odpływającej ze zlewni do Jeziora Wulpińskiego wynosiło 8,09 mg Na · dm^{-3}. Do jeziora spływało rocznie 4706 kg sodu, co dało spływ jednostkowy 3,4 kg Na z 1 ha zlewni. Zbiornik retencyjny w znacznym stopniu zmniejszał ilość sodu odpływającego ze zlewni do jeziora. Główna jego rola polegała na uśrednianiu stężeń. Redukcja ładunku sodu wynosiła ok. 50 %

Słowa kluczowe: redukcja ładunku, sód, ciek, staw

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Ewa STANISŁAWSKA-GLUBIAK[1]
and Jolanta KORZENIOWSKA[1]

TOLERANCE OF WHITE MUSTARD (*Sinapsis alba* L.) TO SOIL POLLUTION WITH SEVERAL HEAVY METALS

TOLERANCJA GORCZYCY BIAŁEJ NA SKAŻENIE GLEBY WYBRANYMI METALAMI CIĘŻKIMI

Abstrakt: A strict experiment on simulated copper, zinc and nickel soil contamination has been performed in concrete-framed microplots, 1 m^3 in capacity.

White mustard proved to be the most sensitive to nickel contamination of soil and most tolerant to excess copper in the substrate. The concentration of nickel in aerial parts of white mustard increased up to 30-fold, whereas the level of copper was twice as much as in the control. The translocation factors computed for the analysed metals in plants showed that copper was the least transferable from roots to shoots and, as its level rose, increasing quantities of this metal were retained in roots. At higher rates of nickel pollution in soil, white mustard transferred more of this metal to shoots. White mustard is only suitable for phytostabilisation of soils moderately contaminated with copper.

Keywords: soil pollution, Cu, Ni, Zn, phytoremediation, *Sinapsis alba* L.

Phytoremediation, ie recultivation treatments involving plants, is one of the measures taken to remediate soils polluted with heavy metals. One of the phytoremediation techniques is phytostabilisation, which relies on planting contaminated land with plants which are tolerant to high concentrations of toxic substances and can transport considerable amounts of pollutants to their aerial organs. The purpose of phytostabilisation is to lower bioavailability of contaminants occurring in soil, to protect the contaminated soil from further degradation and to reduce the risk of immediate contact of humans and animals with contaminants. Another phytoremediation technique, which attracts much attention and is an object of intensive worldwide research is phytoextraction, ie removal of heavy metals from polluted soils. In this process, plants take up heavy metals through their roots and transport them to shoots, where the pollutants are accumulated. Then the biomass is harvested and processed. For phytoextraction to

[1] Institute of Soil Science and Plant Cultivation – National Research Institute in Pulawy Department of Weed Science and Tillage Systems in Wrocław, ul. Orzechowa 61, 50–540 Wrocław, Poland, phone: +48 71 71 363 87 07, email: e.glubiak@iung.wroclaw.pl

be effective, plants must be highly tolerant to high levels of heavy metals in soil and be able to absorb large amounts of such pollutants per surface area unit. High uptake of metals, in turn, depends primarily on an appropriately high green matter harvest or a very high concentration of a given element in the plant, that in on the level of the so-called hyperaccumulation. Much research is conducted with an aim of finding plant species that will fulfil these conditions, including studies on plants belonging to the family *Brassicaceae* [1–5], which are considered to be tolerant to excessive quantities of heavy metals in substratum, although their tolerance to particular metals varies [6]. This paper discusses tolerance to white mustard (*Sinaspis alba* L.), of the family *Brassicaeae*, to soil contamination with copper, nickel and zinc.

Material and methods

A strict microplot experiment, designed as completely randomised trials with 4 replications, has been conducted on three white mustard cultivars: Barka, Rota and Tango. Concrete-framed 1 × 1 × 1 m microplots, set below the ground level, were filled, in the top 0–30 cm horizon, with Haplic Luvoisols soil (pH_{KCl} = 5.5; fraction < 0.02 mm: 16 %; C_{org}: 0.8 %), containing 75 mg · kg^{-1} P i 160 mg · kg^{-1} K and 50 mg · kg^{-1} Mg.

Simulated soil contamination with copper, zinc and nickel was applied according to the following design: 1) control, 2) Cu_1 – 50 mg · kg^{-1}, 3) Cu_2 – 100 mg · kg^{-1}, 4) Cu_3 – 200 mg · kg^{-1}, 5) Zn_1 – 200 mg · kg^{-1}, 6) Zn_2 – 400 mg · kg^{-1}, 7) Zn_3 – 800 mg · kg^{-1}, 8) Ni_1 – 40 mg · kg^{-1}, 9) Ni_2 – 80 mg · kg^{-1}, 10) Ni_3 – 160 mg · kg^{-1}. The metals in the form of sulphates were dissolved in water and applied to plots using a watering can. The metals were first introduced to the 15–30 cm layer and mixed with soil. Next, they were added to the 0–15 cm layer and also thoroughly mixed. White mustard was sown after 3 weeks and grown until the early flowering stage.

After harvesting the plants, average plot samples of shoots and roots of plants from each cultivar were taken to analyse the concentration of Cu, Zn and Ni, using the AAS technique after dry mineralisation of the samples in a muffle furnace and dilution in hydrochloric acid.

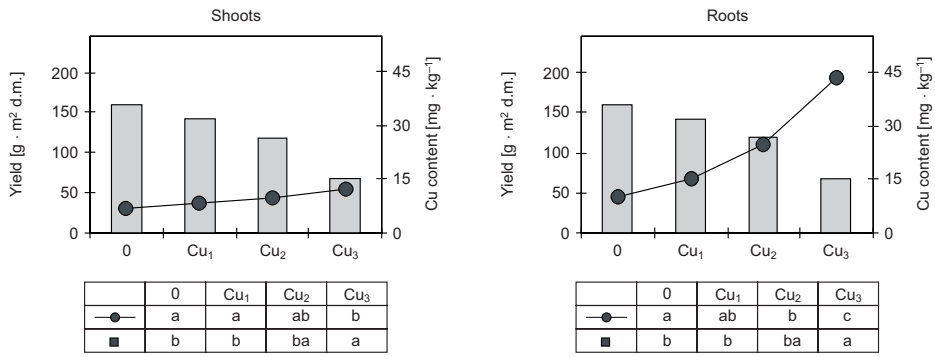
The results of the chemical analyses are given as means from 3 cultivars.

The data on yields of white mustard are means for the three cultivars, too. However, in the statistical computations, each experimental object is represented by 12 replications (4 replications for each cultivar × 3 cultivars).

Results and discussion

White mustard responded to soil pollution by depressing the dry matter yields, with the actual decrease depending on the metal causing pollution. For soil contaminated with Cu (Fig. 1), statistically significant decrease occurred at the level of 100 and 200 mg · kg^{-1} (Cu_2 and Cu_3), reaching 25 % and 60 %, respectively, relative to the control yield. As the yield was on the decrease, the concentration of Cu in shoots increased from 6.8 to 12.2 mg · kg^{-1} d.m., that is two-fold higher at the most compared

with the uncontaminated object. Plants accumulated more Cu in roots than in shoots (Fig. 1). Other authors have also found out that crop roots accumulate more copper than shoots [7, 8]. Studies on phytotoxicity of copper towards crops typically indicate lack of relationship between yield loss caused by excessive copper in soil and copper concentration in crop shoots, which is frequently within the optimum range [9–13]. McBride [14] demonstrated that the content of copper in maize shoots increased as the rates of copper went up, but only to a certain level, after which increasing soil contamination with copper corresponded to small increments of copper levels in shoots.

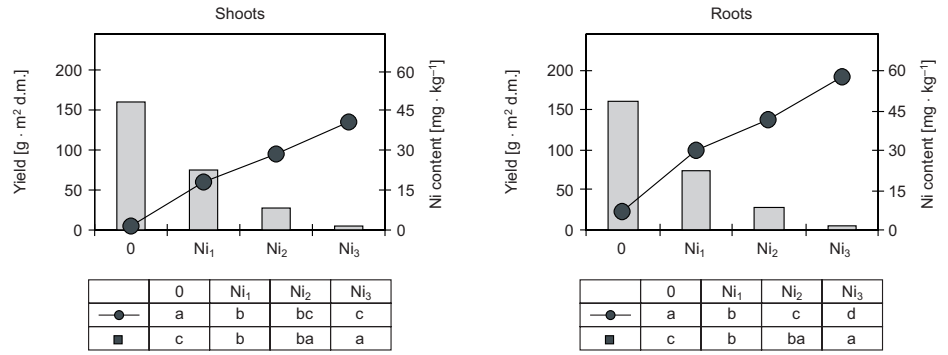


	0	Cu_1	Cu_2	Cu_3
—	a	a	ab	b
■	b	b	ba	a

	0	Cu_1	Cu_2	Cu_3
—	a	ab	b	c
■	b	b	ba	a

Fig. 1. Content of copper in the dry matter of shoots and roots (line) against the background of the biomass (bars). Identical letters in the table denote lack of differences tested with Tukey's test ($p < 0.05$)

The negative effect of nickel on white mustard consisted of a 50 and 80 % loss of plant yields at 40 and 80 mg · kg^{-1} of nickel added to soil (Ni_1 and Ni_2). At the highest rate of nickel, the plants nearly completely died out (Fig. 2). The concentration of this element in shoots increased, depending on the dose of nickel, by about 10-, 20- and 30-fold relative to the control, reaching 41 mg · kg^{-1} d.m. for the Ni_3 polluted object. Increasing soil pollution with nickel also caused an increasing accumulation of this metal in roots, which was about 1.5-fold higher than in shoots (Fig. 2). Spiak [15] demonstrated that at a rate of Ni equal 80 mg · kg^{-1} and a loss of green mass produced by field pea and horse bean reaching over 80 %, the concentration of nickel in field pea increased 48-fold and in horse bean – 75-fold versus the control. In contrast, millet, which contained 27-fold more nickel in green matter, did not lower yields in response to soil contamination with this metal. Ciecko and Wyszkowski [16] found a 5 % loss of oats and maize yield when 30 mg · kg^{-1} of nickel was introduced to soil, with the amount of nickel in green matter increasing 15-fold in oats and 20-fold in maize. According to Kabata-Pendias and Pendias [6], nickel is a very mobile element and can readily transfer to aerial parts of plants, especially to seeds or grains. Poulik [17] determined a 5–11 % increase in the concentration of nickel in oats grain on soil contaminated with this element. The above studies suggest that, irrespective of the toxicity of nickel to a given crop species, the metal is easily transported to the parts of plants above the ground.

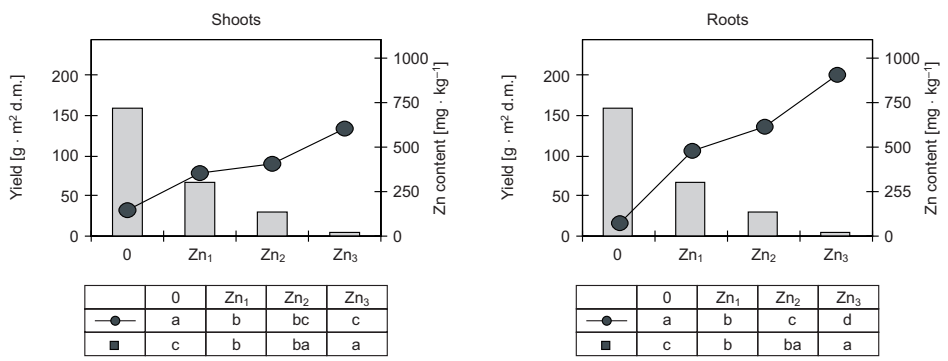


	0	Ni_1	Ni_2	Ni_3
—●—	a	b	bc	c
■	c	b	ba	a

	0	Ni_1	Ni_2	Ni_3
—●—	a	b	c	d
■	c	b	ba	a

Fig. 2. Content of nickel in the dry matter of shoots and roots (line) against the background of the biomass (bars). Identical letters in the table denote lack of differences tested with Tukey's test ($p < 0.05$)

Contamination of soil with zinc added at 200 and 400 mg · kg^{-1} (Zn_1 and Zn_2) resulted in a 60 and 80 % decrease of biomass yields, respectively, as well as nearly complete loss of plants when the highest rate of Zn_3 (800 mg · kg^{-1}) was applied (Fig. 3). White mustard growing on the zinc contaminated objects accumulated the metal in shoots at levels 2- to 4-fold higher than on the control objects. The roots, in turn, were found to contain 6- to 12-fold more zinc than roots of control plants (Fig. 3). When the soil contamination with zinc reached 200 mg · kg^{-1}, the concentration of zinc in shoots rose from 148 to 360 mg · kg^{-1} d.m. the pot experiments performed by Spiak et al [18] evidenced that the amount of 120 mg Ni · kg^{-1} of light soil is harmful to mustard. The concentration of Zn observed under such conditions was 100–300 mg · kg^{-1} d.m. In the pot trials conducted by Wrobel and Nowak [19], high yield loss of mustard growing on light soil was observed as a response to the concentration of zinc in the substratum equal 150 mg · kg^{-1}. The concentration of Zn in shoots was about 2000 mg · kg^{-1} d.m.



	0	Zn_1	Zn_2	Zn_3
—●—	a	b	bc	c
■	c	b	ba	a

	0	Zn_1	Zn_2	Zn_3
—●—	a	b	c	d
■	c	b	ba	a

Fig. 3. Fig. 3. Content of zinc in the dry matter of shoots and roots (line) against the background of the biomass (bars). Identical letters in the table denote lack of differences tested with Tukey's test ($p < 0.05$)

In this study, similarly to the work presented by Marchiol et al [3], the so-called translocation factor ($TF = (C_{aerial}/C_{root}) \cdot 100$) was used to determine the ability of white mustard to transfer metals from roots to aerial parts (Table 1).

Table 1

Translocation factor of metals [$TF = (C_{aerial}/C_{root}) \cdot 100$] calculated for plants of *Sinapsis alba* L.

Contamination level	Metal		
	Cu	Ni	Zn
0	68.7	22.7	197.3
1	54.7	59.7	74.7
2	40.8	68.5	75.8
3	28.2	70.7	66.6
Mean	48.1	55.4	103.6

Most translocation factors have suggested that, out of the three heavy metals examined, copper was most unreadily transferred from roots to shoots. At the same time, as the level of soil pollution with copper increased, more of this metal was kept in roots. In contrast, when the soil contamination of nickel increased, white mustard was able to transfer more of this metal to shoots. By analogy, in the pot trials completed by Gupta et al [20], plants of *Brassica juncea* (L.) accumulated heavy metals in aerial part in the following order: Ni > Zn > Cu.

Plants are generally tolerant to high levels of copper in soil. Kabata-Pendias and Pendias [6] claim that *Brasssicaceae* are tolerant to excessive amounts of nickel in soil. In the present study, it was excess nickel that proved to be the most harmful to white mustard of the three metals. The yield gathered at 160 mg $Ni \cdot kg^{-1}$ was barely 4 % of the control yield, whereas at the level of contamination with copper or zinc equal 200 mg $\cdot kg^{-1}$ slightly over 40 % of the dry matter yield was harvested compared with the uncontaminated objects. White mustard, therefore, was comparably tolerant to excess zinc and copper in soil, although it could more easily transport zinc than copper to shoots.

Nonetheless, when confronted with the threshold value, which define the degree of soil contamination with trace metals [21], white mustard was weakly tolerant event to the lowest degree of soil pollution with nickel and zinc (to so-called raised content), or to the moderate contamination with copper.

Conclusions

1. White mustard (*Sinapsis alba* L.), of the family *Brassicaceae*, growing in soil polluted with copper, nickel and zinc proved to be more tolerant to excess copper and the most sensitive to raised quantities of nickel in the substratum.

2. Using white mustard for remediation of soil contaminated with copper is feasible at the most under a moderate level of soil contamination with this metal.

3. Due to insufficiently low white mustard biomass, it is impossible to use this crop for phytoextraction. However, white mustard can be considered for phytostabilisation of soil contaminated with copper, owing to its ability to retain this element in the roots.

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TOLERANCJA GORCZYCY BIAŁEJ NA SKAŻENIE GLEBY WYBRANYMI METALAMI CIĘŻKIMI

Instytut Uprawy Nawożenia i Gleboznawstwa – Państwowy Instytut Badawczy w Puławach,
Zakład Herbologii i Technik Uprawy Roli we Wrocławiu

Abstrakt: Przeprowadzono doświadczenie ścisłe w obetonowanych mikropoletkach, o pojemności 1 m^3, z symulowanym zanieczyszczeniem gleby miedzią, cynkiem oraz niklem.

Gorczyca biała okazała się najbardziej wrażliwa na zanieczyszczenie gleby niklem, a najbardziej tolerancyjna na nadmiar miedzi w podłożu. Zawartość niklu w częściach nadziemnych wzrastała nawet trzydziestokrotnie, podczas gdy zawartość miedzi zaledwie dwukrotnie w stosunku do kontroli. Na podstawie obliczonych współczynników translokacji badanych metali w roślinie stwierdzono, że miedź najtrudniej przemieszczała się z korzeni do pędów i wraz ze wzrostem poziomu zanieczyszczenia gleby była zatrzymywana w korzeniach w coraz większym stopniu. W przypadku wzrastającego poziomu zanieczyszczenia gleby niklem gorczyca przemieszczała ten pierwiastek w coraz większym stopniu do pędów. Gorczyca biała nadaje się do wykorzystania jedynie w procesie fitostabilizacji gleb średnio zanieczyszczonych miedzią.

Słowa kluczowe: zanieczyszczenie gleby, Cu, Ni, Zn, fitoremediacja, gorczyca biała

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Karolina STEINDOR[1] and Bernard PALOWSKI[1]

CADMIUM AND LEAD ACCUMULATION PATTERNS IN ORGANS OF CHOSEN URBAN TREE SPECIES

AKUMULACJA KADMU I OŁOWIU W ORGANACH WYBRANYCH GATUNKÓW DRZEW MIEJSKICH

Abstract: The concentration of Cd and Pb in leaves/needles, twigs, seeds and fruit coverings of: horse-chestnut (*Aesculus hippocastanum* L.), yew-tree (*Taxus baccata* L.), European ash (*Fraxinus excelsior* L.), and in the soil at the base of the same trees was investigated. The ability of metal accumulation was determined in all investigated tree species as well as different partitioning in examined plant tissues. The lowest Pb concentration was found in fruit parts of all tree species: in seeds or fruit covering. The lowest Cd concentration in unpolluted regions was determined in leaves/needles or twigs.

The highest Cd and Pb concentration in investigated tissues depends on the species and the pollution level of the place where plants were growing. The obtained results could be used to determine the most suitable organs for Pb and Cd biomonitoring in the environment.

Keywords: heavy metals, *Aesculus hippocastanum*, *Taxus baccata*, *Fraxinus excelsior*

Still developing industrialization and urbanization, despite the increasing concern about the environment, causes many toxic emissions including heavy metals. The main source of the pollution are domestic heating systems, industry and traffic [1]. Among the non-essential heavy metals for living organisms, the most spread ones are lead and cadmium. These elements also have a highly toxic effect on living organisms, which can lead to intoxications, pathological changes and even increased mortality among people exposed to cadmium [2, 3]. Therefore, constant monitoring of Cd and Pb concentration in the environment is essential.

The use of plant tissues as heavy metal bioindicators is commonly known. As good Pb and Cd bioindicators were found: leaves of European ash (*Fraxinus excelsior* L.) [4], Norway maple (*Acer platanoides* L.) [5], white birch (*Betula pendula* Roth.), crack willow (*Salix fragilis* L.), broad-leaved linden (*Tilia platyphyllos* Scop.) [6], wood of water oak (*Quercus nigra* L.) and black oak (*Q. velutina* Lam.) [7].

In this project three common tree species were chosen: common yew (*Taxus baccata* L.), European ash (*Fraxinus excelsior* L.) and horse-chestnut (*Aesculus hippocastanum* L.)

[1] Department of Ecology, The University of Silesia, ul. Bankowa 9, 40–007 Katowice, Poland, phone: +48 32 359 11 18, email: karolina.steindor@us.edu.pl

and the concentration of Cd and Pb in their leaves/needles, twigs, seeds and fruit coverings was investigated.

The aim of this paper was to determine the ability of the examined tree species to accumulate Cd and Pb, determine the accumulation pattern in the investigated organs and find the organs most suitable for Cd and Pb bioindication.

Material and methods

Leaves/needles, fruits, twigs and the upper 10 cm of soil at the base of the sampled trees were collected in early autumn 2004 and 2006. The sampling was carried out in heavily polluted cities of Upper Silesia Industrial District – southern Poland (Chorzow, Katowice, Myslowice, Siemianowice Slaskie, Sosnowiec, Gliwice). Sampling places located in the Beskid Slaski (south of Upper Silesia), which is comparatively unpolluted (Kety, Porabka, Slemien, Cieszyn, Ustron, Cisownica, Skoczow, Brenna), were chosen as control sites. In this part of Poland the dominant wind direction is south-west and the mean annual precipitation is 650–800 mm for the Upper Silesia Industrial District and 800–1000 mm for the Beskid Slaski [8].

The material was sampled in at least 5 replicates at each location. The soil was stored in linen bags until air dry. The soil samples were passed through a 1 mm mesh sieve. The 10 g soil samples were shaken with 100 cm^3 10 % HNO_3 for one hour.

The plant material was washed in tap and distilled water. The fruits were divided into seeds and fruit covering. The material was ground and dried at 105 ^oC for 24 h. 3 g samples were dry mineralized in a muffle oven at 460 ^oC and then digested in 25 cm^3 10 % HNO_3 and filtrated. The Cd and Pb concentration was measured using the method AAS. All the analyses were carried out in three replicates.

The accumulation factor was calculated as the element mean concentration ratio between the polluted sites and the control [9].

Results

The Pb and Cd accumulation level in the investigated tree organs reveal differences depending on tree species and the pollution level of the place where the plants were growing. The mean element concentrations in the investigated organs are presented in Table 1.

Cadmium. Cd concentrations in the organs of the examined tree species increased as follows:

	a) polluted sites	b) control sites
Ash tree	fruit covering < seeds < leaves < twigs	twigs < seeds < leaves < fruit covering
Yew-tree	seeds < needles < fruit covering < twigs	needles < seeds < fruit covering < twigs
Horse--chestnut	fruit covering < leaves < twigs < seed	leaves < fruit covering < twigs < seeds

Table 1

Mean metal concentrations in investigated tree organs [mg/kg d.m.]

Tree organ		Ash tree		Yew-tree		Horse-chestnut	
		Polluted site	Control site	Polluted site	Control site	Polluted site	Control site
Cd	seeds	0.98 ± 0.14	0.77 ± 0.22	0.80 ± 0.13	0.71 ± 0.11	2.64 ± 0.22	1.81 ± 0.19
	fruit covering	0.85 ± 0.11	0.95 ± 0.17	0.93 ± 0.22	0.79 ± 0.11	1.47 ± 0.20	1.03 ± 0.10
	leaves	1.02 ± 0.28	0.82 ± 0.20	0.83 ± 0.35	0.70 ± 0.21	1.74 ± 0.26	0.95 ± 0.14
	twigs	1.94 ± 2.31	0.46 ± 0.23	1.73 ± 0.48	0.96 ± 0.20	1.84 ± 0.52	1.15 ± 0.40
Pb	seeds	6.63 ± 3.33	4.98 ± 1.86	3.93 ± 1.29	4.69 ± 0.91	12.12 ± 4.22	2.37 ± 1.91
	fruit covering	10.80 ± 3.35	5.36 ± 4.49	8.35 ± 2.35	4.13 ± 1.99	7.04 ± 12.37	3.10 ± 1.65
	leaves	49.09 ± 73.39	11.40 ± 2.93	66.10 ± 112.85	6.96 ± 1.16	101.54 ± 194.99	7.68 ± 3.32
	twigs	225.73 ± 484.09	7.34 ± 2.00	164.45 ± 292.91	9.30 ± 2.95	63.08 ± 122.32	7.94 ± 2.93

The Cd accumulation factor was the highest for the ash tree twigs, the lowest for the fruit coverings of all examined tree species (Fig. 1).

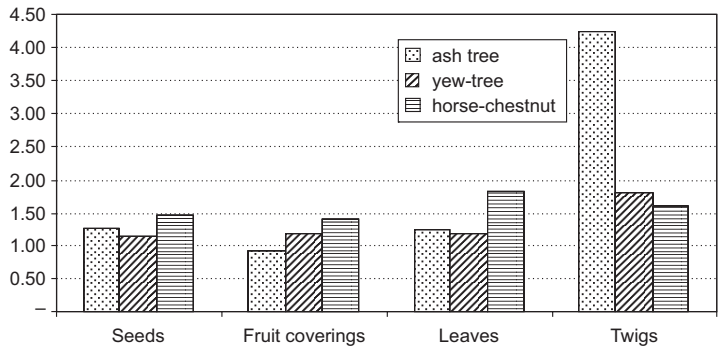

Fig. 1. The Cd accumulation factor for the investigated tree tissues

The Cd concentration among all investigated horse-chestnut organs was positively correlated and significant (Table 2). The correlation coefficient between the Cd level in tissue and in soil was significant except seeds and twigs. A significant correlation between the Cd concentration in yew organs was found between fruit covering and twigs, seeds and twigs, leaves and twigs. The Cd concentration in soil was significantly correlated only with Cd in twigs. The Cd concentration in ash tree was significantly correlated only between leaves and twigs and between soil and fruit covering.

Table 2

The correlation coefficient between the metal concentration in soil and in investigated tree organ and between tree organs

	Ash tree		Yew-tree		Horse-chestnut	
	Cd	Pb	Cd	Pb	Cd	Pb
Fruit covering/seeds	–0.36	0.58***	0.68***	0.38	0.09	–0.13
Fruit covering/leaves	–0.02	0.38	0.94****	0.98****	0.42	–0.70****
Fruit covering/twigs	0.07	0.41	0.53**	0.98****	0.66***	–0.68****
Fruit covering/soil	0.55**	–0.11	0.39	0.97****	0.22	0.07
Seeds/leaves	–0.28	0.50*	0.77****	0.47*	0.60***	0.03
Seed/twigs	–0.24	0.47*	0.62***	0.47*	0.40	0.03
Seeds/soil	0.00	0.24	0.62***	0.49*	0.29	0.42
Leaves/twigs	0.80****	1.00****	0.65***	1.00****	0.49*	1.00****
Leaves/soil	0.07	0.09	0.55**	0.99****	0.05	0.35
Twigs/soil	0.04	0.03	0.26	0.98****	0.65***	0.40

r-correlation significance *p = 0.05; **p = 0.02; ***p = 0,01; ****p = 0.001.

Lead. Pb concentrations in the organs of the examined tree species increased as follows (Table 1):

	a) polluted sites	b) control sites
Ash tree	seeds < fruit covering < leaves < twigs	seeds < fruit covering < twigs < leaves
Yew-tree	seeds < fruit covering < needles < twigs	fruit covering < seeds < needles < twigs
Horse--chestnut	fruit covering < seeds < twigs < leaves	seeds < fruit covering < leaves < twigs

The highest Pb accumulation factor was calculated for ash tree and yew-tree twigs and horse-chestnut leaves, the lowest for the ash tree and yew-tree seeds and for the fruit covering of all investigated species (Fig. 2).

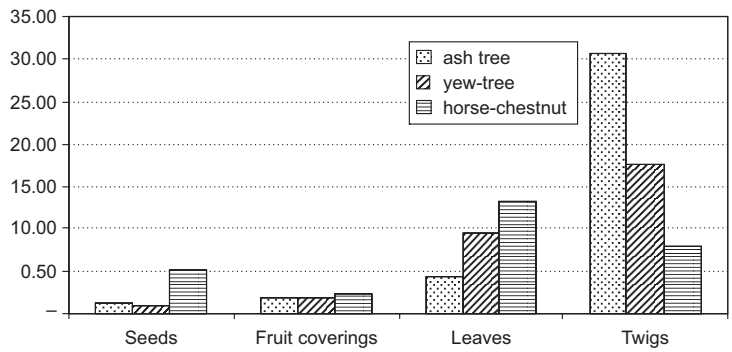

Fig. 2. The Pb accumulation factor for the investigated tree tissues

A strong positive correlation was observed between Pb soil concentration and the level of Pb in the fruit covering, leaves and twigs of yew-tree (Table 2). Insignificant was only the correlation between the Pb concentration in fruit covering and seeds. In ash tree and horse-chestnut the concentration of Pb in leaves and twigs was also strongly correlated. The Pb level in horse-chestnut fruit covering shows a significantly negative correlation between leaves and twigs.

Discussion

The accumulation of Cd and Pb in ash tree twigs was predominant in the whole analyzed plant material. The Cd and Pb concentration in yew-tree twigs was the highest in all analyzed organs of this plant species. Similarly, Rossbach and Jayasekera [10] measured higher Pb concentration in pine twigs than in needles. The highest accumulation factor for the horse-chestnut was calculated for the leaves. Such high metal concentration in twigs may be a result of incorporation of elements in the bark tissues during the perennial deposition [11]. The large surface and the umbrella-like shape of horse-chestnut leaves may protect the twigs against dust deposition in an efficient way and could uptake a majority of dry and wet precipitation. Additionally, the

deposition increases with the appearance of necrotic lesions on leaves [12], caused by feeding of horse-chestnut leaf miner (*Cameraria ohidella*) larva.

The analyses revealed different partitioning of Cd and Pb not only in the examined plant species, but also among organs of the plants and it strongly depends on the pollution level of the environment where the trees were growing. The differences were visible usually in pairs such as twigs and leaves as well as fruit coverings and seeds.

It is considered that heavy metal concentration in plant organs increases with the pattern: seeds < inflorescence < stems < leaves, however this order sometimes varies with plant species [13, 14]. Seeds seem to be the organ best protected from heavy metal infiltration [15, 16]. This thesis finds confirmation in the results of Pb concentrations with an exception of the yew-tree samples from the control sites and horse-chestnut samples from the polluted sites. However, the lower values of Pb were observed only in fruit coverings.

The accumulation pattern observed for Cd was different, eg abnormal results were obtained for the horse-chestnut seeds, in which the Cd concentrations were the highest in both locations. The determined concentrations, even at the comparatively unpolluted stands, were repeatedly higher than results obtained by Lukasiewicz [17] in the horse-chestnut seeds from Poznan. The accumulation factor for both investigated metals in horse-chestnut seeds was higher than in yew-tree and ash-tree.

The correlation coefficient was statistically significant for Cd concentrations in soil and seeds, leaves/twigs and seeds ($p < 0.01$). It may suggest the soil origin and relocation of Cd between organs, because of its high mobility in plant organism [18].

The seeds, fruit coverings and leaves of the horse-chestnut showed a higher Cd accumulation factor than in the comparable organs of ash tree and yew-tree. This points to the suitability of horse-chestnut in environmental biomonitoring of this metal. Moreover, the highest Pb accumulation factor for horse-chestnut leaves and seeds shows that using these organs for monitoring the environment contamination with Pb is possible. Suitable for Cd and Pb biomonitoring are also ash tree twigs and as Pb bioindicators – yew-tree twigs. The statistically significant correlation factor of Pb and Cd concentration between leaves/needles and twigs of investigated tree species suggests their atmospheric origin, which confirms their bioindicative usability.

Conclusions

1. On the basis of the differences in Cd and Pb concentrations in tree tissues from the heavily polluted and comparatively unpolluted sites, the ability to accumulate these elements by the investigated tree species has been stated.

2. The Pb accumulation pattern in the examined plant organs does not essentially vary from the usually used patterns: the lowest concentrations were measured in seeds and fruit coverings and the highest in leaves and twigs. The differences are revealed between this pairs depending on the species and the location.

3. The Cd accumulation pattern in the plant organs shows greater divergences depending on the species. Exceptionally high was the Cd concentration in horse-chestnut seeds in relation to other investigated tree organs.

4. Ash tree twigs, horse-chestnut seeds, fruit covering and leaves could be suitable in the biomonitoring of Cd contamination of the environment and ash tree and yew-tree twigs, horse-chestnut leaves and seeds – in Pb biomonitorng.

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AKUMULACJA METALI CIĘŻKICH W ORGANACH WYBRANYCH GATUNKÓW DRZEW MIEJSKICH

Katedra Ekologii, Wydział Biologii i Ochrony Środowiska
Uniwersytet Śląski

Abstrakt: Oznaczono stężenie Cd i Pb w liściach, pędach, nasionach oraz części płonej owocu trzech gatunków drzew popularnie nasadzanych w miastach: kasztanowcu zwyczajnym (*Aesculus hippocastanum* L.),

cisie pospolitym (*Taxus baccata* L.) i jesionie wyniosłym (*Fraxinus excelsior* L.) oraz w materiale glebowym zebranym pod wybranymi osobnikami. Stwierdzono zdolność do akumulacji metali przez badane gatunki oraz zróżnicowane stężenie metali w poszczególnych organach. Najniższe stężenie Pb oznaczono u wszystkich trzech badanych gatunków w częściach owocu – nasionach lub części płonej, w przypadku Cd dla drzew z rejonów stosunkowo słabo zanieczyszczonych niższe zawartości kadmu notowano w liściach/szpilkach lub pędach.

Organy o najwyższym stopniu koncentracji Cd i Pb w tkankach różnią się w zależności od gatunku drzewa, badanego metalu oraz stopnia zanieczyszczenia środowiska, z którego pobierano próbki. Uzyskane dane mogą posłużyć do wytypowania organów przydatnych w biomonitoringu kadmu i ołowiu w środowisku.

Słowa kluczowe: metale ciężkie, *Aesculus hippocastanum*, *Taxus baccata*, *Fraxinus excelsior*

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Barbara ŚCIGALSKA[1] and Bernadetta ŁABUZ[1]

CONTENT OF HEAVY METALS IN THE TOP LAYER OF SOILS WITH TRITICALE GROWING THEREON IN ONE-CROP SYSTEMS

ZAWARTOŚĆ METALI CIĘŻKICH W WIERZCHNIEJ WARSTWIE GLEBY W UPRAWIE PSZENŻYTA W MONOKULTURACH ZBOŻOWYCH

Abstract: The research was carried out in the period from 2002 to 2008 on the basis of a strict field experiment located at the Experimental Station of the Department of Soil and Plant Cultivation, University of Agriculture in Krakow, in the locality of Mydlniki near Krakow. A two-factor experiment was set up using a method of random sub-blocks, in 4 repetitions, on a typical, good wheat complex soil of a slightly acid reaction (pH_{Cl} = 5.7). Two triticale species were grown: Woltario winter triticale and Matejko spring triticale, using three crop sequences: Norfolk crop rotation, two species, one-crop system with the addition of oats, and one species, one-crop system with the addition of triticale. The objective of the research was to assess the heavy metal contamination level (Cr, Zn, Pb, Cu, Cd, Ni, and Mn) of the top soil layer (0–20 cm) when triticale was grown thereon using a one-crop system method in comparison with the cultivation of triticale using crop rotation. In the soil material, the following was determined: pH, organic matter, and total content of heavy metals. The research experiments showed that the analyzed soil on which triticale was grown during a period of 6 years using a one-crop system was characterized by an averagely higher content of Cr, Zn, Pb, Cu, Ni, and Mn compared with the crop rotation system, and by a higher content of Cd. In the objects, where a two species, one-crop system was applied with oats as a forecrop for triticale, it was reported that the content of Cr, Zn, Pb, Fe, and Ni tended to decrease whereas the Cu content to increase. Except for Cu, the highest increase in the content of heavy metals in the soil was reported for the one species, one-crop system used to grow this cereal.

Keywords: heavy metals, triticale, arable layer, and one-crop system

The content of heavy metals is a very important factor for soils meant for plant production. Agricultural soils are particularly exposed to the contamination by heavy metals. For the majority of soils in Poland, it is characteristic that heavy metals constitute their naturally occurring components [1–3]. The increased content of heavy metals in the soils in Poland is the effect of using crop protection chemicals and mineral & natural fertilizers, which contain heavy metals [4]. Two agricultural measures:

[1] Department of General Soil and Plant Cultivation, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 43 67, fax: +48 12 662 43 66, email: bscigalska@ar.krakow.pl

introducing new plant varieties into field production and increasing their amounts in the disposition of crops can play a part in exhausting reserves of phytoavailable forms of microorganisms in soils. Acidification of soils and reduction of organic manuring [5] are two of a number of factors impacting the negative balance among microorganisms in soils. Thus, the research into soil microorganisms appears very important and is gaining in importance in modern agriculture & farming.

The objective of the present research was to assess and compare the contamination degree of the top layer of soils (arable layer of soils) by Cr, Zn, Pb, Cu, Cd, Ni, Fe, and Mn when growing triticale using a six-year, one-crop system and a crop rotation system.

Material and methods

The research was carried out in a period from 2002 to 2008 on the basis of a strict field experiment, in a field located at the Experimental Station of the Department of Soil and Plant Cultivation, University of Agriculture in Krakow, in the locality of Mydlniki near Krakow. A two-factor experiment was set up using the method of random sub-blocks, in 4 repetitions, on a typical, good wheat complex brown soil. The soil in the experimental field showed a slightly acid reaction (pH_{KCl} = 5.7) and its content of organic matter was 1.40 % (Table 2).

The granulometric composition of the field soil under experiment was: sand – 34 %; dust – 42 %; silt and clay fraction – 25 %; therefore, this soil was texturally classified as a medium soil. Two triticale speciess: Woltario winter triticale and Matejko spring triticale were grown using three crop sequences: Norfolk crop rotation, two-species, one-cropsystem with the addition of oats, and one-species, one-crop system with the addition of triticale.

A general soil sample was taken from the soil layer, its thickness being 0–20 cm, using a soil auger; this general soil sample consisted of six (6) primary samples in four (4) repetitions from the object. The samples collected were dried and screened through a 2 mm- mesh screen. Next, pH of the soil was determined in 1 mol · dm^{-3} KCl using the potentiometric method, and the content of organic carbon (C_{org}) using the Tiurin method. The per cent content [%] of organic matter was calculated by multiplying the content of C_{org} by 1.72. The total content of heavy metals: Cr, Zn, Pb, Cd, Cu, Fe, Ni, and Mn was determined using the ICP-AES method (ie *Inductively Coupled Plasma – Atomic Emission Spectroscopy*) with JY 238 ULTRACE apparatus manufactured by Jobin Von Emission. The assessment of the content level of those metals was based on the standards indicating their limit values. The occurrence stability of the microelements examined was measured by applying a Coefficient of Variation (CV [%]). In the statistical calculations, the method of variance analysis was applied, and the statistical hypothesis was verified using the multiple Tukey's test at the statistical significance level of $\alpha = 0.05$.

Results and discussion

The content of heavy metals studied was expressed in mg · kg^{-1}. In the three experimental objects, the average content of those heavy metals was as follows: Cr –

1.06; Zn – 27.90; Pb – 20.90; Cu – 4.27; Cd – 0.59; Ni – 1.20; Fe – 1139.40; and Mn – 134.90 (Table 1). According to the relevant standard [6] ref. to medium soils, the average content value of Cd and Zn should be, respectively: for Cd – 0.31 mg · kg^{-1}, and as Zn – 33.2 mg · kg^{-1}. The results of the analysis performed by the authors of this paper showed that the average content of Cd varied between 0.55 and 0.67 mg · kg^{-1}, and the content of Zn between 25.93 and 30.98 mg · kg^{-1}. Therefore, the average content of Zn in the soils analyzed was below the standard level. However, according to Straczynski [7], the standard content of Zn in medium soils should be 10.44 mg · kg^{-1}; thus, the content of this metal in the soils under experiment was three (3) times as high as recommended by Straczynski. It is generally assumed that 10 % of soils located in the Krakow region (ie where the experiment took place) are characterized by a low content of Zn, 62 % – by a medium content of Zn, and 28 % – by a high content of Zn. According to the authors' own studies, the content of Cd ranged from 0.55 to 0.66 mg · kg^{-1} and exceeded the value as indicated in the relevant standard. The highest concentrations of Cd are found in the western part of the former province of Krakow [8]. The soils in the Krakow region show a high ability to accumulate cadmium because they are relatively heavy [9]. Soils which strongly accumulate cadmium predominate in the NW part of the province in question. The variations quoted for the concentrations of Cd and Zn in soils are connected with different parent rocks from which they derive and with chemical properties of soils, and the availability of heavy metals to plants increases with the rising level of soil acidification [10].

The following was found during the experiment: compared with the soil where a crop rotation system was applied to grow triticale, the soil used to grow triticale in a 6-year, the one crop system was on average characterized by a higher content of Cr, Zn, Pb, Cu, Ni, and Mn, as well as by a higher amount of Cd. In the objects, where two species were grown using the one-crop system, and oats was used as a forecrop for triticale, it was found that the contents of Cr, Zn, Pb, Fe, and Ni tended to drop, and the content of Cu – to rise. When triticale was cultivated in the one-species system, the highest increase in the content of heavy metals in the soil was reported, except for Cu. Furthermore, a significantly higher content of Cr, Zn, Pb, Cd, and Mn (Table 1) was reported in the object with the triticale grown using the one-species, one-crop system compared with the object with a crop rotation system. In the case of Ni, Cu, and Fe, their content values were insignificant, but the results obtained show that the content values of those heavy metals tend to grow in the soil used to grow triticale in the one-species system. The content of Ni varies widely in the soils in the Krakow region and ranges from 0.60 to 47.8 mg · kg^{-1}, with the average content in the soils in Poland being 12.12 mg · kg^{-1}. Organic matter strongly binds nickel, and its solubility increases along with the increase in the acidity of soil [11]. The majority of arable soils in this region (58 %) show a high ability to accumulate Ni, and only 35 % of the soils are characterized by a low ability to bind this metal [8]. During the authors' own research, they did not find any significant variations in the pH values of soils in the experimental objects. Yet, they found the organic matter content to tend to rise when the one-species, one-crop system of growing triticale was applied (Table 2). The content of Ni was higher in the objects where the triticale was cultivated using the one-species, one-crop system.

Table 1

Contents of heavy metals and their variance (CV [%]) in soils on which triticale is grown using crop rotation and one-crop systems (Mydlniki 2008)

Objects	Forecrop	Form	Cr	Zn	Pb	Cu	Cd	Ni	Fe	Mn
			[mg · kg^{-1}]							
Crop rotation	*Horse-bean*	*Winter triticale*	0.92	25.98	18.18	4.13	0.56	1.18	1043.80	102.93
	Sugar beet^{++}	*Spring triticale*	0.94	27.10	18.30	4.12	0.55	1.21	1046.80	110.93
Two-species one-crop system	*Winter triticale*	*Spring triticale*	1.08	26.88	19.63	4.16	0.59	1.18	1112.00	125.20
	Oat	*Winter triticale*	0.98	25.93	19.20	4.33	0.56	1.19	1046.30	125.00
One-species one-crop system	*Spring triticale*	*Winter triticale*	1.21	30.98	20.35	4.42	0.67	1.22	1287.50	168.75
	Winter triticale	*Spring triticale*	1.21	30.55	20.90	4.46	0.66	1.25	1300.00	176.50
Average Value			1.06	27.90	19.40	4.27	0.59	1.20	1139.40	134.90
$LSD_{\alpha = 0.05}$ for the objects			0.19	3.67	1.68	n.s.**	0.06	n.s.	n.s.	34.2
CV [%]*			14.0	10.9	3.4	11.5	23.7	1.7	55.5	27.4

* CV [%] – variation coefficient; ** n.s. – non-significant differences; ++ manure rate.

Table 2

Contents of organic matter (expressed in %) and pH of the soils in three objects of the experiment (Mydlniki 2008)

Objects	Forecrop	Form	pH_{KCl}	Organic matter [%]
Crop rotation	*Horse-bean*	*Winter triticale*	5.7	1.45
	Sugar beet $^{++}$	*Spring triticale*	5.8	1.38
One-crop, two-species system	*Winter triticale*	*Spring triticale*	5.7	1.38
	Oat	*Winter triticale*	5.7	1.38
One-crop, one-species system	*Spring triticale*	*Winter triticale*	5.8	1.36
	Winter triticale	*Spring triticale*	5.7	1.43
Average Value			5.7	1.40
CV [%]			0.8	3.9

In the soils in Poland, the content of Pb is, on average, 13.8 mg · kg^{-1}. With regard to the content of this element in the entire Krakow region, the soils in the western part of this region are the most Pb-contaminated. The authors' own studies revealed that the standard value of Pb content was exceeded as this content ranged from 18.18 to 20.9 mg · kg^{-1}. No Cu-contamination of the soils was found; the content of Cu in the soil in three experimental objects varied between 4.12 and 4.46 mg · kg^{-1}. The average content of Cu in the soils in Poland is from 0.2 to 293 mg · kg^{-1}. During the experiment, the authors found that the content of Cu was higher in the soils in the object where oats and the one-species, one-crop system of triticale were grown. Similarly, it was reported that the content of Fe and Mn tended to increase in these objects.

In some other regions of Poland, top layers of soils used for agricultural purposes are contaminated by heavy metals: zinc, lead, copper, and cadmium [12]. The contamination degree of those soils varies. In the Krakow region, the majority of soils belong to medium and heavy classes of soils. Brown soils predominate (47.7 %). Zn and Pb are main elements to contaminate soils in the Krakow region [8].

The results presented in this paper correspond with the results of research obtained by other authors [8, 9, 11] in the Krakow region. In the region where this experiment was carried out, the elements Zn, Cd, and Pb contaminate the soils to a high degree and their contents exceed the recommended standard levels. The results obtained by the authors of this paper proved the crop sequence of cereals grown on arable soils had an effect on the content of heavy metals in the soils. It was found that the soil contamination by heavy metals in the objects with the triticale grown under one-species system was higher compared with the soils in the objects with crop rotation. Compared with the soils with triticale grown using the crop rotation system, soils with the one-species system showed a significant increase in the content of Cr, Zn, Pb, Cd, and Mn. Additionally, compared with soils with crop rotation, the content of Cr significantly increased in the soils where the one-species, one-crop system of growing triticale was used. The content of Cr and Zn tended to decrease in the soils where the two-species, one crop-system with oat was applied to grow cereals; the content of those two elements was close to their content in soils where crop rotation was applied (Table 1). Thus,

a presumption can be made that the content of oats in the two-species, one-crop system can moderate negative effects of this specific crop sequence. And, in this case, the soil contamination by heavy metals was reported to be lower than in the soils where the one-species, one-crop system was used; also, this contamination level was reported to be comparable with the contamination degree in soils where the crop rotation system was applied. Furthermore, a higher variation (V [%]) in the contents of cadmium, manganese, and iron in soils was reported. Nickel and lead showed the lowest variation in their contents in the soils.

Conclusions

1. Under the conditions of the experiment described, the levels of soil contamination by heavy metals in the objects used in the experiment mostly did not exceed the ranges of the standards adopted. Those ranges were exceeded only in the case of cadmium, zinc, and lead.

2. The crop sequence impacted the variations in the heavy metal contents in the soils analyzed. It was found that the contents of heavy metals increased in the soils used to grow triticale with the multiyear, one-species & one-crop system applied.

3. When triticale was grown using a two-species, one-crop system with oats, the negative effects of this sequence could be moderated and it was possible to keep the content values of heavy metals at a level close to that reported for the soils used to grow triticale under the crop rotation system.

4. It was found that the variance (V%) in the content of cadmium, manganese, and iron in the soils analyzed was higher than in the content of the remaining heavy metals. The lowest variation in the content level was reported for nickel and lead.

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ZAWARTOŚĆ METALI CIĘŻKICH W WIERZCHNIEJ WARSTWIE GLEBY W UPRAWIE PSZENŻYTA W MONOKULTURACH ZBOŻOWYCH

Katedra Agrotechniki i Ekologii Rolniczej
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Badania realizowano w latach 2002–2008 na bazie ścisłego doświadczenia polowego, zlokalizowanego w Stacji Doświadczalnej Katedry Ogólnej Uprawy Roli i Roślin Uniwersytetu Rolniczego w Mydlikach koło Krakowa. Dwuczynnikowe doświadczenie założono metodą losowych podbloków, w 4 powtórzeniach, na glebie brunatnej właściwej, kompleksu pszennego dobrego o odczynie lekko kwaśnym (pH_{KCl} = 5,7). Uprawiano dwie odmiany pszenżyta – ozimą Woltario i jarą – Matejko w trzech wariantach następstwa roślin: płodozmian Norfolk, monokultura zbożowa 2-gatunkowa z udziałem owsa i monokultura 1-gatunkowa z udziałem pszenżyta. Celem badań była ocena stopnia zanieczyszczenia wierzchniej warstwy gleby (0–20 cm) Cr, Zn, Pb, Cd, Cu, Fe, Ni i Mn w uprawie pszenżyta w monokulturach zbożowych w porównaniu z uprawą tego zboża w płodozmianie. W materiale glebowym oznaczono: pH, materię organiczną oraz całkowitą zawartość metali ciężkich. Wyniki badań wykazały, że gleba spod uprawy w 6-letniej monokulturze zbożowej pszenżyta charakteryzowała się średnio wyższą w porównaniu z płodozmianem zawartością Cr, Zn, Pb, Cu, Ni i Mn oraz podwyższoną zawartością Cd. W monokulturze 2-gatunkowej w obiektach, gdzie przedplonem dla pszenżyta był owies, obserwowano tendencję do spadku zawartości Cr, Zn, Pb, Fe, Ni i wzrostu zawartości Cu. Natomiast w 1-gatunkowej monokulturze tego zboża zanotowano największy wzrost zawartości metali ciężkich w glebie – z wyjątkiem Cu.

Słowa kluczowe: metale ciężkie, pszenżyto, warstwa orna, monokultura

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Maciej WALCZAK[1] and Aleksandra BURKOWSKA[1]

UVB RADIATION IMPACT ON ACTIVITY OF DNA AND CELLULAR PROTEIN SYNTHESIS OF WATER ENVIRONMENT BACTERIA

WPŁYW UVB NA AKTYWNOŚĆ SYNTEZY DNA I BIAŁEK KOMÓRKOWYCH PRZEZ BAKTERIE ŚRODOWISKA WODNEGO

Abstract: The studies were carried out in 2007 based on water samples collected from SM and SW of pelagic zone of Brzezno lake. For the purpose of further research, representative collection of bacterial strains was isolated from collected samples. The progress of the research included estimation of the DNA and cellular protein synthesis activity of the bacteria subjected to UVB radiation. The survey was conducted in two experimental layouts: with and without *humic substances* (HS) playing role of compounds potentially protective from UV radiation. Conducted research demonstrated that under influence of UVB radiation the SM and SW bacteria activity of DNA and cellular protein synthesis were strongly inhibited. On the other hand, presence of HS have another impact on DNA and cellular protein synthesis.

Keywords: UV, DNA synthesis, protein synthesis, humic substances, surface microlayer

In the vertical plane, the external layer of water body is so-called *surface microlayer* (SM). It comprises as little as several hundred micrometers of the water body surface. Hence, the amount of solar radiation reaching SM is virtually the same as the mainland surface. The most biologically significant solar radiation component reaching SM, on account of harmful impact, is the mean UV radiation, so-called UVB λ = 290–320 nm and UVA λ = 320–400 nm [1]. It causes DNA defects (lethal effect) or hinders organisms' growth by inhibiting enzyme synthesis, reducing active transport and inducing mutations, which result in sublethal effect [1]. Therefore, insolation is one of the major factors determining number and activity of bacteria inhabiting that peculiar environment. Strong solar radiation as is known limits numbers of all microorganisms

[1] Department of Environmental Microbiology and Biotechnology, Institute of Ecology and Environment Protection, Nicolaus Copernicus University, ul. J. Gagarina 9, 87–100 Toruń, Poland, phone: +48 56 611 44 33, email: walczak@umk.pl

in the water body, however the amount of harmful UV radiation reaching deeper than SM water layers is definitely smaller because of absorption and diffuse. SM compared to *subsurface water* (SSW), for extreme temperature values or solar energy doses, is unstable environment, extreme in a sense.

There are studies reporting harmful impact of solar radiation, UVB in particular, on production of bacterial biomass and exoenzymes activity [2, 3]. On the other hand however, it is common knowledge that in SM water up to 50 % of *dissolved organic matter* (DOM) make up *humic substances* (HS) [4]. As is known, HS effectively absorb UV radiation protecting microorganisms from that radiation [5, 6]. During the exposure to radiation they undergo photooxidation and decomposition into simpler compounds, which become additional amount of easily assimilable organic matter that is likely to stimulate heterotrophic bacteria growth [7, 8]. Exact measurements of synthesis activity of particular cellular macromolecules in in situ conditions are extremely difficult to perform. It is due to the impossibility of control of particular physicochemical factors affecting organisms' activity in natural conditions. Therefore, this study examined UVB impact on activity of DNA synthesis and cellular proteins in manageable laboratory conditions. Furthermore, present work sought answer to the question whether or not HS can play role of protective agent from UVB radiation for bacteria.

Material and methods

The surveys were based on heterotrophic bacteria strains, isolated from the surface microlayer water and subsurface water.

Water samples collection. Water meant for analyses was collected in the summer, in pelagic zone of the Brzezno lake (latitude 53°57.5′; longitude 17°48.6′), which lies within the Tuchola Forest area. The surface area of the lake equals 71.6 ha, with a maximal depth of 9.7 m, length of 2405 m, and width of 560 m. It is situated at 139.8 m above sea level and is rated among eutrophic water bodies.

SM water samples were collected by a Garrett [9] technique using a Plexiglas plate, which collects a 150 μm water layer. Subsurface water was sampled from a depth of 25 cm using an automatic pump. Taken water samples were poured into sterile glass containers.

Isolation of bacterial strains. In order to isolate bacterial strains from accordingly dissolved water samples there was a surface screening carried out in three simultaneous experiments with 0.1 cm^3 on the *Tryptone Soy Agar* (TSA) (Difco) medium surface. After 6 days of incubation at 20 °C a representative strains collection was detached and transferred onto TSA medium bevels.

Preparation of test bacterial strains suspension. Isolated bacteria strains were generated for 3 days at 20 °C in 50 cm^3 of liquid *Tryptone Soy Broth* (TSB) medium. Afterward, from each culture taken was 30 cm^3 and spun for 5 minutes at 10K rpm, temperature of 10 °C. After supernants have been spun bacterial deposit was removed and suspended in 30 cm^3 of sterile Ringer's solution. Optic density of each strain bacterial

suspension was driven to equal value of 0.5 applying sterile physiological sodium salt solution as a diluting agent.

Exposure to UVB radiation. Prepared bacterial suspension of given strain was divided into 3 parts, 10 cm^3 each, and transferred into three parallel sterile Petri dishes. First one was a controlling agent and was not subjected to UVB radiation. Two other dishes containing bacterial suspension were exposed to UVB radiation (lamp Philips; 15 min, 50 $\mu W/cm^2$), while before exposure to radiation there was 0.1 cm^3 of HS (final concentration 100 mg $\cdot$ cm^{-3}) added to one of them.

DNA synthesis estimation. After exposure to radiation from all dishes (including the control one) 9 cm^3 of bacterial suspension was transferred to test-tube and 1 cm^3 of radioactive thymidine [^{3}H] solution was added. Thymidine concentration in a sample was 14 nM. Assimilation of radioactive thymidine by bacteria was estimated by Fuhrman and Azam method [10]. The amounts of taken thymidine formed a basis for estimation of DNA synthesis activity given that there is $2.5 \cdot 10^{-15}$g of DNA to one bacterial cell on average.

Estimation of cellular proteins synthesis. This procedure was carried out in comparable manner as DNA synthesis activity estimation, using labelled [^{3}H] leucine. Applied in the analysis leucine concentration was 15 nM. Assimilation of radioactive leucine by bacteria was estimated by Kirchman method [11]. The amount of leucine incorporated by bacterial cells formed a basis for measurement of produced protein. With this in view it was assumed that 1 mole of leucine constitutes 131.2 g and on this basis number of incorporated leucine moles was converted into leucine grams. Subsequently, obtained number of incorporated leucine grams was converted into grams of synthesized bacterial cells protein. The calculations were based on the assumption that leucine makes up 0.073 of bacterial protein mass on average [12].

Results and discussion

Results presented in this study regarding the UVB impact on DNA synthesis (Table 1) demonstrate that UVB radiation had hampering effect on DNA synthesis concerning all analysed strains of SM and SSW (Table 1). Experiments with HS application as potentially protective agent proved that DNA synthesis was still hampered, but inhibition amount was smaller than in case with no HS presence.

Among SM strains considerable decreased of inhibition at HS presence was observed in cases of 7 strains (2–7 and 9). For strains no. 8 and 10 noticed inhibition decrease was very slight. Strain no. 1 revealed continued further decrease of DNA synthesis activity at HS presence.

SSW strains response was very similar. Substantial inhibition decrease at HS presence was observed for 6 strains (11, 12, and 15–18). Strain no. 19 also demonstrated decreased inhibition at HS presence, but the difference was small. For strain no. 13 no difference was made, whereas for strains no. 14 and 20 the differences were insignificant.

Table 1

Influence of UVB radiation on activity of DNA synthesis

Strain No.	Control	UVB	UVB + HS
SM			
1	0.72*	0.08	0.06
2	**0.54**	**0.04**	**0.40**
3	**1.49**	**0.27**	**0.76**
4	**0.66**	**0.08**	**0.60**
5	**0.69**	**0.09**	**0.36**
6	**0.19**	**0.08**	**0.18**
7	**2.28**	**0.28**	**2.15**
8	2.29	1.04	1.14
9	**3.42**	**0.28**	**2.21**
10	3.05	1.32	1.86
SSW			
11	**0.52**	**0.18**	**0.32**
12	**1.62**	**0.02**	**0.31**
13	0.48	0.16	0.16
14	0.77	0.36	0.30
15	**1.30**	**0.19**	**0.96**
16	**4.27**	**0.08**	**0.90**
17	**1.07**	**0.20**	**1.00**
18	**6.30**	**0.07**	**2.21**
19	0.81	0.22	0.32
20	1.58	0.78	0.75

* – μg DNA.

Comparison of mean values (Fig. 1) received for SM and SSW strains confirms that regardless of the water layer of surveyed strains origination UVB radiation similarly hampered DNA synthesis. Data presented in the figure demonstrate also substantial differences of DNA synthesis inhibition degree depending on HS presence. Similar results concerning UV impact on DNA synthesis activity and/or secondary production of bacteria were published by Davidson [13], Herndl et al [3, 8] and Kaiser and Herndl [2].

The above-mentioned works comprise evidences of DNA synthesis activity decrease based on exposure of bacteria to UV radiation up to more than 50 % compared with a control sample [3], which remains in a full accordance with results of present study. The experiments using various fractions of organic matter as protective agents proved thymidine incorporation activity reduction based on UV effect and HS presence at as small degree as 20 % [8]. Also, investigations carried out in *in situ* conditions demonstrated significant limitations of bacterioneuston DNA synthesis activity [14].

Scully et al [7] reported that bacteria exposure to UVB radiation causes substantial decrease of survival. The same author proved that the survival was less hindered when fulvic acids solution was added to bacterial suspension. A study conducted by Vosjan

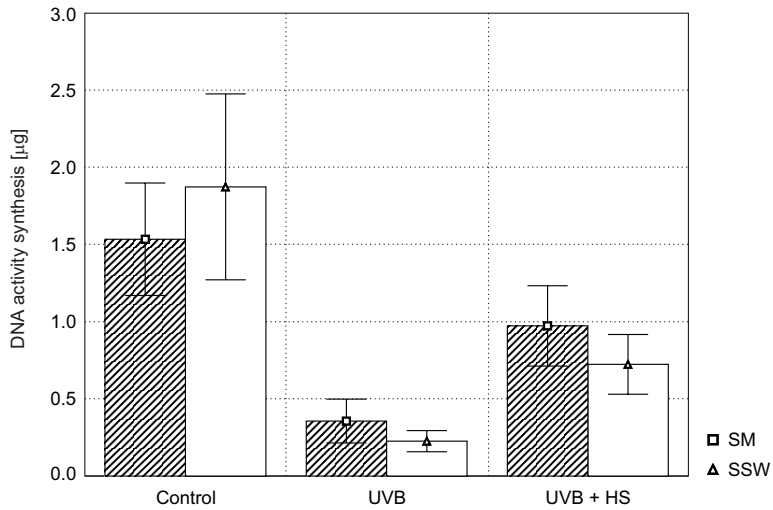

Fig. 1. Influence of UVB on DNA activity synthesis (verticalal bars represent standard error; SM – surface microlayer; SSW – subsurface water)

and Zdanowski [15] demonstrated that UV significantly hampered bacteria metabolic activity expressed in amounts of synthesized ATP.

Results of this study proved that UVB radiation has hampering impact on cellular proteins synthesis (Table 2).

Table 2

Influence of UVB radiation on activity of cellular protein synthesis

Strain No.	Control	UVB	UVB + SH
SM			
1	14.45*	7.49	0.57
2	19.00	12.17	5.05
3	2.62	1.43	1.07
4	59.23	30.95	6.51
5	156.34	41.74	3.78
6	14.25	5.90	5.31
7	44.84	34.92	27.20
SSW			
11	4.82	2.24	**2.64**
12	50.20	50.16	4.97
13	17.88	3.35	1.67
14	4.37	3.66	1.17
15	9.88	5.54	2.21
16	295.72	31.51	7.78
17	8.00	8.07	2.81

* – µg of protein.

For SM strains limitation of proteins synthesis under influence of UVB was noted in cases of nearly all strains, except for strain no. 7. Nevertheless, comparison of UVB operation impact on DNA and proteins synthesis activities clearly shows that proteins synthesis process is hindered to much lower degree than DNA. Similar results pertain to SSW strains. With respect to them two strains did not demonstrate any respond to UVB radiation (strains no. 12 and no. 17), whereas strain no. 14 reaction was very slight. Moreover, as previously stated, activity of cellular proteins synthesis by SSW strains exposed to UVB radiation was significantly less hindered that DNA synthesis process. Also, Denward's et al study [16] proves that leucine incorporation process was not significantly disturbed by UV radiation. There is also a study, which suggests that bacteria proteins synthesis process would undergo more intensively when affected by UV radiation [17]. That author, however exposed to radiation water sampled from a lake, not excluding bacteria. In such an experiment UVB operation resulted among other things in organic matter photooxidation. Low-molecular matter, produced in this way could have caused increase in general cells activity, including protein synthesis.

Obtained results of experiments with application of HS as potentially protective substance from UVB are completely different from those received in DNA synthesis investigations. Use of HS did not cause increase in protein synthesis activity and even further collapse of that activity was observed (Fig. 2). These statements pertain virtually to all surveyed bacterial strains, except for strain no. 11. Presented results indicate that HS can play a role of protective agent, but only for some selected kinds of metabolic activity. For others they not only do not constitute protective substances, but have even further hampering effect (Fig. 2).

Similar results making up an evidence for stronger UV impact on DNA than protein synthesis were published in connection with survey conducted *in situ* [14, 18]. Chrost and Faust [18] noticed substantial limitation of bacteria secondary production in the

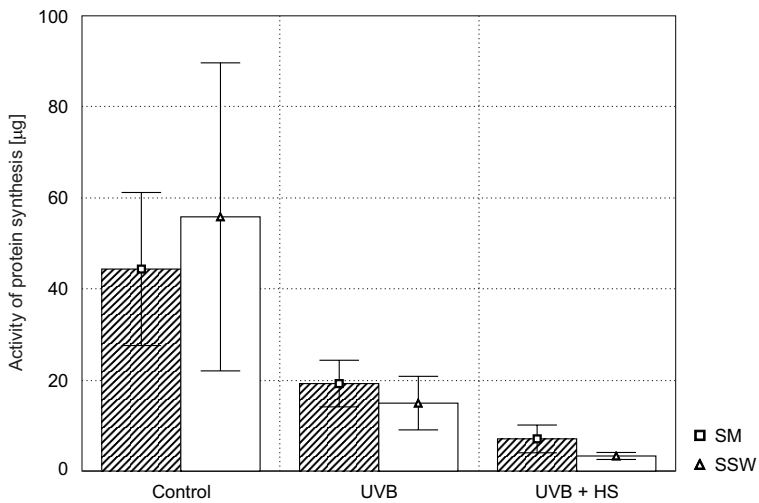

Fig. 2. Influence of UVB on cellular protein activity synthesis (verticalal bars represent standard error; SM – surface microlayer; SSW – subsurface water)

afternoon that is after strong solar radiation periods. Simultaneously the protein production level was stable. Analogous results signifying strong UV impact on DNA synthesis and weak impact on proteins synthesis of SM bacteria were registered during experiments conducted in twenty-four hour cycle [14].

To conclude it should be emphasised that UVB radiation has unfavourable impact on activity of DNA and cellular proteins synthesis. However, DNA synthesis process is much more sensitive to this radiation exposure. In addition, presented results prove that HS not always operate as protective substances. HS regarded as a filter of a kind, which delivers protection from UV [5, 6] demonstrate such impact only for some sorts of bacterial cells activity. For others they can act like additional inhibitor.

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WPŁYW UVB NA AKTYWNOŚĆ SYNTEZY DNA I BIAŁEK KOMÓRKOWYCH PRZEZ BAKTERIE ŚRODOWISKA WODNEGO

Zakład Mikrobiologii Środowiskowej i Biotechnologii
Uniwersytet Mikołaja Kopernika w Toruniu

Abstrakt: Badania prowadzono w 2007 r., pobierając próbki wody z MP i WPP w strefie pelagialu jeziora Brzeźno. Z pobranych próbkach wody izolowano reprezentatywną kolekcję szczepów, na których wykonywano dalsze badania. W toku badań oznaczano aktywność syntezy DNA i białek komórkowych bakterii poddanych promieniowaniu UVB. Badania prowadzono w dwóch układach doświadczalnych: bez substancji humusowych (SH) i w obecności tych substancji jako związków o działaniu potencjalnie ochronnych przed UV. Przeprowadzone badania wykazały, że pod wpływem promieniowania UVB aktywność syntezy DNA i białek komórkowych ulegały silnej inhibicji. Natomiast obecność SH inaczej wpływa na syntezę DNA i białek.

Słowa kluczowe: UV, synteza DNA, synteza białek, substancje humusowe, mikrowarstwa powierzchniowa

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