SOCIETY OF ECOLOGICAL CHEMISTRY AND ENGINEERING

ECOLOGICAL CHEMISTRY AND ENGINEERING A

CHEMIA I IN¯YNIERIA EKOLOGICZNA A

Vol. 18 No. 8

OPOLE 2011

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

ISSN 1898–6188

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SPIS TRECI

Wiêkszoœæ artyku³ów opublikowanych w tym zeszycie by³a przedstawiona w czasie XIII lub XIV Miêdzynarodowych Konferencji Naukowych METAL IONS AND OTHER ABIOTIC FACTORS IN THE ENVIRONMENT.

Spotkania te by³y zorganizowane przez Katedrê Ochrony Œrodowiska Uniwersytetu Rolniczego w Krakowie, w 2008 i 2009 roku.

Majority of papers published in the issue have been presented during the 13th or 14th Scientific Conferences on METAL IONS AND OTHER ABIOTIC FACTORS IN THE ENVIRONMENT, Krakow, in 2008 or 2009.

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 8 2011

Beata GRYGIERZEC[1]

EFFECT OF NITROGEN FERTILIZATION ON THE QUANTITY OF SEED YIELD OF SELECTED *Poa pratensis* L. CULTIVARS

WP£YW NAWO¯ENIA AZOTEM NA WIELKOŒÆ PLONU NASION WYBRANYCH ODMIAN *Poa pratensis* L.

Abstract: The paper presents a compilation of results obtained from field and laboratory experiments conducted in 2005–2007 at the Malopolska Plant Breeding Station in Skrzeszowice near Krakow (220 m a.s.l) on three forage cultivars of *Poa pratensis* (Skrzeszowicka, Duna and Balin). The investigations aimed at determining the effect of mineral fertilization mainly with nitrogen on the quantity of seed. The studies analysed three nitrogen fertilizer doses (60, 90 and 110 kg N · ha^{-1}) which were used as a whole or divided into two or three parts. Moreover assessed were several dates of nitrogen application: early spring, "under the panicle" and the autumn date.

Both the dose of nitrogen and the date of its application significantly affected the number of plants per 1 m^2. Mineral fertilization with nitrogen dosed 110 kg N · ha^{-1} and divided into two parts resulted in about 5 times greater seed yields than in the objects without fertilization obtained from Balin cultivar (577.4 kg · ha^{-1}) and Skrzeszowicka cultivar (543.9 kg · ha^{-1}). A significantly lower crop yield was produced by Duna cultivar (245.2 kg · ha^{-1}).

Keywords: *Poa pratensis*, cultivars, fertilization, seed yield

In the group of non-cereal grasses meadow grass is considered a species of high economic importance. High potential of its utilization for pasture and non-feed purposes creates demand for sowing material [1, 2]. The source which supplies the sowing material to cover the national demand is cultivar reproduction and import, which currently constitutes even 67 %. The current scale of grass seed imports is definitely too high. It results mainly from low productivity of native seed grass plantations and therefore low profitability of seed production. The key to improve the profitability of seed production is increasing seed yields whereas the element most determining the productivity is fertilization [3]. A particular role in this respect has been ascribed to nitrogen fertilizers. In order to prevent plant lodging, nitrogen plant fertilization should

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be optimized considering its amount, frequency as well as the date of the dose application with simultaneous regard to species characteristics [4]. The investigations attempted at an assessment the impact of nitrogen fertilization dose and the date of its application on the amount of seed yield of three meadow grass cultivars.

Materials and methods

The research was conducted in 2005–2007 at the Malopolska Plant Breeding Experimental Station – HPB in Skrzeszowice (220 a.s.l) near Krakow, on degraded chernozem developed from loess.

The soil revealed the following chemical properties: pH_{KCl} – 6.9; available P – 54, K – 124 and Mg – 46 g · kg^{-1}; organic N – 1.7 and total carbon – 15.7 g · kg^{-1} of soil.

The experiment was set up in the autumn 2004 using the randomised block method in four replications. The area of each plot was 10 m^2 (1 × 10).

The object of investigation was a seed plantation of three fodder cultivars of meadow grass (*Poa pratensis*): Skrzeszowicka (Eska 46SE), Duna and Balin.

Phosphorus, dosed 35 kg P · ha^{-1} as triple superphosphate (46 % P_2O_4) was used for fertilization conducted once in spring. Potassium, in the amount of 83 kg K · ha^{-1} as high grade potassium salt (60 % K_2O) was applied once, also in spring.

Nitrogen fertilization in the doses of 60, 90 and 110 kg N · ha^{-1} was applied on the following dates:

– once in the early spring;

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

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

Chemical weed control on the seed plantation, with a dose of 1 dm^3 · ha^{-1} of Aminopielik Gold, was conducted each year at the beginning of April (when the vegetation started) and in September. Before the seed harvesting, single weeds were hand removed. Passages and single annual meadow grasses (*Poa annua*) counted as persistent weeds of meadow grass seed plantations were point sprayed with a Roundup dose of 4 dm^3 · ha^{-1}.

In every year of the investigations, field observations and biometric plant measurements were conducted at the beginning of August. The number of plants was counted on individual plots on the length of 1 m, the length and width of panicles and the flag leaf were measured. The harvesting of the seed plantations was carried out in two stages. The first stage – hand cutting for cuts (cutting height 15 cm) was done at full kernel maturity and about five days later the cut cultivars were threshed using the Wintersteiger plot combined harvester. Harvested seed material was dried in a store-room. Dried kernels with water content below 14 % were rubbed to remove the hairs growing at the base of seedlings. The seeds prepared in this way were cleared on the winnower. The cleaning process involved separating kernels from chaff and spikelets unbroken during the earlier process. Subsequently, cleaned grains were weighed, the obtained yield was calculated per 1 ha and one thousand grain weight was determined.

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

The annual precipitation total for the period of the investigations ranged from 463.8 to 615.9 mm, whereas the precipitation total for the six months (April–September) from 345.2 to 537.6 mm. Average annual temperature reached from 6.5 to 6.8 ^{o}C and between 11.8 and 12.6 ^{o}C during the vegetation season.

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

Results and discussion

The level of nitrogen fertilization is a factor influencing generative shoot formation in meadow grass (*Poa pratensis*) seed plantations. In the Author's own research it significantly modified the number of generative shoots per area unit (Table 1). The greatest shoot density was registered after nitrogen application in a dose of 110 kg $N \cdot ha^{-1}$ divided into two portions (in the early spring and at the start of earing) in Skrzeszowicka c.v. – 346 shoots/m, which was 64 % more in comparison with the control object, 251 shoots/m in Duna c.v. (31 % more than in the control) and 341 shoots/m in Balin c.v., ie 56 % more than on the unfertilized object.

Table 1

The mean number of selected *Poa pratensis* cultivars (units)

Specification	Skrzeszowicka	Duna	Balin
Control	211	192	218
N_{60} – in one dose + $P_{35}K_{83}$	261	217	232
N_{60} – in two doses + $P_{35}K_{83}$	253	231	239
N_{60} – in three doses + $P_{35}K_{83}$	268	223	226
N_{90} – in one dose + $P_{35}K_{83}$	275	239	257
N_{90} – in two doses + $P_{35}K_{83}$	289	248	273
N_{90} – in three doses + $P_{35}K_{83}$	278	242	264
N_{110} – in one dose + $P_{35}K_{83}$	329	236	297
N_{110} – in two doses + $P_{35}K_{83}$	346	251	341
N_{110} – in three doses + $P_{35}K_{83}$	338	249	326
LSD (p = 0.05)	21.9	14.1	15.9

As stated by Marshall and Hades [5], grass seed plantations require nitrogen in the first place to form the adequate number of generative shoots. This thesis was confirmed by numerous literature sources [3, 6–8]. Interdependence of these features expressed by the correlation coefficient in the Wielkopolska region is very high, on the level r = 0.78 [8].

Nitrogen fertilization modifies also panicle structure, which beside generative shoot density is the most important factor determining the amount of seed yield on the plantation [3, 9]. It is evidenced by the correlation obtained in the Author's own

Table 2

The mean length and width of panicle of selected *Poa pratensis* cultivars [cm]

Specification	Skrzeszowicka		Duna		Balin	
	length	width	length	width	length	width
Control	4.2	4.6	5.0	5.8	4.7	5.0
N_{60} – in one dose + $P_{35}K_{83}$	5.7	6.5	6.6	5.9	5.8	5.2
N_{60} – in two doses + $P_{35}K_{83}$	6.4	6.9	7.0	6.2	7.0	5.6
N_{60} – in three doses + $P_{35}K_{83}$	6.0	6.9	7.4	6.0	6.1	6.5
N_{90} – in one dose + $P_{35}K_{83}$	7.0	7.1	8.0	6.1	8.5	6.9
N_{90} – in two doses + $P_{35}K_{83}$	7.6	7.1	9.2	6.4	9.0	6.8
N_{90} – in three doses + $P_{35}K_{83}$	8.7	7.5	8.3	6.5	8.4	7.0
N_{110} – in one dose + $P_{35}K_{83}$	8.4	8.2	9.1	5.9	9.3	6.8
N_{110} – in two doses + $P_{35}K_{83}$	8.8	8.5	9.7	6.5	9.7	7.5
N_{110} – in three doses + $P_{35}K_{83}$	8.0	8.3	9.3	6.6	9.0	7.3
LSD (p = 0.05)	0.83	0.77	0.88	0.99	0.64	0.89

Table 3

The mean yield [kg · ha^{-1}] and mass of thousand seeds [g] of selected *Poa pratensis* cultivars

Specification	Skrzeszowicka		Duna		Balin	
	yield	mass of thousand seeds	yield	mass of thousand seeds	yield	mass of thousand seeds
Control	117.5	0.235	98.1	0.221	121.7	0.231
N_{60} – in one dose + $P_{35}K_{83}$	254.9	0.263	134.7	0.278	276.4	0.284
N_{60} – in two doses + $P_{35}K_{83}$	279.8	0.308	156.3	0.349	295.1	0.320
N_{60} – in three doses + $P_{35}K_{83}$	269.3	0.295	147.2	0.300	280.3	0.286
N_{90} – in one dose + $P_{35}K_{83}$	346.7	0.331	161.9	0.313	357.8	0.330
N_{90} – in two doses + $P_{35}K_{83}$	395.4	0.351	208.5	0.350	412.5	0.358
N_{90} – in three doses + $P_{35}K_{83}$	357.2	0.335	186.3	0.332	367.9	0.346
N_{110} – in one dose + $P_{35}K_{83}$	487.4	0.340	214.5	0.339	536.2	0.343
N_{110} – in two doses + $P_{35}K_{83}$	543.9	0.344	245.2	0.346	587.4	0.350
N_{110} – in three doses + $P_{35}K_{83}$	497.1	0.348	223.4	0.352	567.3	0.351
LSD (p = 0.05)	15.2	0.017	9.3	0.016	8.9	0.017

research between the seed yield and the length and width of panicles (Table 4). In the Author's own research nitrogen fertilization significantly increased the length and width of panicles (Table 2). It was demonstrated that the elongation and width of panicles in *Poa pratensis* were most affected by nitrogen fertilization in an aggregate dose of 110 kg N · ha^{-1} applied in the early spring and at the start of earing. Under the influence of this dose panicles in Skrzeszowicka and Balin c.v. were elongated over twice and in Duna c.v. almost twice. Moreover, the width of panicles increased from 12 % in Duna c.v. to 85 % in Skrzeszowicka c.v.

Table 4

The value of corrleation coeffieient of selected features

Cultivars	Length and width of panicle	Number of plants per 1 m and seed yield	Length of panicle and seed yield	Width of panicle and seed yield	Seed yield and mass of thousand seeds	Length of panicle and mass of thousand seeds	Width of panicle and mass of thousand seeds
Eska 46	0.801	0.950	0.878	0.874	0.870	0.847	0.796
Duna	0.279	0.850	0.919	0.380	0.820	0.800	0.360
Balin	0.779	0.942	0.902	0.744	0.832	0.912	0.744

Poa pratensis seed yield is a synthesis of morphological and biological traits, influenced by nitrogen fertilization. It is also a cultivar feature [8, 10] so the production potential of cultivars results mainly from a considerable diversification in their ability to form the inflorescence shoots [11]. In the Author's own investigations, each dose of nitrogen fertilizers significantly diversified the seed yield (Table 3). The strongest response was registered under the influence of 100 kg N · ha^{-1} dose applied in the early spring and at the start of earing. On unfertilized treatments almost 5-fold higher seed yields were obtained from Balin c.v. (587.4 kg · ha^{-1}) and from Skrzeszowicka c.v (543.9 kg · ha^{-1}) and over twice higher from Duna c.v. (245.2 kg · ha^{-1}). On the other hand the highest thousand grain weight (about 50 % more than on the control) was registered as a result effect of the aggregate dose 110 kg N · ha^{-1}) applied three times: in the early spring, at the earing stage and in autumn.

Conclusions

1. Nitrogen fertilization in a dose of 110 kg N · ha^{-1}, applied on *Poa pratensis* seed plantations in the early spring and at the start of earing stage increased: generative shoot density by 31–64 %, width of panicles by 12–85 %, the length of panicles about twice and the seed yield from over 2 to almost 5-fold.

2. The highest seed yield per 1 m was generated owing to the inflorescence shoot density from 251 in Duna c.v. to 346 in Skrzeszowicka c.v.

3. The seed yield was positively correlated with the number of generative shoots per 1 m and also with the length and width of panicles.

4. The highest one thousand grain weight was registered under the influence of an aggregate nitrogen dose 110 kg N · ha^{-1} applied in the early spring, at the start of earing and in autumn.

5. The one thousand grain weight was positively correlated with yield and the length and width of panicles.

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WP£YW NAWO¯ENIA AZOTEM NA WIELKOŒÆ PLONU NASION WYBRANYCH ODMIAN ***Poa pratensis*** L.

Katedra £¹karstwa
Uniwersytet Rolniczy im. Hugona Ko³³¹taja w Krakowie

Abstrakt: Praca zawiera zestawienie wyników badañ polowych i laboratoryjnych przeprowadzonych w Stacji Ma³opolskiej Hodowli Roœlin – HBP w Skrzeszowicach ko³o Krakowa (220 m n.p.m.) w latach 2005–2007 z trzema pastewnymi odmianami wiechliny ³¹kowej (Skrzeszowicka, Duna, Balin). Badania mia³y na celu okreœlenie wp³ywu nawo¿enia mineralnego, g³ównie azotem, na wielkoœæ plonu nasion. W badaniach uwzglêdniono trzy dawki nawo¿enia azotem (60, 90 i 110 kg N · ha^{-1}), które stosowano jednorazowo b¹dŸ dzielono na dwie lub trzy czêœci. Ponadto ocenie poddano kilka terminów stosowania azotu: wczesnowiosenny, na pocz¹tku k³oszenia i termin jesienny.

Wielkoœæ dawki azotu, jak równie¿ termin jego stosowania wp³yn¹³ na iloœci roœlin na 1 m^2. Pod wp³ywem nawo¿enia mineralnego azotem w dawce 110 kg N · ha^{-1} dzielonej na dwie czêœci uzyskano w odniesieniu do obiektów nie nawo¿onych oko³o 5-krotnie wy¿sze plony nasion u odmiany Balin (587,4 kg · ha^{-1}) oraz u odmiany Skrzeszowickiej (543,9 kg · ha^{-1}). Istotnie ni¿szym plonowaniem odznaczy³a siê odmiana Duna (245,2 kg · ha^{-1}).

S³owa kluczowe: *Poa pratensis*, odmiany, nawo¿enie, plon nasion

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Vol. 18, No. 8 2011

Barbara HERMAN[1], Robert BICZAK[1]
and Piotr RYCHTER[1]

ROOT CELERY REACTION ON NaCl AND $CaCl_2$ SALINITY

REAKCJA SELERA KORZENIOWEGO NA ZASOLENIE NaCl i $CaCl_2$

Abstract: The aim of this research was to determine the effect of diverse salinity levels of the soil by NaCl and $CaCl_2$ on the chlorophyll, protein, sugars as well as ascorbic acid content in the root celery (*Apium graveolens* L.var. *rapaceum*. (Mill.) DC) leaves. In the pot experiment the plants were watered with a solution of sodium chloride and calcium chloride containing (100 and 300 mM NaCl) and (50 and 150 mM $CaCl_2$), respectively. The results have shown that contents of tested components in celery leaves to a large extent were dependent upon both the levels of basic salinity and used salt. Basic salinity of both NaCl and $CaCl_2$ caused decreased content of the total chlorophyll, protein and soluble sugars in leaves of celery; the stronger the soil salinity was the greater the change was. Soil salinity to large extend influenced also ascorbic acid level in the leaves of celery. Salinity of the soil by $CaCl_2$ at both doses caused an increase of ascorbic acid level of the celery leaves. The other reaction appeared using NaCl, at lower salinity concentration an increase of ascorbic acid was observed, whilst at higher salinity of the soil a decrease of this vitamin has been noticed.

Keywords: salinity, celery, chlorophyll, protein, sugars, ascorbic acid

Plants are subject to various biotic or abiotic stresses coming from their surrounding natural environment including heavy metals, unutilized wastes such as long degrading polymers as well as salinity [1–3]. Salinity is one of the major abiotic stresses affecting germination [4, 5] and plant growth [6, 8]. Plant growth inhibition, resulting in reduced crop yield, has been reported by many investigators [9–14]. Salinity inhibits plant growth in three principle ways: by ion toxicity (mainly of Na^+ and Cl^-), osmotic stress, and nutritional imbalance [15–17]. All of these cause effects on plant growth at physiological and biochemical levels [17].

An unfortunate consequence of salinity stress in plants is the excessive production of *reactive oxygen species* (ROS), such as superoxide, hydrogen peroxide and hydroxyl

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radical. These ROS can seriously disrupt normal metabolism through oxidative damage of macromolecules such as photosynthetic pigments, proteins, nucleic acids and lipids [6, 18–20]. Plants have evolved specific protective mechanisms, involving antioxidant molecules such as ascorbic acid, glutathione, carotenes, α-tocopherol, and enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase [4, 6, 19, 21].

Reactive oxygen species play a role in lipid peroxidation, membranes damage and consequently in leaf senescence [21]. Long-term exposure of plants to salinity leads to ionic stress, which can accelerate premature senescence of adult leaves, and also a reduction in the photosynthetic area available to support continued growth [22, 23]. Leaf senescence is marked by decreases in chlorophyll and protein concentration [11, 21, 24]. A decrease in protein content has often been reported as a response of plants to salinization [9, 10, 16, 22, 24–26]. Various researchers have also reported a decrease in chlorophyll content under salt stress [11, 16, 18, 22, 24, 27, 28]. Salinity has an adverse impact on photosynthetic processes inhibiting photosynthetic production (eg sugar) [22].

Calcium plays a crucial role in many plant physiological processes and is essential for plant growth [29]. High NaCl salinity has been shown to induce calcium deficiencies in different plants [15, 30, 31]. In this way, it is known that external application of calcium can ameliorate the adverse effects of salinity on diverse plants species [9, 11, 15, 30, 32].

In the present study, changes in chlorophyll, sugars, ascorbic acid and protein contents of leaves of root celery grown in NaCl and $CaCl_2$ spiked soils are described.

Experimental procedures

Root celery (*Apium graveolens* L.var. *rapaceum.* (Mill.) DC) Odrzanski variety was used in a pot experiment. Tests were conducted under greenhouse conditions using plastic pots with a capacity of almost 5 dm^3. Background parameters of medium were as follows: pH (KCl) = 6.7, 1.6 % humus, as well as macro element contents: 106 mg N, 125 mg P_2O_5, 155 mg K_2O per 1 kg of soil.

Four celery seedlings in the phase of 3–4 leaves were potted per pot in the second half of May. In the first half of July the soil in the pots was treated with sodium and calcium chloride solutions containing 100 and 300 mM NaCl and 50 and 150 mM $CaCl_2$, respectively. The soil of every pot watered maintaining average humidity to 70 % of water holding capacity

Fresh plant material was taken three times with weekly intervals (14, 28, 42 days after medium salinity). Total chlorophyll and ascorbic acid were determined in fresh plant material [33, 34]. In dry matter of plants contents of total soluble sugars according to the Luff-Schoorl method and total nitrogen (accounted onto protein) according to the Kjeldahl method were determined [34, 35].

Statistical analysis was performed on all the quantitative results. Analysis of variance was performed using the Fischer-test F and Tukey's test. The least significant differences among mean values were calculated at $p < 0.05$ confidence level.

Results and discussion

In the experiments applied soil salinity levels using both sodium chloride and calcium chloride affected total chlorophyll, soluble sugars, ascorbic acid and protein contents of root celery leaves (Tables 1–4, Fig. 1).

Measurements of total chlorophyll levels in leaves during the experiment showed changes in the contents of these pigments (Table 1). Changes were dependent upon both time of analysis and levels of soil salinity. Initially during plant growth total chlorophyll content has been observed to increase and thereafter to decrease. During the whole test period total chlorophyll content per fresh matter of leaves of root celery growing in media with both saline sodium chloride and calcium chloride were maintained at lower levels as compared with background soil. A decrease of total chlorophyll in celery leaves have been observed, related to the strength of the soil salinity (Fig. 1). Taking into account average values of total chlorophyll contents of all analysis periods, for

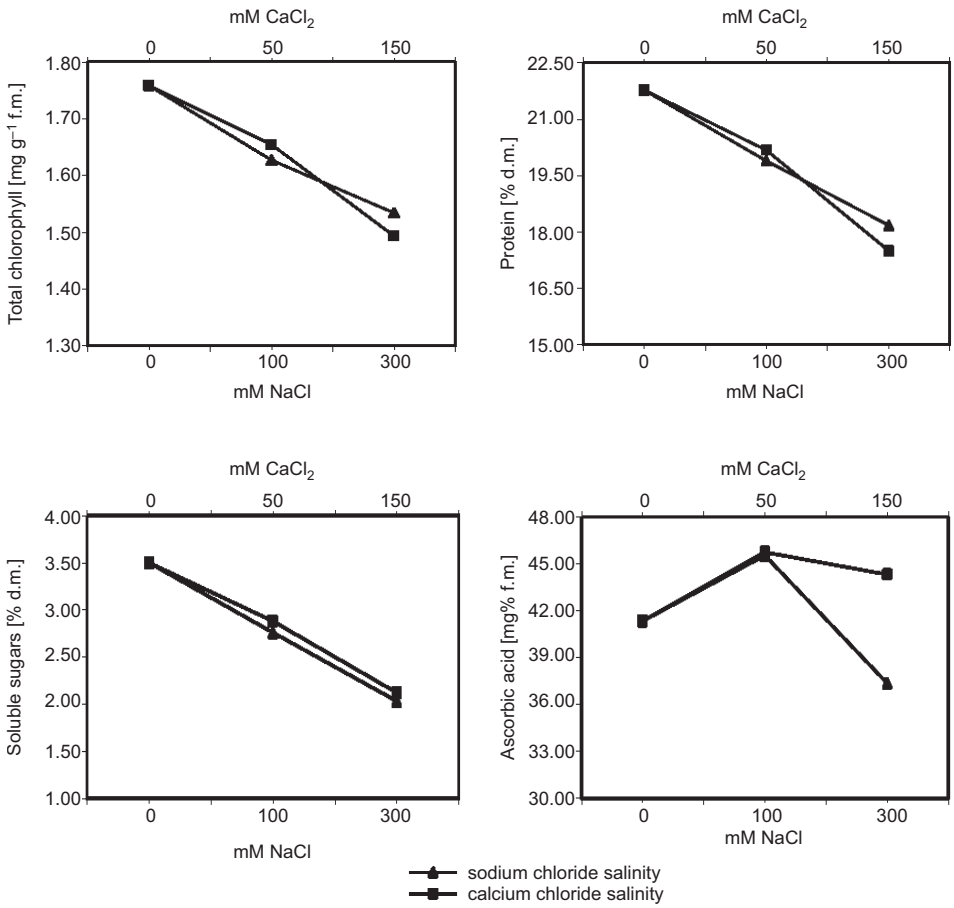

Fig. 1. Changes in nutrition values during exposure plant to salinity. Comparison of both applied salts

higher levels of salinity (300 mM NaCl and 150 mM $CaCl_2$) decreases close to 13 and 15 %, respectively have been noticed as compared with control plants.

Table 1

Changes of total chlorophyll content [mg g^{-1} f.m.] in leaves of root celery during salt treatments

Days	Salinity level of NaCl			Salinity level of $CaCl_2$		
	Background	100 mM	300 mM	Background	50 mM	150 mM
14	1.780	1.671	1.544	1.780	1.645	1.482
28	1.810	1.644	1.588	1.810	1.804	1.525
42	1.683	1.566	1.470	1.683	1.514	1.472
Average value	1.758	1.627	1.534	1.758	1.654	1.493
$LSD_{0.05}$	For time periods – 0.089 For salinity levels – 0.089			For time periods – 0.074 For salinity levels – 0.074		

Among reports concerning reactions of cultivated plants to medium salinity many indicate chlorophyll as an important factor for changes in physiology. The majority of the reports include affects of NaCl on soil, whilst barely a few concern $CaCl_2$ as a factor of salinity. Our results coincide with those obtained by many researches which found that soil salinity activated by NaCl lead to a decrease of total chlorophyll in plants [4, 5, 8, 9, 18, 22, 24, 27, 28, 36–39]. Unexpectedly addition of small amounts of $CaCl_2$ reduce the decrease of total chlorophyll content in leaves of crop plants [11]. Jimenez et al [40] have shown that a mixture of equal amounts of NaCl and $CaCl_2$ leads to a decrease of total chlorophyll content. Our results revealed that high levels of $CaCl_2$ strongly resulted in lowering total chlorophyll of root celery. A decrease of chlorophyll levels induces acceleration of senescence processes of leaves [11, 24].

Moreover, Lutts et al [24] report that salinity causing untimely senescence of leaves besides decreasing chlorophyll levels also induced lowering of protein content. Decrease of protein content is proportional to the extent of medium salinity as a result of NaCl stress [10, 16, 22, 25, 26, 41, 42], which is in agreement with results obtained in the present paper. The applied medium salinity using both sodium chloride and calcium chloride caused a lowering of protein levels in tested plants leaves (Table 2).

Table 2

Changes of protein content [% d.m.] in leaves of root celery during salt treatments

Days	Salinity level of NaCl			Salinity level of $CaCl_2$		
	Background	100 mM	300 mM	Background	50 mM	150 mM
14	22.98	19.59	16.58	22.98	19.64	16.55
28	21.12	19.01	17.69	21.12	19.44	16.68
42	21.22	21.04	20.26	21.22	21.44	19.23
Average value	21.77	19.88	18.17	21.77	20.17	17.49
$LSD_{0.05}$	For time periods – 0.83 For salinity levels – 0.83			For time periods – 0.81 For salinity levels – 0.81		

The changes observed were proportional to the extent of soil salinity (Fig. 1). Taking into account average values of protein levels of all analysis periods, for higher levels of salinity (300 mM NaCl and 150 mM $CaCl_2$) decreases of protein content of 17 and 20 %, respectively, in celery leaves have been noticed as compared with background.

It is commonly known that medium salinity influences crop quality [11, 12, 32, 43–45]. Vegetable nutrition includes not only protein content, but really important are also bioorganic factors such as carbohydrates and vitamins.

The results obtained for soluble sugar contents in leaves of root celery have shown that applied salinity levels of soil using both natrium chloride and calcium chloride significantly have decreased contents of these compounds. During long-term exposure to salinity the sugar content was the lower the higher medium salinity was (Fig. 1). Taking average values, sugar levels for all analysis periods at lower levels of salinity (100 mM NaCl and 50 mM $CaCl_2$) decrease by 21 and 18 %, respectively, in celery leaves as compared to background plants (Table 3). For higher medium salinity levels of $CaCl_2$ (150 mM) and NaCl (300 mM) lowering of sugar content was much more significant (40 and 42 %, respectively).

Table 3

Changes of soluble sugar contents [% d.m.] in leaves of root celery during salt treatments

Days	Salinity level of NaCl			Salinity level of $CaCl_2$		
	Background	100 mM	300 mM	Background	50 mM	150 mM
14	3.78	3.13	2.40	3.78	2.98	2.25
28	3.28	2.86	1.54	3.28	2.82	1.87
42	3.48	2.30	2.16	3.48	2.83	2.17
Average value	3.50	2.76	2.03	3.50	2.88	2.12
$LSD_{0.05}$	For time periods – 0.13 For salinity levels – 0.13			For time periods – 0.16 For salinity levels – 0.16		

A large numbers of publications are available focusing on changes in sugar metabolism of plants under medium salinity [4, 5, 16, 22, 23, 32, 44, 46]. However, no general rule can be derived from the data presented there. In one case sugar level decreased during increasing doses of salinity, on the other hand, it was also found these, the higher the salinity level the higher the sugar content. From the present investigations the results presented coincide with those obtained by Sultana et al [22] in which a decrease of soluble sugars under salinity has been noticed.

According to our results, soil salinity to a large extent, also influenced vitamin C levels of root celery leaves (Table 4). In case of this component varied results were observed. To a large extent it was dependent on salt type and soil salinity level (Fig. 1). For average values of ascorbic acid contents of all analysis periods for lower levels of salinity of both NaCl and $CaCl_2$ a 10 % increase as compared with background has been noticed. For higher levels of salinity changes were variable. Soil salinity stress due to NaCl caused an almost 10 % decrease of vitamin C whilst stress induced by $CaCl_2$ caused a 7 % increase. These results coincide with some earlier ones. Sairam and Srivastava [18] observed a decrease whilst Keles and Öncel [47] and Eraslan et al [7]

found an increase of the content of ascorbic acid under salt stress. Plants tolerance of salinity may induce changes in ascorbic acid levels. Vaidyanathan et al [6] have shown an increase of vitamin C in tolerant species independently of medium NaCl salinity level. In case of susceptible species increases of that component for lower levels of soil salinity have been observed.

Table 4

Changes of ascorbic acid content [mg^{-1} f.m.] in leaves of root celery during salt treatments

Days	Salinity level of NaCl			Salinity level of $CaCl_2$		
	Background	100 mM	300 mM	Background	50 mM	150 mM
14	33.14	39.90	29.33	33.14	39.24	36.19
28	40.66	43.22	37.33	40.66	46.65	43.59
42	50.18	53.48	45.42	50.18	51.28	53.12
Average value	41.33	45.53	37.36	41.33	45.72	44.30
$LSD_{0.05}$	For time periods – 3.51 For salinity levels – 3.51			For time periods – 2.79 For salinity levels – 2.79		

Conclusions

Medium salinity caused by sodium chloride and calcium chloride induced changes of total chlorophyll, protein, soluble sugars and ascorbic acid levels in leaves of root celery.

Changes of contents of the components determined were to a large extent dependent on soil salinity level.

Salt stress caused by both NaCl and $CaCl_2$ induced decreases of chlorophyll, protein and sugar contents in the tested vegetable. The stronger the soil salinity was the greater the decrease was.

Changes of levels of ascorbic acid in celery leaves were variable. For lower level salinity $CaCl_2$ and NaCl increases of that compound has been noticed.

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REAKCJA SELERA KORZENIOWEGO NA ZASOLENIE NaCl i $CaCl_2$

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Abstrakt: Przeprowadzone badania mia³y na celu okre¶lenie wp³ywu zró¿nicowanego zasolenia pod³o¿a NaCl i $CaCl_2$ na zawarto¶æ chlorofilu, bia³ka, cukrów i kwasu askorbinowego w li¶ciach selera korzeniowego (*Apium graveolens* L. var. *rapaceum.* (Mill.) DC). W eksperymencie wazonowym ro¶liny potraktowano doglebowo roztworami chlorku sodu (100 i 300 mM NaCl) i chlorku wapnia (50 i 150 mM $CaCl_2$). Uzyskane wyniki pozwalaj¹ wnioskowaæ, ¿e zawarto¶æ analizowanych sk³adników w li¶ciach selera by³a uzale¿niona od poziomu zasolenia pod³o¿a oraz od soli u¿ytej do zasalania. Zasolenie pod³o¿a zarówno NaCl, jak i $CaCl_2$ prowadzi³o do obni¿enia zawarto¶ci chlorofilu ca³kowitego, bia³ka i cukrów rozpuszczalnych w li¶ciach selera, przy czym odnotowane spadki zawarto¶ci tych sk³adników by³y tym wiêksze, im wy¿szy by³ poziom zasolenia gleby. Zasolenie gleby w du¿ym stopniu wp³ynê³o tak¿e na poziom kwasu askorbinowego

w li±ciach selera. Zasolenie gleby $CaCl_2$, przy obu dawkach, powodowa³o wzrost poziomu kwasu askorbinowego. Inna reakcja wyst¹pi³a pod wp³ywem NaCl, przy ni¿szym poziomie zasolenia obserwowano wzrost zawarto±ci witaminy C, podczas gdy przy wy¿szym poziomie zasolenia gleby odnotowano spadek zawarto±ci tej witaminy.

S³owa kluczowe: zasolenie, seler, chlorofil, bia³ko, cukry, kwas askorbinowy

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 8 2011

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EFFECT OF THE WAY OF UTILIZATION AND THE LEVEL OF FERTILIZATION ON THE QUALITY OF LEACHATE WATER PART I. THE CONCENTRATION OF MINERAL COMPONENTS IN LEACHATE WATER

WP£YW SPOSOBU U¯YTKOWANIA I POZIOMU NAWO¯ENIA NA JAKOŒÆ WÓD ODCIEKOWYCH CZ. I. STÊ¯ENIE SK£ADNIKÓW MINERALNYCH W WODACH ODCIEKOWYCH

Abstract: The investigations were conducted in Czarny Potok on the slope of Jaworzyna Krynicka (650 m above sea level, 20°55′32″ E, 49°24′51″ N). The experimental field was located on the brown, acid soil of the loamy sand granulometric composition, characterized with medium potassium concentration and very poor in phosphorus. Three different parts, as regards plant coverage, were distinguished in the experimental area: the grassland, the arable land and the forest. Analysing the monthly course, higher ammonia and nitrate nitrogen concentration could be noticed during the autumn, when the vegetation process is inhibited and the nitrate uptake less intensive. The phosphorus level in water was very low and ranged from several to a few dozen hundredth part of mg · dm^{-3}. When potassium, calcium and magnesium content in leachate water is taken into consideration it can be stated that it is affected by the amount of rainfall. The calcium and magnesium level in leachate water during the summer months is also influenced by the more intensive nitrification. Hydrogen ions from the sorptive complex displace the calcium and magnesium ions, which are transported with the rain water deep into the soil profile. Leachate waters from the particular variants differed as regards nitrate nitrogen, potassium, calcium and magnesium content. The highest level of these components was observed for leachate water from the arable land and from the forest.

Keywords: fertilization, plant coverage of the soil, water, macroelements

The agricultural and forest management in the mountain area, especially the proportions of certain cultivations in the total area as well as the kind and amount of fertilizers should be performed with respect to their influence on the natural environment. Special attention needs to be paid to the water reservoirs formed in this region, as

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they cover above 30 % of the total requirements for water in our country [1]. Inconsiderate agricultural activity can cause many negative changes, eg: soil erosion, anthropogenic eutrophisation, changes in landscape etc. The permanent grasslands, due to yearly plant coverage, protect soil against erosion, have purifying effect as regards water and air and constitute a part of mountain landscape. Hydrological functions of grasslands are especially important in respect of the significance of mountain regions as water resources [2, 3].

The aim of the conducted study was an estimation of the effect of soil plant coverage on the content of mineral compounds in leachate waters.

Materials and methods

The investigations were conducted in Czarny Potok on the slope of Jaworzyna Krynicka (650 m above the sea level, $20^{o}55'32''$ E, $49^{o}24'51''$ N). The experimental field was located on the brown, acid soil of the loamy sand granulometric composition. The maximum water capacity measured for undisturbed soil amounted to 59 %. The soil was characterized with medium potassium concentration and was very poor in phosphorus. The inclination of the slope, where the investigation was located was 4 % NE. The vegetation period in this region lasts from 150 to 180 days and the duration of snow cover was about 150 days. The rainfalls in 2007 amounted to 504.6 mm – for vegetation period and 930.5 mm – for the whole year and in the year 2008 – 480.3 mm and 781.9 mm, respectively.

Three different parts, as regards plant coverage, were distinguished in the experimental area: the grassland, the arable land and the forest.

The following variants were found on the permanent grassland:

– the meadow which was not fertilized and not mown (non-utilized meadow);

– the fertilized and mown $P_{25}K_{50}N_{120}$ meadow (utilized meadow).

The following crop rotation with $P_{25}K_{50}N_{120}$ fertilization was practiced on the arable land: root crops, cereals, cereals with undercrops and legumes:

– oat was sown in 2007 and potatoes planted in 2008.

Three parts were divided from the forest area:

– beech, spruce and mixed forests.

Not fertilized and not mown meadow is the object where no pratotechnical practices were done. The dominating species of its sward were tufted hairgrass (*Deschampsia caespitosa* L.), red fescue (*Festuca rubra* L.) and lady's mantle (*Alchemilla pastoralis* Bus.). The fertilized and mown meadow was every year fertilized with mineral fertilizers: phosphorus – once in the spring as triple superphosphate 46 %; potassium in the form of potassium salt 56 % in equal doses for the first and second regrowth; nitrogen as ammonium saltpetre 34 % – two doses: for the first regrowth – 60 % of the total nitrogen fertilizer and for the second regrowth – 40 %. Meadow fescue (*Festuca pratensis* Huds.) and kentucky bluegrass (*Poa pratensis* L.) were dominating species in the sward. The first regrowth was collected at the turn of II and III decade of June and the second during the III decade of August. The following doses of pre-sown fertilizers were applied for the arable land in 2007: 25 kg P $\cdot$ ha^{-1} phosphorus as triple

superphosphate 46 %; 50 kg K · ha^{-1} as potassium salt 56 % and 120 kg N · ha^{-1} in the form of ammonium saltpetre 34 %. The amount of nitrogen was divided into two doses: one was utilized before sowing and the second as top dressing at the stage of oat tillering. The sowing rate was 160 kg · ha^{-1}. In 2008 on the arable land intended for growing potatoes, cattle-sheep manure in an amount of 20 Mg · ha^{-1} was applied. The concentrations of nutrients in manure used were as follows: total N – 0.69 %; P – 0.14 %; K – 0.60 %; Ca – 0.25 %; Mg – 0.08 %; Na – 0.06 %. The forest experimental field was left without any operations except the measurements of leachate water.

Lysimeters collecting leachate water were installed at the beginning of the spring 2005 in three replicants for each of the above-mentioned experimental variants. Because of the low level of soil thickness lysimeters were installed by digging to a depth of 40 cm and the summary area of each of them was round-shaped of 50 cm diameter (1963 cm^2). The funnel bottoms of lysimeters were filled with gravel, water was removed using a tube to plastic containers located in the cellar. The rainfalls were measured using the Helman rain-gauge and measurements were conducted after each rainfall. In leachate water the content of the following components were evaluated: $N\text{-}NH_4$, $N\text{-}NO_3$ – using LF 205 microprocessor photometer (Slangi); P, K, Ca, Mg and Na – by ICP-AES method using JY 238 Ultrace apparatus. The obtained results were subjected to statistical analysis of one-way variance and LSD test at the significance level of $\alpha < 0.05$, using Statistica 7 software.

The results of investigations and discussion

Table 1 presents the concentrations of the particular mineral components in leachate waters collected in 2007 and 2008. Analysis of the results obtained for the leachate water from the leafy, coniferous and mixed forest area revealed that concentrations of all components were almost equal and the differences were not statistically significant. For these three variants the arithmetic mean was calculated and presented as “forest”.

On the meadow, the average ammonium nitrogen ($N\text{-}NH_4$) content for the vegetation period in water flowing through the soil profile was similar and the lowest on the level of 0.10–0.11 mg · dm^{-3}. The arable land and forest were characterized by a significantly higher $N\text{-}NH_4$ content (by above 50 %). High concentration of ammonium nitrogen in leachates observed during different seasons of the year for all variants can result from the high level of rainfalls and in consequence faster infiltration into the ground, limiting the proceeding of biochemical transformations of ammonium ions. However, the concentration of this component was close to that reported in literature [4–6].

The content of nitrate nitrogen ($N\text{-}NO_3$) in leachate water for both meadow objects was usually at the level of tenth parts of mg · dm^{-3}, less frequently it reached the level of few mg · dm^{-3}. Distinctly higher concentration (7–9 times higher) was observed for leachates from the arable land, and a dozen times higher for the forest area. Large variability of the concentration during the whole plant vegetation period can be noticed. The lowest values were characteristic for spring and winter months, whereas the highest were stated in summer and autumn. The low level of $N\text{-}NO_3$ in leachate water in spring resulted from intensive plant development and utilization of nitrate nitrogen for biomass

Table 1

The concentration of mineral components in leachate water [mg · dm^{-3}]

Variant	Year/Season	[mg · dm^{-3}]													
		$N\text{-}NH_4$		$N\text{-}NO_3$		P		K		Ca		Mg		Na	
	Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Non-utilized meadow	Spring	0.20	0.10	0.63	0.47	0.21	0.03	1.25	0.32	11.58	43.2	1.50	1.61	0.58	0.71
	Summer	0.20	0.12	1.63	1.24	0.13	0.03	1.08	0.62	19.37	68.8	1.98	2.45	0.93	1.05
	Autumn	0.08	0.06	0.61	0.14	0.28	0.02	1.09	0.25	61.59	69.2	3.03	1.58	1.14	0.74
	Winter	0.00	0.00	0.47	0.18	0.02	0.02	0.35	0.19	8.96	42.8	1.6	1.64	0.80	0.71
	Average	0.10^{a}		0.67^{a}		0.09^{b}		0.64^{a}		40.69^{bc}		1.92^{a}		0.83^{a}	
Fertilized meadow	Spring	0.12	0.10	0.41	0.88	0.43	0.05	2.39	0.18	9.65	9.28	1.05	1.09	0.67	0.67
	Summer	0.16	0.10	1.35	1.35	0.06	0.03	0.75	0.24	11.25	9.44	1.27	1.29	1.13	0.66
	Autumn	0.16	0.20	0.77	2.10	0.12	0.02	0.55	0.19	10.92	9.56	1.18	1.6	0.93	0.89
	Winter	0.00	0.08	1.11	0.43	0.04	0.02	0.56	0.53	7.88	8.32	1.04	1.09	0.72	0.57
	Average	0.11^{a}		1.05^{b}		0.10^{b}		0.67^{a}		9.54^{a}		1.20^{a}		0.78^{a}	
Arable land	Spring	0.28	0.29	2.26	0.88	0.16	0.03	1.04	0.54	24.87	16.76	2.34	2.08	0.81	0.97
	Summer	0.23	0.15	10.39	6.10	0.10	0.06	2.02	0.79	63.88	19.44	6.55	3.40	2.92	2.48
	Autumn	0.16	0.24	7.68	18.08	0.08	0.03	1.37	1.48	61.14	23.32	6.15	7.45	2.64	7.67
	Winter	0.00	0.00	4.75	0.68	0.03	0.02	0.96	0.42	33.52	10.40	2.94	2.34	1.90	1.02
	Average	0.17^{ab}		6.35^{c}		0.06^{a}		1.08^{b}		31.67^{b}		4.16^{b}		2.55^{c}	
Forest	Spring	0.18	0.14	10.85	8.81	0.09	0.03	3.95	3.32	38.52	31.32	3.68	2.95	1.99	1.28
	Summer	0.16	0.17	17.17	10.62	0.07	0.04	5.19	3.88	49.18	36.96	4.32	2.62	2.13	1.44
	Autumn	0.39	0.19	18.30	19.66	0.09	0.02	3.58	2.50	83.41	29.00	7.46	1.96	3.31	1.00
	Winter	0.00	0.03	3.62	6.33	0.06	0.03	2.75	2.68	41.00	27.36	3.03	3.28	1.98	1.56
	Average	0.16^{ab}		11.92^{d}		0.05^{a}		3.48^{c}		42.09^{bc}		3.66^{b}		1.84^{b}	

* Homogeneous groups according to LSD test, $\alpha < 0.05$, s.i. – statistically insignificant.

production. During the summer and autumn period the vegetation process proceeds slower than during the spring and in the autumn it is stopped, which results in less intensive uptake of this form of nitrogen. Additionally during the summer period the nitrification process is more intensive. During the autumn of 2007, concentration of $N\text{-}NO_3$ in leachate water from the arable land was stated to be 57 % lower than in the respective period of the following year. It was probably due to high C:N rate in post-harvest remains (oat straw), microorganisms which hydrolyze organic matter used up nitrogen, whereas in the winter higher level of $N\text{-}NO_3$ in leachate water from that object was observed because of microorganism decomposition.

The content of phosphorus in water was very low and ranged from several to a dozen parts of mg · dm^{-3}. Its average content for the meadow objects amounted to 0.1 mg · dm^{-3}, whereas for leachates from the arable land and forest it was 50 % lower. It results from the very low soil pH level (forest – pH_{KCl} 2.95). Such low pH causes the withdrawal of phosphorus compounds (chemical sorption), the P concentration in soil is also very low. During the experiment there was stated no effect of fertilization or kind of plant coverage on the increase of the phosphate level in leachate water, which is consistent with the results obtained by Czerwinski and Pracz [7].

The lowest concentration of potassium was stated for the water derived from the meadow objects, whereas water from the arable land was characterized by almost twice higher K content. The level of potassium in water from the forest amounted to 3.46 mg · dm^{-3} and was almost 6 times higher than the lowest stated value. When estimating the potassium level it can be found that it is affected by the amount of rainfall, because higher soil moisture favours the replacement and washing out of potassium ions [6]. Another factor influencing the level of this parameter is the level of potassium assimilation by plants.

Among all analysed components, calcium was present in the highest amounts. Similar results were obtained by Szymanska [8]. The lowest Ca level was observed in water from the utilized meadow – 9.54 mg · dm^{-3}. Water from the arable land contained 31.67 mg · dm^{-3} of this element. Ca concentration in leachates from the non-utilized meadow and the forest was above 4 times higher. Generally, the lowest concentration of calcium was observed in the spring and winter period.

The mean Mg content in water was 10–20 times lower when compared with the Ca content, but the major relationships between variants and seasons of the year were very similar. The level of Ca and Mg in leachate water was affected by the total amount of rainfall. Litynski and Jurkowska [9] in their study proved that rainfall is responsible for the greatest losses of these components in soil, because they are removed and washed out. The nitrification, more intensive during the summer period, also affects the washing out. Hydrogen ions displace calcium and magnesium ions from the sorptive complex and transport them with rain water deep into the soil profile.

The average sodium content in leachate water was below 1 mg · dm^{-3} for both meadows and above 2.5 mg · dm^{-3} for the arable land. Water obtained from the forest contained a twice higher level of this element when compared with the meadow variants. The highest concentration of sodium in leachate water (7.67 mg · dm^{-3}) was observed in the autumn 2008 in the arable land. This large amount of Na resulted from

decomposition of cattle-sheep manure, which is rich in this element (0.06 mg · kg). Manure was applied on this object at the end of April and during the spring and summer it was assimilated by potatoes.

The high level of minerals in leachate water for the forest object is elaborated in the second part of the present work entitled “The loads of components in leachate water”.

Conclusions

1. Intensive rainfall is the major factor influencing higher concentration of $N\text{-}NH_3$ in leachate water.

2. $N\text{-}NO_3$ content in leachate water is affected by the soil plant coverage as well as intensity of plant development.

3. The low phosphorus content and low pH of the soil determined the low level of this element in water.

4. Potassium, calcium and magnesium concentration in leachates was affected by the plant coverage of the soil as well as intensity of rainfall.

5. Sodium content in water is influenced by its content in the soil.

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WP£YW SPOSOBU U¯YTKOWANIA I POZIOMU NAWO¯ENIA NA JAKOŒÆ WÓD ODCIEKOWYCH CZ. I. STÊ¯ENIE SK£ADNIKÓW MINERALNYCH W WODACH ODCIEKOWYCH

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Abstrakt: Badania prowadzono w Czarnym Potoku u podnó¿a Jaworzyny Krynickiej (650 m n.p.m., 20°55′34″ E, 49°24′35″ N). Na polu doœwiadczalnym wystêpowa³a gleba brunatna o sk³adzie granulometrycz-

nym piasku gliniastego. By³a to gleba kwaœna, œrednio zasobna w potas, a bardzo uboga w fosfor. Na terenie doœwiadczalnym wydzielono trzy czêœci (ró¿ni¹ce siê okryw¹ roœlinn¹ gleby): u¿ytek zielony, grunt orny i las. Analizuj¹c przebieg miesiêczny, mo¿na zauwa¿yæ wiêksze stê¿enie azotu amonowego i azotanowego w miesi¹cach jesiennych, kiedy to proces wegetacji zostaje zahamowany, wiêc i pobieranie azotanów jest ma³o intensywne. Zawartoœæ fosforu w wodzie by³a bardzo niska i kszta³towa³a siê na poziomie kilku lub kilkunastu setnych czêœci mg · dm^{-3}. Oceniaj¹c stê¿enie potasu, wapnia i magnezu w wodzie odciekowej mo¿na stwierdziæ, i¿ zale¿y ono od iloœci opadów atmosferycznych. Na zawartoœæ wapnia i magnezu w wodzie odciekowej w miesi¹cach letnich ma wp³yw równie¿ nitryfikacja nasilona w tym okresie. Jony wodorowe wypieraj¹ z kompleksu sorpcyjnego jony wapnia i magnezu i przemieszczaj¹ je z wodami opadowymi w g³¹b profilu glebowego. Wody odciekowe z poszczególnych wariantów znacznie siê ró¿ni³y pod wzglêdem zwartoœci azotu azotanowego, potasu wapnia i magnezu. Najwiêcej tych sk³adników zawiera³y wody odciekowe z gruntu ornego i lasu.

S³owa kluczowe: nawo¿enie, okrywa roœlinna gleby, woda, makrosk³adniki

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ESTIMATION OF THE PERSISTENCE OF LIMING ACTION ON THE MOUNTAIN MEADOW

OCENA D£UGOTRWA£OŒCI DZIA£ANIA WAPNOWANIA NA £¥CE GÓRSKIEJ

Abstract: The study was conducted on a mountain meadow (altitude of 640 m) in the years 1999–2008. The investigations were located on the brown soil of the loamy sand granulometric composition. The soil was very acidic, the pH value amounted to 5.20 in H_2O and 4.28 in KCl. Two kinds of lime were used for liming in the autumn of 1998: calcium-magnesium carbonate in the amount of 1.5 Mg CaO and calcium oxide in the dose of 0.5 Mg CaO · ha^{-1}. Four variants were subjected to the study: control and 3 limed objects. Moreover, two kinds of fertilizers were also applied for two limed fields: phosphate-potassium – PK and phosphate--potassium-nitrogen – PKN. The meadow was mown twice a year. The evaluation of liming was carried out annually on the basis of the meadow yielding and soil pH after 3, 6 and 10 years from the treatment. Additionally, during the last year of the experiment, manganese, copper and cadmium content was determined in the soil from the control object and from the field where only liming was applied. The soil samples were collected from the layer of 0–15 cm. The investigations revealed that liming had no effect on the meadow yielding. On the other hand, it improved soil pH reaction, which increased by 0.4 – pH in H_2O and 0.4–0.7 – pH in KCl. The latter effect lasted for the following three years. The soil evaluation conducted after 10 years from the liming revealed significant decrease of soil pH. The lowest decrease was stated in the case of the object where only liming was applied, whereas the highest change was noticed for the object with full PKN fertilization. In the latter case the pH reached the value close to the level stated before liming. Liming had a beneficial influence as regards the manganese and cadmium concentration in the soil decreasing their levels by approximately 2.5 times. On the contrary, the copper level slightly increased under the action of liming.

Keywords: mountain meadow, liming, fertilization, yielding, soil pH

The meadow-pasture flora is quite tolerant as regards the soil pH reaction. Therefore, the efficiency of liming on the grasslands expressed as yielding is low [1–3]. The study of Gorlach and Curylo [1] revealed that the results of liming are visible in yielding only in the case of very acidic soils, when pH_{KCl} is below 4.0. The lack of liming effect on the plant yielding on the permanent grasslands can be explained by a high concentration of organic matter in the soil, which lowers the toxic action of aluminium and

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manganese. However, the forages produced on the acidic soils are characterized with weak quality and simultaneously are susceptible to the development of fungi as well as formation of nitrosamines. These facts fully justify the necessity of liming on the permanent grasslands.

The aim of this work was to estimate the effectiveness and persistence of liming effect on the mountain meadow.

Materials and methods

The study was conducted on a mountain meadow (altitude of 640 m) in the years 1999–2008. The investigations were located on the brown soil of the loamy sand granulometric composition. The soil was very acidic – the pH_{KCl} = 4.3. The experimental fields, except the control, were limed using 1.5 Mg CaO · ha^{-1} in the form of carbonate and 0.5 Mg CaO · ha^{-1} in the form of oxide. The dose of lime was calculated on the basis of the half of the hydrolytic acidity value of the soil (0.5 Hh). Four variants were subjected to the study: control and 3 limed objects. Table 1 and 2 present the scheme of the experiment. The following fertilization was applied in the meadow: 18 kg P – once in the spring, 66 kg K – in two equal doses – in the spring and for the second regrowth. The 100 kg · ha^{-1} dosage of nitrogen was divided: 60 % was used for the first and 40 % for the second regrowth. The meadow was mown twice a year. For the estimation of the soil pH as well as concentration of heavy metals the soil samples were collected from the layer of 0–15 cm. The soil reaction was determined after 3, 6 and 10 years and the content of heavy metals after 6 and 10 years from the liming. The dry matter content was evaluated by the drying method at 105 ^{o}C, crude protein content by the Kjeldahl method, and the content of heavy metals by ICP-AES (*inductively coupled plasma*) method.

Results

The productivity of the meadow was influenced mainly by the level of nitrogen, phosphorus and potassium fertilization (Table 1).

Table 1

Combined yield of dry matter and crude protein for 10 years [Mg · ha^{-1}]

Variant		Dry matter	Crude protein
Control		32.7	3.34
+ Ca	"0"	33.8	3.61
	$P_{18}K_{66}$	50.3	5.82
	$P_{18}K_{66}N_{100}$	64.8	7.51
LSD (p = 0.05)		6.92	—

The action of liming alone was not visible in the dry matter and crude protein yield during 10 years after it was applied. The fertilization with phosphorus and potassium

together affected 54 % higher dry matter yields and 74 % higher crude protein yield. On the other hand, the applied nitrogen dosage together with phosphate-potassium fertilization increase the yield of these components by 29 % as regards phosphate--potassium fertilization and 98 and 125 % in relation to the control.

The analyses of the soil conducted three years after liming revealed a distinct growth of the soil pH (Table 2). This pH reaction persisted on a similar level during the following three years and after the next four years it significantly decreased. The lowest pH decrease in respect to the value noticed 3 and 6 years earlier was stated for the object limed but not fertilized – 0.12 for pH in H_2O and 0.15 for pH in KCl. A higher difference was found for the object fertilized with phosphorus and potassium together, and the highest change – in the object where fertilization with three components ie nitrogen, phosphorus and potassium was applied. In the latter case the soil pH decreased to the level close to that stated 10 years earlier at the beginning of the experiment.

Table 2

Soil pH after 3th, 6th and 10th year of study

Variant		pH value					
		2001		2004		2008	
		H_2O	KCl	H_2O	KCl	H_2O	KCl
Control		5.20	4.28	5.20	4.25	5.19	4.26
+ Ca	"0"	5.60	4.90	5.90	4.85	5.78	4.70
	$P_{18}K_{66}$	5.60	5.00	5.60	4.96	5.50	4.52
	$P_{18}K_{66}N_{100}$	5.56	4.60	5.66	4.61	5.22	4.38

The effect of liming was also stated as regards the amount of some heavy metals assimilated in the soil (Fig. 1). The soil of the object limed, non-fertilized with nitrogen, phosphorus and potassium contained the same amount of copper and approximately 2.5

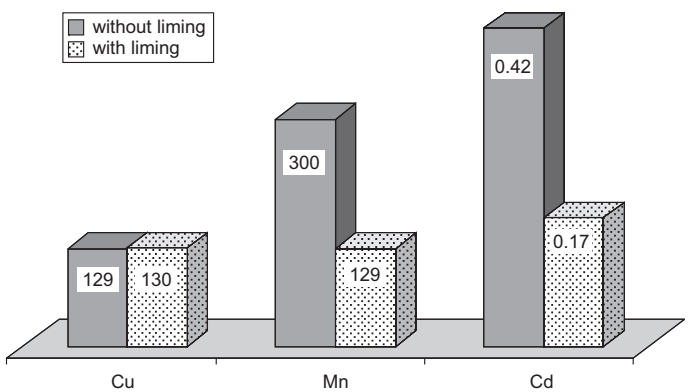

Fig. 1. The content of heavy metals as affected by the liming

times lower levels of manganese and cadmium when compared with the soil of the non-limed, control object. The concentrations of these elements were on a similar level when measured 6 years and 10 years after treatment [4].

Also other authors reported the lack of liming effect on the yielding of pastures and meadows [1, 2]. The stated lime action as a factor decreasing the soil acidity was close to the results obtained in the same region by Kopec [2]. The persistence of soil pH reaction on a similar level for 6 years after liming suggests that this phenomenon resulted from the slow movement of calcium deep to the soil profile [4]. 10 years after liming the greatest decrease of the soil pH in the object fertilized with phosphorus, nitrogen and potassium can be connected with the acidifying action of ammonium nitrate. The decreased level of assimilable cadmium and manganese, which show toxic action against soil, observed under the liming condition, should be recognized as a very positive phenomenon and consistent with the data found in literature [2, 5, 6]. On the contrary, liming did not affect the content of copper.

Conclusions

1. On the mountain meadow, the liming applied in a dose 2 Mg CaO · ha^{-1} did not influence the yielding but significantly affected and improved soil pH reaction, and decreased the amount of assimilable manganese and cadmium.

2. The persistence of liming effect was affected by the level of fertilization with other components. The application of nitrogen as ammonium saltpetre significantly shortened the effect of liming.

3. The effect of the lime dose in the amount of 2 Mg CaO · ha^{-1} on the chemical characteristics of the soil was significant even 10 years after treatment in the objects not fertilized with ammonium saltpetre, whereas the pH reaction of the soil from the object fertilized with ammonium saltpetre decreased at the same time to the level stated before liming.

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OCENA D£UGOTRWA£OŒCI DZIA£ANIA WAPNOWANIA NA £¥CE GÓRSKIEJ

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Abstrakt: Badania przeprowadzono na ³1ce górskiej (640 m n.p.m.) w latach 1998–2008. By³y one zlokalizowane na glebie brunatnej o sk³adzie granulometrycznym piasku gliniastego. By³a to gleba bardzo kwaœna, jej pH wynosi³o w H_2O – 5,20, a w KCl – 4,28. Do wapnowania ³1ki u¿yto dwojakiego rodzaju wapna: wêglanowo-magnezowego w iloœci 1,5 Mg CaO i tlenkowego 0,5 Mg CaO · ha^{-1}. Zabieg ten wykonano jesieni¹ 1998 r. W sumie badania obejmowa³y 4 warianty: kontrolê i 3 obiekty wapnowane. Ocenê wapnowania przeprowadzano corocznie na podstawie plonowania ³1ki i odczynu gleby po 3, 6 i 10. roku od zabiegu. W szóstym i ostatnim roku w glebie obiektów kontrolnego i z samym wapnowaniem dodatkowo oznaczono zawartoœæ manganu, miedzi i kadmu. Próbki gleby do analizy pobierano z warstwy 0–15 cm. Wapnowanie nie wywar³o ¿adnego wp³ywu na plonowanie ³1ki. Natomiast poprawi³o odczyn gleb zwiêkszaj¹c po 3 latach od wapnowania pH w H_2O o 0,4, a w KCl o 0,4–0,7 jednostki. Taki efekt tego zabiegu utrzyma³ siê przez dalsze 3 lata. Natomiast ocena gleby wykonana po 10 latach od wapnowania wykaza³a znaczne zmniejszenie pH gleby. Najmniejsza by³a ona w obiekcie wy³31cznie wapnowanym, a najwiêksza w obiekcie z pe³nym nawo¿eniem (PKN). W tym ostatnim obiekcie pH gleby zbli¿y³o siê do stanu sprzed wapnowania. Wapnowanie korzystnie wp³ynê³o na zawartoœæ w glebie manganu i kadmu zmniejszaj¹c ich iloœci oko³o 2,5-krotnie. Natomiast wapnowanie nie mia³o wp³ywu na zawartoœæ miedzi.

S³owa kluczowe: ³1ka górska, wapnowanie, nawo¿enie, plonowanie, pH gleby

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SELENIUM CONCENTRATION IN VARIOUS CARP (*Cyprinus carpio* L.) ORGANS

ZRÓ¯NICOWANIE ZAWARTOŒCI SELENU W NARZ¥DACH KARPI (*Cyprinus carpio* L.)

Abstract: In 2007 samples taken from carps (*Cyprinus carpio* L.) aged between one and two years with an average weight of 430 g were tested for selenium concentration. The fish was obtained from a 5 ha pond owned by the MILICZ Fish Breeding Farm, located in the Barycz River catchment. The following organs were taken from each 15 fishes: muscles, trunk kidneys, hepatopancrea, gills, intestine and spleen. Selenium concentrations were determined using the hydride vapour generation method. The highest selenium content was found in spleen (on average 0.848 mg Se · kg^{-1}), intestine (on average 0.651 mg Se · kg^{-1}) and kidney (on average 0.603 mg Se · kg^{-1}). The medium concentration, at 0.37 mg Se · kg^{-1}, was measured in hepatopancrea. Selenium was at its lowest concentration in gills and muscles.

Keywords: selenium, carp, tissues

Microelements are involved in important processes occurring inside the organism. Their presence is necessary for the proper functioning of the body and their lack may lead to various disorders. Selenium (Se) is one of such microelements. Its role was underestimated for a time; it attracted more attention when it was discovered that its deficiency brings about serious disorders, such as the Keshan or Kasin-Beck diseases [1]. Se deficiency has been found to be responsible for a number of ailments connected with various organs, tissues or systems, eg it weakens the immune system, causes necrosis, bone diseases, thyroid malfunctions, may lead to hypertension or cancer [2].

People in Poland consume insufficient amounts of Se [3]. The environment in most regions of country is poor in this element. A low Se concentration in soil reflected its poor amount in plants and herbivorous animals. The average person in Poland is estimated to eat about 40 µg Se per day, while the requirement is twice as high [3]. The amount of Se in the body depends on its uptake with food; in the case of fish – absorption from water through gills. Poland's inland waters contain on average 0.05 µg

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Se · dm^{-3} (0.01–0.08). In the region of Lower Silesia, near the Milicz town, there operates the biggest Cyprinidae production facility in Europe. Nowadays about 10 % of eaten carps stock is produced there. The milickie ponds receive water from local rivers. Se concentrations in this water are recognized – in accordance with the USEPA standards – as low [4].

Studies involving carps of various ages have proved that the carp's organs are capable of accumulating Se [5]. In this study the highest amounts were found in the kidneys only of two-year-old carps (K_2). Consequently it influenced the aim of our examination. It is interesting to find out whether it is a normal tendency to accumulate Se in two-year-old carps kidneys or it was caused by individual variety.

Low fish consumption is a typical tendency both in Poland and in the rest of the world, although this trend could be changed by popularization of health benefits of fish consumption. In beef, pork or poultry Se concentrations are lower [6, 7]. Considerably more Se was found in various fishes – striped Snake-head fish (on average 335 μg Se · kg^{-1} of wet mass), batrachian walking catfish (on average 473 μg Se · kg^{-1} of wet mass) [6]. People with microelement deficiency are commonly recommended to eat fish.

Studies regarding Se content have been conducted in respect of the liver, kidneys, heart, diaphragm, pancreas of various animals [8] but nothing is known about carp intestine or spleen.

The aim of work was determination of total Se concentration (II, IV, VI oxidation state) in the K_2 carp organs (*Cyprinus carpio* L.): muscles, gills, hepatopancreas, kidneys including intestine and spleen, and comparison of this microelement content between them.

Material and methods

Fresh fish were bought from the MILICZ Fish Breeding Farm. They were caught by means of fishing nets. The fishing nets were cast three times on three successive days in order to obtain a sample containing 15 carps (*Cyprinus carpio* L.) aged one-two years. Three water samples were taken from the pond to determine the basic physico-chemical parameters of the water. The following parameters were checked: reaction (204 pH-meter); electrolytic conductivity PN-EN 27888:1999; hardness – the Versenate method PN-ISO 6059; alkalinity PN-90/C-04540.03; calcium – the Versenate method PN-ISO 6058; magnesium PN-ISO 6059; copper, zinc – atomic absorption spectrometry with flame atomization PN-ISO 8288:2002 by means of a VARIAN 220FS atomic absorption spectrometer; sulfates – the ionite titration method; nitrates(III) – particle absorption spectrometry PN-EN 26777; nitrates(V) – particle absorption spectrometry PN-82/C-04576.08; ammonia – the direct Nessler method PN-C-04576-4; phosphates PN-EN 1189.

The following were obtained from the fish: gill arches from the left-hand side, entire hepatopancreas, muscles, intestine and spleen. After being rinsed in distilled water the organs were placed in a freezer at –18 ^{o}C; next they were wet mineralized with nitrac(III) acid [9] under a high-pressure, closed CEM MARS-5 microwave oven (USA). The pond water samples were also wet mineralized with nitrac(III) acid [9].

Se concentrations were determined using hydride generation atomic absorption spectrophotometry (HG AAS) by means of a VARIAN Spectra 220 FS. The methodology used followed that described by Diaz-Alarcon [10], in a closed system. The results were verified against DORM-2 reference material. The reference Se concentration in DORM-2 amounted to 1.40 ± 0.09 mg kg^{-1}, and in the applied analytic procedure: 1.32 ± 0.1 mg Se · kg^{-1}. Se concentrations were quoted in µg Se · kg^{-1} of wet mass – for carp organs. Correlations between the concentration of Se in individual organs were calculated and the significance of the differences was determined while studying fish from breeding ponds.

The results were presented by means of a box and whiskey plot. The centrally placed rectangle covers 50 % of the results, while the sides of the box are the lower and upper quartiles. The horizontal line in the rectangle stands for the median, with the whiskers extending from the minimum to the maximum data values.

The results were verified statistically (calculation of average values, standard deviations, significance of differences) using Statistica ver. 8.0.

Results and discussion

The Se concentrations in the water samples did not exceed 0.001 mg Se · dm^{-3}. The values must be regarded as low, as they fall within the standards prescribed for surface waters. The average values of the determined water parameters: water reaction – 7.4; electrolytic conductivity – 428 µS · dm^{-3}; hardness – 185.64 mg $CaCO_3$ · dm^{-3}; alkalinity – 140 mg $CaCO_3$ · dm^{-3}; calcium – 55 mg Ca · dm^{-3}; magnesium – 7.3 mg Mg · dm^{-3}; copper – 0.002 mg Cu · dm^{-3}, zinc – 0.003 mg Zn · dm^{-3}; sulfates – 43 mg SO_4 · dm^{-3}; nitrate(V) nitrogen – 0.04 mg N-NO_3 · dm^{-3}; nitrate(III) nitrogen – 0.05 mg N-NO_2 · dm^{-3}; ammonia nitrogen – 0.14 mg N-NH_4 · dm^{-3}; phosphates – 0.04 mg PO_4 · dm^{-3}.

In terms of the growing mean Se concentration, the organs can be ordered as follows:

gills < muscles < hepatopancreas < kidney < intestine < spleen [Table 1, Fig. 1]

High Se concentrations in the spleen may be connected with its impact of the activity of cytotoxic NT cells, which are produced in this organ [11] or its presence in the form of selenoprotein W [7].

Se is removed from the body via the kidneys in urine or via the intestine in faeces. The intestine is also involved in absorbing Se and transporting it to lipoproteids and albumines [7]; for this reason the amount of Se accumulated in its walls may be the resultant of absorption and secretion of the element.

Table 1

Selenium concentration [µg · kg^{-1}] in various carp organs

	Gills	Muscle	Hepatopancreas	Kidney	Intestine	Spleen
Max	331.50	668.66	781.85	1265.69	857.06	1291.97
Min	52.73	88.52	163.17	195.34	391.75	429.13
Medium	159.26	200.71	370.00	602.97	651.52	848.53
SD	85.39	146.55	181.70	312.25	156.48	198.84

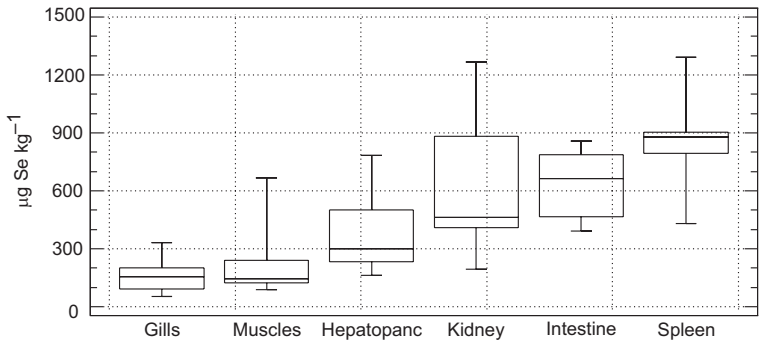

Fig. 1. Selenium concentration in various carp organs

Se was concentrated more in the kidneys than in the hepatopancreas. This tendency seems to occur in other animals as well. Research into Se concentration in the liver and kidneys of lambs indicated that the mean Se content was higher in the kidneys than in the liver [12]. Most probably the element is present in kidneys in the form of extracellular glutathione peroxidase or deiodinase [7] and, similarly to Se in the intestine, its amount may be linked to the secreting processes. Se concentration in the kidney was most strictly correlated with the amount of Se in muscles (0.84), gills (0.54) and hepatopancreas (0.47). The ratio of the kidney medium Se concentration to the hepatopancreas one was calculated as 1.6. This value was lower for 2-years carps than in the previous Author's research (2,7) [5] and was close to that of adult carps (1,4) [5]. It suggests a large individual differentiation of Se concentration in carp organs. Because of low Se concentration in water the amount of this element in gills is the lowest.

In muscles Se most probably forms part of the protein – selenomethionine W [7]. In the carps under study the content of Se in muscles was statistically much lower than in all of the studied organs except for gills. Similar results were obtained for the organs of chickens [13]. No differences were discovered in the order of organs accumulating highest Se concentrations. The organs were ordered as follows:

$$\text{muscles } (190 \pm 26\ \mu g\ Se \cdot kg^{-1}) < \text{pancreas } (240 \pm 35\ \mu g\ Se \cdot kg^{-1}) < \text{liver } (340 \pm 41\ \mu g\ Se \cdot kg^{-1}) < \text{kidney } (360 \pm 30\ \mu g\ Se \cdot kg^{-1})\ [13]$$

Although the lowest Se concentration was found in the muscles of the studied carps, it was similar to that determined for carps caught in Slovakia (243 µg Se · kg^{-1}), higher than that in Slovakian trouts (196 µg Se · kg^{-1}) [14], and similar to other fishes, eg the sea bass (*Dicentrarchus labrax*) – 250 µg Se · kg^{-1} of wet weight, in the case of which no Se supplementation was used [15], while by an order higher than Se concentration in lamb meat, which only confirms the opinion that fish should be eaten if Se deficiency is diagnosed.

Conclusions

The fact that the lowest concentration of selenium is to have been found in gills indicates that water is not the most important intake path of the element. Althought the selenium concentration in water is low, fish meet is able to accumulate significant amounts of selenium. The Se concentration in carp organs is almost the lowest in muscles but it is on a higher level than in other animals' meat.

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ZRÓ¯NICOWANIE ZAWARTOŒCI SELENU W NARZ¥DACH KARPI (*Cyprinus carpio* L.)

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Abstrakt: W 2007 roku zbadano próbki pochodz¹ce od kroczków karpia (*Cyprinus carpio* L.) o œredniej masie 430 g. Ryby pozyskano z 5 ha stawu Gospodarstwa Rybackiego MILICZ, po³o¿onego w zlewni rzeki Baryczy. Z ka¿dej z 15 ryb pobrano nastêpuj¹ce narz¹dy: miêœnie, nerkê tu³owiow¹, w¹trobotrzustkê, skrzela, jelito i œledzionê. Okreœlono zawartoœæ selenu metod¹ generacji par wodorków (HG AAS). W wyniku przeprowadzonych badañ stwierdzono najwy¿sz¹ zawartoœæ selenu w œledzionie (œrednio 0,848 mg Se · kg^{-1}), jelicie (œrednio 0,651 mg Se · kg^{-1}) oraz nerce (œrednio 0,603 mg Se · kg^{-1}). Œredni¹ koncentracj¹ selenu na poziomie 0,37 mg Se · kg^{-1} charakteryzowa³a siê w¹trobotrzustka. Najmniej selenu stwierdzono w skrzelach i miêœniach ryb.

S³owa kluczowe: selen, karpie, tkanki

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SELENIUM CONTENT IN HARD AND SOFT HAIR OF SILESIAN AND HOLSTEIN RACE HORSES

ZAWARTOŒÆ SELENU W SIERŒCI TWARDEJ I MIÊKKIEJ KONI RASY ŒL¥SKIEJ I HOLSZTYÑSKIEJ

Abstract: Hair samples were collected from the Silesian (Poland) and Holstein (Germany) horses. The samples were taken from tail and mane as well as sides of the body. Total and assimilated content of selenium(Se) were determined. Element content was from 76.1 to 1343.7 $\mu g \cdot kg^{-1}$.

The obtained results showed a significant difference between content of element among hard and soft hair of both types of horses. It was not found however the exceeding of Se content reference value in horses' hair.

Keywords: horses, hair, selenium

Selenium(Se) is the element which is a component of many enzymes participating in important metabolic processes in animals. The biological function of Se is connected with its presence in glutathionperoxidase, being an cellular membranes lipid anti-oxidant, playing similar role to vitamin E (α-tocopherol). Selenium is also the component of other oxidoreductive enzymes and cytochromes taking part in cells metabolism [1].

Selenium deficiency decreases the circulatory and immunological systems efficiency, causes liver necrosis and thyroid hypofunction. It can also be the factor enlarging the risk of neoplasmic diseases. Se excess causes anemia, atrophy, damage of skin formation (hair, nails) or blindness. All of them are the symptoms of alkaline disease – selenosis [2].

The quantity of element is correlated with its content in air, soil or food (fodder). It is possible to detect its presence on animals' body surface. Selenium is also accumulated

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"inside" – blood, in pulpous organs tissues or in hair. Persons who are vegetarians or pastured animals have usually small content of Se in their internal tissues [3, 4].

The toxicological analysis of classic biological materials, like blood or pulpous organs fragments gives only temporary values of studied elements [5]. The time aspect of their concentration changeability is lost in such investigations. The epidermis products like feather or hair have not got this "defect", because of keratin protection. It prevents the loss of components as well as the penetration of the dirt. Thanks to this the hair is "chemically solid" [6, 7].

Another advantage of hair analysis is the low invasiveness of material collecting process, which permits easy opinion about animals' health. Hair guarantees good accumulation of the elements (tissue deposit). There is mainly slow and one-way transport of mineral compounds. Hair mineral composition is not changed in short terms like hours or days [8].

The hair investigations can be a reliable source of information, alternative for blood, urine, milk or special liquids investigation [9].

Aim of work was estimation of Se content in hard and soft fur of Silesian and Holstein horses in different breeding environments.

Material and methods

Hair samples derived from hard and soft hair of Silesian and Holstein horses. Animals came from a farm on the suburbs of Wroclaw (Poland) – Silesian horses and from a farm near Schlezwig (northern Germany) – Holstein horses. The investigations were carried out on 12 Silesian and 10 Holstein horses.

The animals were kept in boxes, on bedding, with possibility of everyday playground on circular. Feeding was typical – hay, oat, pithy mixture and vitamin-mineral supplements [10]. Horses were used for riding and recreation. All animals represented a group of young horses (below 7 years old).

Two kinds of hair were taken from each animal – from tail and mane (hard and long hairs, irreplaceable annually) as well as from body sides (soft and short hair, replaceable every year). Hair samples were packed in paper envelopes and marked by codes.

Samples were cleaned initially as well as divided into 2 groups. One of them was washed, fat was removed in demineralised water with detergent addition, and finally material was rinsed 3 times. Washed hair was kept in normal temperature under perforated cover to dry. It was assumed, that Se quantity determined in it was assimilated quantity (related to hair structure).

Second part of hair samples was not washed. Se concentration in this group was qualified as total (the finding element in this type of hair was related both to the hair structure and to "external" environmental).

All samples were additionally crumbled and homogenized. Finally, prepared materials were ready for mineralization in microwave stove MARS-5.

1500.0 mg of material was put to special teflon containers. 5 cm^3 of concentrated nitric(V) acid 1:1 (Suprapur by SIGMA) was poured into all containers. After

mineralization liquid samples were transferred to clean PP test-tubes (Falcon type). Addition of HCl (Suprapur by SIGMA) caused Se reduction to 4th oxidation state.

Se concentration in investigated solutions was measured by the pair of hydrides generation method in the SpectrAA 220 FS apparatus (Varian).

The results were verified statistically (calculation of average values, standard deviations, significance of differences) using Statgraphic ver. 5.0 and GraphPad Prism ver. 5.1.

Results and discussion

The Se contents in hard and soft fur were given in Tables 1 and 2.

Table 1

Content of Se in hard fur [$\mu g \cdot kg^{-1}$]

	Total content		Assimilated content	
	x ± s	range	x ± s	range
Silesian horses	$356.9^{a} \pm 84.6$	220.0–498.8	$264.8^{a} \pm 63.2$	157.0–376.8
Holsein horses	$847.2^{b} \pm 232.7$	503.1–1343.7	$661.8^{b} \pm 218.7$	400.9–1024.5

a-b – $p \leq 0.01$ (between races).

Table 2

Content of Se in soft fur [$\mu g \cdot kg^{-1}$]

	Total content		Assimilated content	
	x ± s	range	x ± s	range
Silesian horses	$298.9^{a} \pm 34.7$	212.9–329.8	$224.9^{a} \pm 34.7$	195.0–309.9
Holsein horses	$156.0^{b} \pm 59.8$	104.6–278.5	$111.6^{b} \pm 47.0$	76.1–224.6

a-b – $p \leq 0.01$ (between races).

The selenium content in hard hair (from tail and mane) was from 220.0 to 1343.7 $\mu g \cdot kg^{-1}$. Holstein horses hair had average Se total quantity twice higher than Silesian ones ($p \leq 0.01$). Average of element assimilated content was from 157.0 to 1024.4 $\mu g \cdot kg^{-1}$. This quantity was also twice higher in Holstein horses' hair ($p \leq 0.01$). It is important that differences between total and assimilated content of Se for both races were 25.8 % and 21.9 % respectively.

In soft hair (from body sides) Se content was from 104.6 to 329.7 $\mu g \cdot kg^{-1}$. Se average total quantity in Holstein horses' hair was much lower than in Silesian ones ($p \leq 0.01$). Average of element assimilated content was from 76.1 to 309.9 $\mu g \cdot kg^{-1}$. This quantity was twice higher in Silesian horses' hair ($p \leq 0.01$). Differences between total and assimilated content of Se for both races were 24.8 % and 28.5 % respectively. It results from environmental influences.

Comparison of Se total and assimilated concentration between hard and soft hair does not show any significant differences for Silesian race ($p \leq 0.05$), but for Holstein horses this kind of differences were highly significant ($p \leq 0.01$). Moreover, average assimilated Se content for both kind of hair was varied for Silesian horses (244.8 $\mu g \cdot kg^{-1}$) and Holstein ones (386.6 $\mu g \cdot kg^{-1}$). It confirms better Se supply in German horses.

Detected Se contents in horse hair were approximate to values given for healthy horses living in non contaminated regions by Dunnett and Lees [5] – 370 $\mu g \cdot kg^{-1}$, Goullé et al [9] – 870 $\mu g \cdot kg^{-1}$, Hintz [11] – 700 $\mu g \cdot kg^{-1}$ as well as by Asano et al [12] – 581 $\mu g \cdot kg^{-1}$.

However Polish scientists Budzynska et al [13] and Budzynski et al [14] give much lower values – 160–162 $\mu g \cdot kg^{-1}$ in hair of Arabian horses (it shows probably deficiency of Se in feed and environment).

The analysis of Se content in hair of both horse groups shows an essential difference of the content of analysed element between them. It shows influence of environment, feeding and race (genotype). It was not affirmed overcrossing of Se content reference value in horses' hair passed by Asano et al [15] – 1302.5 $\mu g \cdot kg^{-1}$.

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ZAWARTOŒÆ SELENU(Se) W SIERŒCI TWARDEJ I MIÊKKIEJ KONI RASY ŒL¥SKIEJ I HOLSZTYÑSKIEJ

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Abstrakt: Zebrano i zbadano próbki w³osów z ogona i grzywy (w³osy twarde) oraz boków cia³a (w³osy miêkkie) koni rasy œl¹skiej (Polska) i holsztyñskiej (Niemcy). Oznaczono w nich ca³kowit¹ i przyswojon¹ zawartoœæ selenu (Se). Stwierdzono stê¿enia pierwiastka w zakresie 76,1–1343,7 $\mu g \cdot kg^{-1}$.

Otrzymane wyniki wskazuj¹ na statystycznie istotn¹ ró¿nicê zawartoœci Se wœród w³osów twardych, jak i miêkkich koni obu grup. Nie stwierdzono jednak przekroczenia wartoœci referencyjnych zawartoœci Se we w³osach koni.

S³owa kluczowe: konie, w³osy, selen

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CONTENT OF SELECTED ELEMENTS IN SEEDLINGS OF INBRED LINES OF WINTER RYE (*Secale cereale* L.)

ZAWARTOŒÆ WYBRANYCH PIERWIASTKÓW W SIEWKACH LINII WSOBNYCH ¯YTA OZIMEGO (*Secale cereale* L.)

Abstract: 9 inbred lines were chosen for the experiment (L176, L230, CH_7, L154, M353, L4, L299, L310, L28) of the winter rye S_{25} generation (*Secale cereale* L.). The 5 day-old seedlings of the line were treated with a cadmium sulfate solution at a concentration of 10^{-6} and 10^{-4} M for 36 h, and then placed on the Hoagland nutrient. The control materials were comprised of seedlings of the line growing on the same nutrient. The twenty-one day old seedlings were mineralized and the content of the following elements was marked: cadmium, magnesium, zinc, calcium, manganese and potassium, using the Perkin-Elmer1100 atomic absorption spectrophotometer. The lowest content of cadmium amounting to several mg/kg was observed in the control combination. The content of chemical elements in all inbred lines was higher at a lower concentration of cadmium 10^{-6} M/36 h (with the exception of L230 and L299 line).

Keywords: cadmium, rye, *Secale cereale* L, magnesium, potassium, calcium, zinc, manganese

Heavy metals are considered to be one of the main sources of pollution in the environment and are classified into two categories: essential (Fe, Cu, and Zn) and toxic metals (Pb, Cd, Ni and Cr) [1]. The excess of heavy metals in nature causes permanent damage of organisms. The elements of a very large degree of threat: Cd, Pb, Hg, Cr and Ni, accumulate in the kidneys, liver, brain and fat tissue of animals, interfering with their organisms' balance. In the case of plants their content in nature has increased mainly in cellular walls and vacuoles leading to a stunt in growth and development. The plants are characterized by a constant cation equilibrium, which is expressed by the stability and chemical ingredients. The changes in the equilibrium may be caused by the

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intake of heavy metals from the environment [2]. According to Kozanecka [3] and Krawczyk [4] plants growing in the same conditions accumulate heavy metals in various ways. This is determined by the features of a given species, which have been defined as the "chemical fingerprints" of plants [5].

The aim of the present work was to establish if the genetically differentiated inbred lines of winter rye, exposed to the action of cadmium stress, differ on account of the content of chosen chemical elements in the seedling stage.

Materials and methods

9 inbred lines of winter rye (*Secale cereale* L.) of the S_{25} generation were chosen for this experiment (L299, L230, L176, CH7, L154, M353, L4, L310, L29). Next, 25 (5-day) seedlings of each line were placed in three combinations: the control – the Hoagland nutrient (macroelements: $Ca(NO_3)_2 \cdot 4H_2O$, KNO_3, $MgSO_4 \cdot 7H_2O$, $NH_4H_2PO_4$, 1 % $Fe_3(C_6H_5O_7)_2 \cdot 6H_2O$ and microelements: H_3BO_3, $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, NaCl, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$), the same nutrient at a two concentrations of cadmium sulphate:10^{-6} M and 10^{-4} M for 36 hours. After this time all seedlings were placed on the Hoagland nutrient. The 21-day seedlings of each combination were dried, mineralized and the content of the following elements was marked: cadmium, magnesium, zinc, calcium, manganese, and potassium with the application of the atomic spectrophotometric absorption technique.

In total, 27 combinations were analyzed. The results were given as mean values of 3 experiments.

Results and discussion

The influence of the toxic action of cadmium was seen of seedlings treated with this metal (especially at the cadmium concentration of 10^{-4} M/36 h). These seedlings were characterized by a reduced turgor, a lighter colour of leaves, and the browning of roots. This is confirmed in the work [6]. In the seedlings of inbred lines of rye, the growth of cadmium content was observed with of the increased at concentrations of this element in the nutrient (Table 1).

Table 1

The content of cadmium ions [mg/kg] in inbred lines of rye

Combination	Inbred lines of rye [mg/kg]								
	L176	L230	CH_7	L154	M353	L4	L299	L310	L29
Control	0.64	0.6	0.3	0.17	0.4	0.98	0.34	0.5	0.07
Cd 10^{-6}, 36 h	16.5	14.6	35.2	20.2	22.9	23	16.4	26.6	35
Cd 10^{-4}, 36 h	540.4	664.8	419.5	465.3	627.3	525.6	596.3	675.5	540.9

The most cadmium was accumulated by the lines at the highest concentration of this metal 10^{-4} M/36 h and amounted to 419.5 mg/kg (CH_7) to 675.5 mg/kg (L310). A considerably lower content of cadmium in seedlings of inbred lines of rye was noted

at a concentration of 10^{-6} M/36 h, which ranged from 14.6 (L230) to 35.2 mg/kg (CH_7). The CH_7 line also had the lowest content of ions of cadmium at the highest concentration. However, in the control, traces of this element, were observed at acceptable levels (0.05–1 mg/kg). The rye lines were characterized by a varying sensitivity to the action of cadmium ions.

The content of the biogenic elements (Mg, Zn, Mn, K and Ca) in seedlings of the inbred line of rye was several times higher in combinations with cadmium in comparison with the control (Fig. 1–5). Differences in the accumulation of these elements in seedlings depended on the dose of cadmium. Most of the lines accumulated more zinc, magnesium, calcium, manganese and potassium with a lower concentration of cadmium 10^{-6} M/36 h. An exception to this were lines L299 and L230, which contained the most zinc at a concentration of $Cd10^{-4}$ M/36 h. According to Jang et al [7] and McKenna et al [8] the most probable reason for such a high content of zinc may be connected with joint penetrating canals for both elements and their mobility in plants.

A similar dependency took place in the case of calcium and manganese, whose highest content was observed in combinations of cadmium at a concentration of

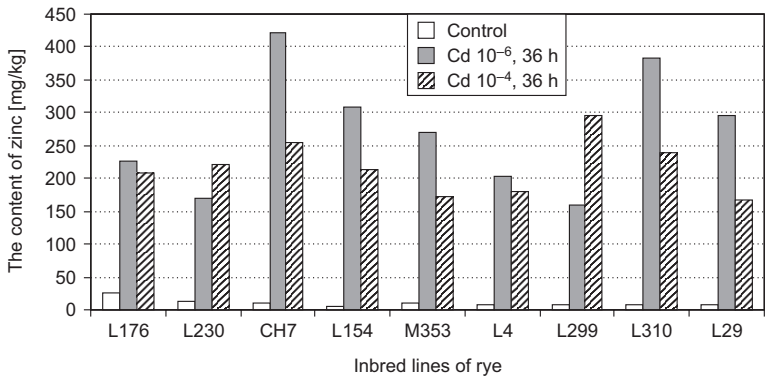

Fig. 1. The content of zinc ions [mg/kg] in inbred lines of rye

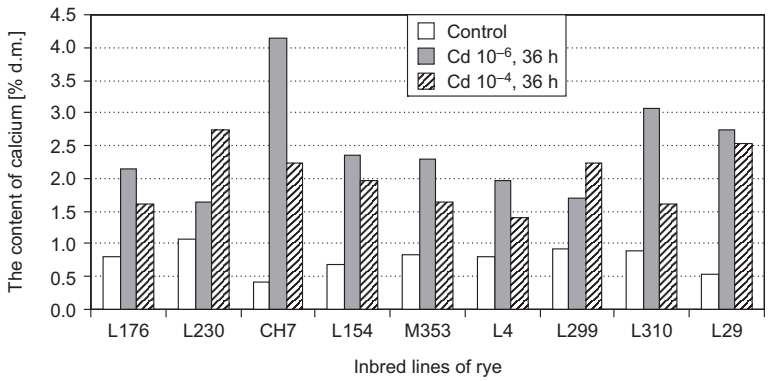

Fig. 2. The content of calcium ions [% d.m.] in inbred lines of rye

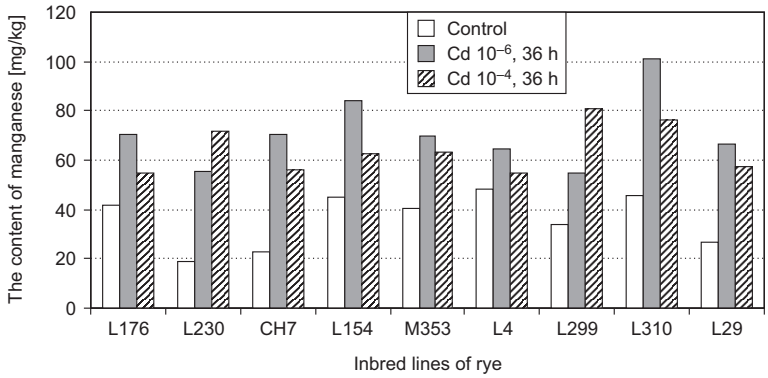

Fig. 3. The content of manganese ions [mg/kg] in inbred lines of rye

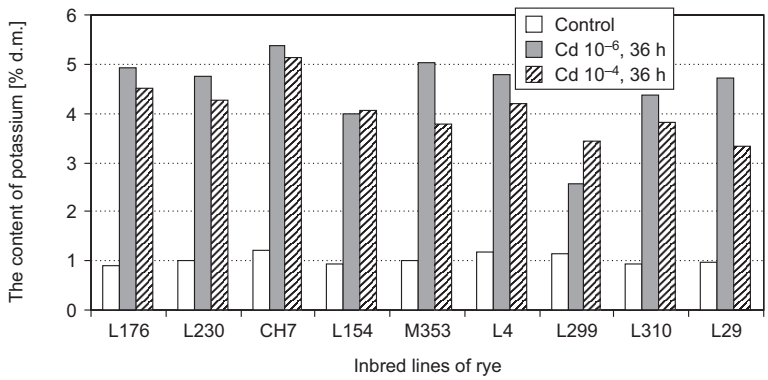

Fig. 4. The content of potassium ions [% d.m.] in inbred lines of rye

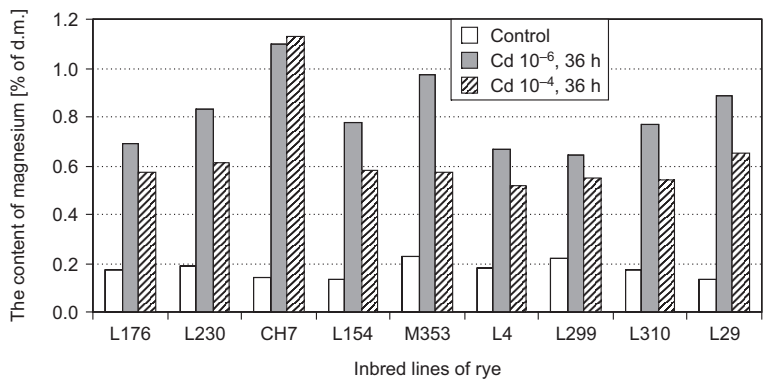

Fig. 5. The content of magnesium ions [% d.m.] in inbred lines of rye

10^{-6} M/36 h, whilst lines L299 and L230 constituted an exception (Fig. 2 and 3). At a lower concentration of cadmium the content of potassium increased several times in most lines. However, the growth of this element at the Cd concentration (10^{-4} M/36 h) was not observed in line L299 only (Fig. 4).

In the case of magnesium, a 6-fold increase of its content was observed in rye seedlings treated with cadmium at concentrations of 10^{-6} M/36 h, whereas at concentrations of 10^{-4} M/36 h only in line CH_7 (Fig. 5). In most lines the content of potassium and magnesium lessened with the growth of the dose of cadmium. Availability of biogenic elements and cadmium in the nutrient probably led to their excess intake by seedlings. The quite short time of their growth (21 days) did not allow the plants to remove or bind the ions of these elements.

In general, plants are more resistant to excess microelements concentration than to their deficit. In the case of heavy metals eg cadmium it increases over critical values in soil led to chlorotic leaves, deformity of roots and the consequent the growth stunt of rye lines. According to Kabata-Pendias and Pendias [9], the sensitivity of plants to excess heavy metals is associated with damage to the photosynthesis apparatus, which in effect disrupts the metabolic process.

On the basis of the results obtained it can be said that rye inbred lines of various genotypes accumulated the researched elements in seedlings in various ways. This is confirmed in the results of Kozanecka [3] and Krawczyk [4], according to whom plants growing in the same environment react differently to the action of heavy metals.

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ZAWARTOŒÆ WYBRANYCH PIERWIASTKÓW W SIEWKACH LINII WSOBNYCH ¯YTA OZIMEGO (***Secale cereale*** L.)

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Abstrakt: Do doœwiadczenia wybrano 9 linii wsobnych (L176, L230, CH_7, L154, M353, L4, L299, L310 i L29) pokolenia S_{25} ¿yta ozimego (*Secale cereale* L.). Piêciodniowe siewki linii traktowano roztworem siarczanu kadmu o stê¿eniach 10^{-6} i 10^{-4} M przez 36 h, a nastêpnie przenoszono je na po¿ywkê. Materia³ kontrolny stanowi³y siewki linii rosn¹ce na po¿ywce Hoaglanda. Dwudziestojednodniowe siewki mineralizowano i oznaczono zawartoœæ nastêpuj¹cych pierwiastków: kadm, magnez, cynk, wapñ, mangan i potas, przy

u¿yciu spektrofotometru absorpcji atomowej firmy Perkin-Elmer1100. Najni¿sz¹ zawartoœæ kadmu wynosz¹c¹ kilka mg/kg zanotowano w kombinacji kontrolnej. Zawartoœæ biogennych pierwiastków by³a wy¿sza u wszystkich linii wsobnych ¿yta w ni¿szym stê¿eniu kadmu 10^{-6} M/36 h (z wyj¹tkiem linii L230 i L299).

S³owa kluczowe: kadm, ¿yto, *Secale cereale* L., potas, mangan, wapñ, cynk, magnez

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EFFECT OF SILVER NANOPARTICLES ON THE MORTALITY AND PATHOGENICITY OF ENTOMOPATHOGENIC NEMATODES

WP£YW NANOCZ¥STEK SREBRA NA ŒMIERTELNOŒÆ I W£AŒCIWOŒCI PATOGENNE NICIENI ENTOMOPATOGENNYCH

Abstract: The effect of silver nanoparticles on the mortality of entomopathogenic nematodes *Heterorhabditis bacteriophora* from Nematop biopreparation and *Steinernema feltiae* from Owinema biopreparation was researched. It was found that mortality depends on nano-Ag concentrations and on the time of larval contact with them. In this study the effect of different concentrations of nano-Ag on pathogenic properties of entomopathogenic nematodes was also studied. No significant differences were observed.

Keywords: entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Steinernema feltiae*, Nematop, Owinema, silver nanoparticles, nano-Ag

Entomopathogenic nematodes are the natural component of soil mesofauna and an important factor limiting insect density [1]. Steinernematidae and Heterorhabditidae are associated with mutualistic bacteria *Xenorhabdus* and *Photorhabdus*, respectively [2]. Preparations made from entomopathogenic nematodes are the safest means of pest control. Nematodes have many advantages including simple and cheap productive cultures, a wide range of hosts and safety for the environment and higher organisms [3].

The development of nanotechnologies is now being observed worldwide. Nanotechnology is a discipline dealing with particles of 1 to 100 nm (1 nm = 1×10^{-9} m) which are named nanoparticles. Nanotechnology has a great impact on biological sciences and more and more nanomaterials are used in medicine, pharmacy and agriculture [4, 5]. Silver is a noble metal whose antibacterial properties have been known since the ancient times. In the ionic form silver might be toxic for organisms but

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silver nanoparticles have a broad spectrum of biological properties even at low concentrations [6].

Material and methods

The effect of silver nanoparticles on the mortality and pathogenic properties of entomopathogenic nematodes *Heterorhabditis bacteriophora* (Poinar 1976) and *Steinernema feltiae* (Filipjev 1934) was studied in experimental conditions. Colloidal silver nanoparticles came from the firm Nano-tech Polska Sp. zo.o. Silver nanoparticles suspended in deionised water in concentrations of 5 ppm and 0.5 ppm were used in the experiments. *H. bacteriophora* originated from biopreparation Nematop of the German firm E-nema and *S. feltiae* came from Owinema made by OWIPLANT in Owinska near Poznan.

Experiment 1 was carried out during 5 days under laboratory conditions at a temperature of 25 ^{o}C. Larvae of the 3rd invasive growth stage (IJs) were placed in water solutions containing the appropriate concentration of nano-Ag. The control group consisted of larvae kept in distilled water. Samples of solution were taken and nematodes mortality was estimated every day. Tests were performed in 5 repetitions. After 5 days the nematodes that survived the contact with nano-Ag were separated by sedimentation. Nematodes *H. bacteriophora* obtained from nano-Ag solution of 5 ppm were neglected since their number was insufficient for further experiments. Live nematodes obtained in that way were used to infect the caterpillars of *Galleria mellonella* of a mean weight of 140–160 mg.

Experiment 2 was performed in Petri dishes of a diameter of 9 cm lined with filter paper in which 10 insects were placed. Five hundred invasive larvae (IJs) of the appropriate nematode species were added to each dish, which made 50 IJs/insect. Tests were made in 5 repetitions. Mortality was controlled during 5 days. Dead insects were transferred to empty dishes and placed in the incubation chamber for 48 h. Then the insects were dissected to check whether nematodes were the cause of their death. The experiment was performed at 25 ^{o}C and 90 % relative humidity of the substratum. The control consisted of insects in the respective growth stage infected by nematodes which had no contact with nano-Ag. The mortality, the extensiveness and intensity of infection of *G. mellonella* larvae by two species of entomopathogenic nematodes were analyzed.

The obtained results were statistically processed with the SPSS 15.0 programme (multifactor ANOVA, Chi^2 test, Tukey test). Statistical significance was tested at $p < 0.05$.

Results and discussion

Nematodes mortality in solutions of silver nanoparticles (5 ppm, 0.5 ppm) was analysed every day during 5 days. The mortality of entomopathogenic nematodes increased with increasing concentration of nano-Ag (Figs. 1 and 2). The highest concentration of nanoparticles (5 ppm) caused 99 and 96 % mortality in *H. bacteriophora* and *S. feltiae*, respectively on the fifth day of experiment. Lower concentration

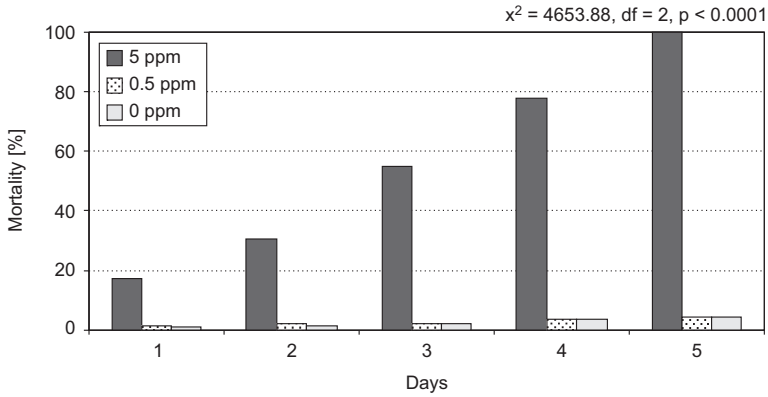

Fig. 1. The effect of nano-Ag on the mortality of the IJs of *Heterorhabditis bacteriophora* (test Chi^2 refers to the last day of experiment)

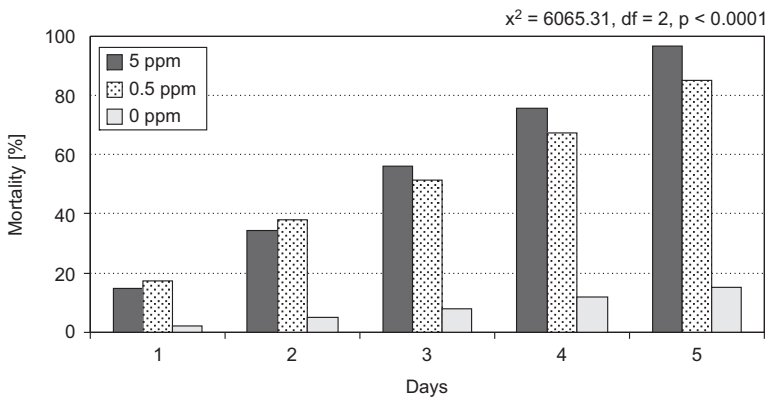

Fig. 2. The effect of nano-Ag on the mortality of the IJs of *Steinernema feltiae* (test Chi^2 refers to the last day of experiment)

caused much lower mortality of 4 % in *H. bacteriophora* but 85 % mortality in *S. feltiae*. Nematodes mortality measured on the last day of experiment in the control was 4 % in *H. bacteriophora* and 15 % in *S. feltiae*. In the nearest future, studies on nano-Ag accumulation in nematodes bodies are planned.

Entomopathogenic nematodes that contacted different concentrations of nano-Ag (5 ppm, 0.5 ppm) solutions did not differ in their ability to kill the host *G. mellonella* which can show that nematodes' symbiotic bacteria are immune to nano-Ag (Table 1). In all cases mortality and the extensiveness of infection after the contact of nematodes with nano-Ag were similar to those in the control when measured on the last day of the experiment. The mortality of insects infected by *H. bacteriophora* that survived 5 days' long contact with 0.5 ppm nano-Ag was 100 % while that in the control was 98 %. On consecutive days insects mortality grew, however, faster with Ag-treated nematodes than in the control.

Table 1

The effect of nano-Ag on pathogenic properties of the nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae* exposed for 5 days to nano-Ag solutions (test of mortality and extensiveness of infection [%] of the *Galleria mellonella* larvae) (test Chi^2)

Nematode species	Nano-Ag concentration						Chi-square test	
	5 ppm		0.5 ppm		0 ppm			
	Mortality	Extensiveness	Mortality	Extensiveness	Mortality	Extensiveness	Mortality	Extensiveness
H. bacteriophora (Nematop)								
1st day	—	—	0	0	6	2	$x^2 = 3.09$; $p > 0.05$	$x^2 = 1.010$; $p > 0.05$
2nd day	—	—	86	52	50	24	$x^2 = 19.385$; $p < 0.05$	$x^2 = 9.653$; $p < 0.05$
3rd day	—	—	96	56	90	40	$x^2 = 12.000$; $p < 0.05$	$x^2 = 4.000$; $p < 0.05$
4th day	—	—	98	56	92	42	$x^2 = 0.000$; $p > 0.05$	$x^2 = 1.010$; $p > 0.05$
5th day	—	—	100	56	98	46	$x^2 = 1.042$; $p > 0.05$	$x^2 = 2.041$; $p > 0.05$
S. feltiae (Owinema)								
1st day	7	7	7	7	13	13	$x^2 = 1.098$; $p > 0.05$	$x^2 = 1.098$; $p > 0.05$
2nd day	97	97	97	93	100	100	$x^2 = 0.225$; $p > 0.05$	$x^2 = 0.207$; $p > 0.05$
3rd day	97	97	100	97	100	100	$x^2 = 2.022$; $p > 0.05$	$x^2 = 2.022$; $p > 0.05$
4th day	100	100	100	97	100	100	$x^2 = 2.022$; $p > 0.05$	$x^2 = 2.022$; $p > 0.05$
5th day	100	100	100	97	100	100	—	—

The extensiveness of insect infection finally achieved 56 % in the experiment and 46 % in the control. The mortality of *G. mellonella* after contact with *S. feltiae* was very high and attained 100 % on the last day in all cases. Similar results were obtained for the extensiveness of infection by *S. feltiae* which contacted 5 ppm solution of nano-Ag and in the control. Slightly lower extensiveness was noted for nematodes originating from 0.5 ppm solution.

The intensity of infection is the mean number of invasive larvae of nematodes that have entered the insect and developed into the L4 form, females, males and hermaphroditic individuals in the case of Heterorhabditidae. In *H. bacteriophora* (Table 2) the intensity of infection was 2.96 at a concentration of 0.5 ppm and 1.58 in the control. Contribution of particular growth stages to the population structure of the parasitic generation is shown in table 2. Hermaphrodites dominated among the studied populations.

Table 2

The effect of nano-Ag on the intensity of infection of *Galleria mellonella* and on the population structure of the parasitic generation (*Heterorhabditis bacteriophora* and *Steinernema feltiae*) (different letters mean statistically significant differences at $p < 0.05$, Tukey test and ANOVA).

Nematode species	Concentrations of nano-Ag	Intensity of infection (Means)	ANOVA	Population structure of the parasitic generation (Means)		
				Female or herm-aphrodite	Male	L4
Heterorhabditis bacteriophora	5 ppm	—	—	—	—	—
	0.5 ppm	2.96	$F_{1.96} = 4.33$, $p < 0.05$	2.60	0	0.35
	0 ppm	1.58		1.28	0	0.30
Steinernema feltiae	5 ppm	12.27 A	$F_{2.87} = 19.44$, $p > 0.05$	8.03	4.17	0.07
	0.5 ppm	9.13 A		6.47	2.57	0.10
	0 ppm	23.70 B		15.90	7.63	0.27

The intensity of infection by *S. feltiae* was 12.27, 9.13 and 23.70 for 5 ppm, 0.5 ppm and 0 ppm nano-Ag, respectively (Table 2). Contribution of particular growth stages to the population structure of the parasitic generation is presented in Table 2. Females were the main component of the studied nematode populations.

Conclusions

1. The mortality of invasive larvae of *H. bacteriophora* and *S. feltiae* exposed to nano-Ag depended on the concentration of nanoparticles and the time of exposure.

2. Mortality and extensiveness of infection of *G. mellonellla* larvae were similar for nematodes that contacted with nano-Ag and those from the control.

3. The intensity of infection was higher in *S. feltiae*.

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INFLUENCE OF THE WEATHER CONDITIONS ON FABA BEAN YIELDING

WPŁYW WARUNKÓW POGODOWYCH NA PLONOWANIE BOBIKU

Abstract: The aim of this work was to determine the relationship between faba bean seeds yield, number of its seeds per sqm (m^2) as well as 1000 seeds weight and meteorological factors and their deviations from optimal values. Results of experinients conducted in 1989–1991, 1993–1995 and 1999–2003 at the Experimental Station Prusy near Krakow were shown in this paper. The yield of faba bean grown on degraded chernozem, ranged between 2.11 and 5.20 $Mg \cdot ha^{-1}$. The seed yield demonstrated high correlation with the number of seeds per sqm, and much lower with weight of 1000 seeds. There were no statistical significant correlation between yield of seeds and meteorological factors. Weight of 1000 seeds have been positively correlated with deviation of precipitation sum from water requirements in the period April–August, and with average air temperature in April. Number of seeds per sqm was significant correlated with deviation of precipitation sum from water requirements in may and average air temperature in April.

Keywords: faba bean, yield, rainfall, temperature, Sielianinov coefficient

Crop yielding depends mainly on the weather course, soil conditions and properly applied agrotechnological measures. Direct unfavourable effect of the weather conditions (such as hail, drought or high temperatures) leads to the occurrence of abiotic stress in plants, which is the cause of low stability of the legume yielding. Moreover they directly influence the features determining the amount of seed yield, ie the plant density, the number of pods per plant, the number of seeds per pod and 1000 seed weight. Faba bean water requirements during the growing period are estimated for between 300–500 mm [1–3] depending on the soil kind. Both deficiency and excess of rainfall negatively affect the amount of seed yield and the yield structure elements [2, 4,

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5]. Faba bean thermal requirements are described as moderate [2]. Low temperatures during the initial period of development favour vernalization and positively influence the development of the root system. Too low temperatures during shooting accompanied by heavy rainfall may be reason of cool-water stress adversely affecting further plant development [6]. High temperature at the ripening time contributes to shortening of this stage, which is unfavourably reflected in the quantity of seed yield. According to Michalska [2] the air amplitudes not exceeding 10 ^{o}C are optimal for faba bean yielding. The effect of the weather conditions, beside the basis agrotechnological factors, is the main source of low stability of faba bean yielding [5, 7, 8–10]. Variability of seed yield caused by the effect of weather conditions may reach between 20 and 40 %.

The research aimed to determine the relationship between the amount of seeds yield, 1000 seed weight and seed number per 1 m^2 of faba bean and the weather conditions, such as the air temperature, rainfall amount, hydrothermal coefficient and rainfall total deviation from faba bean rainfall requirements.

Material and methods

Presented work has used the results obtained in the field experiments conducted in the years 1989–1991, 1993–1995 and 1999–2003 at the experimental Station in Prusy near Krakow. The experiments were localized on degraded chernozem of very good wheat complex and first class quality soil. Meteorological data supplied by the automatic Hardi Metpole meteorological station comprising the air temperature and rainfall amount were used for the statistical analyses conducted. On the basis of these measurements the value of Silianinov hydrothermal coefficient was computed and deviations from rainfall needs of faba bean cultivated under conditions of heavy soil [3]. The relationship between the analyzed variables was determined using linear correlation analysis. The empirical model for estimating 1000 seed weight was determined on the basis of stepwise regression.

Results

The pattern of weather conditions during faba bean vegetation (April–August) over the multiannual period 1961–1990 and in the analogous months during the investigations conducted in 1989–2005 was greatly diversified (Table 1). Average air temperature during the period from April to August was 14.3 ^{o}C and was by 0.9 ^{o}C higher than the multiannual mean, which points to apparent climate warming in the last decade of the 20th century and in the first years of the present century. July and August were particularly warm months. Precipitation total during faba bean vegetation in 1989–2005 was lower by an average of 49 mm than the precipitation total of the analogous period in the years 1961–1990 however, smaller amount of rainfall occurred from May till August.

Table 1

Characteristic of meteorological conditions in the Experimental Station Prusy

Months	Mean		Minimum	Maximum	Standard deviation	CV %
	1961–1990	1989–2005				
Mean air temperature						
IV	7.9	8.6	6.9	11.8	1.3	14.6
V	13.1	13.8	10.8	16.3	1.6	11.8
VI	16.2	16.3	14.7	17.8	0.9	5.5
VII	17.5	18.8	16.4	21.8	1.6	8.6
VIII	16.9	18.4	16.7	19.8	0.9	5.0
IV–VIII	14.3	15.2	14.2	16.1	0.6	4.2
Sum of precipitation						
IV	48.0	60.7	19.8	145.7	36.9	60.8
V	83.0	69.6	27.3	136.4	28.2	40.5
VI	97.0	81.9	32.9	201.0	48.8	59.6
VII	85.0	74.0	30.6	212.7	50.7	68.5
VIII	87.0	64.9	16.2	122.2	31.7	48.8
IV–VIII	400.0	351.1	213.3	522.4	78.0	22.2
Sielianinow coefficient						
IV	2.0	2.4	0.8	5.9	1.5	61.9
V	2.0	1.7	0.7	3.1	0.7	45.0
VI	2.0	1.7	0.7	3.9	1.0	59.6
VII	1.6	1.3	0.5	4.1	1.0	74.5
VIII	1.7	1.1	0.3	2.2	0.6	51.1
IV–VIII	1.8	1.5	0.9	2.2	0.3	21.6

Excess or deficiency of rainfall in the individual months of vegetation and aggregate amounts over the April–August period were shown in Fig. 1. Analysis of this graph shows that the greatest amount of evenly distributed rainfall occurred in 2001, which was connected with maximum seed yield – 5.20 Mg · ha^{-1}. In the other years periods of deficient or excessive rainfall occurred too but their pattern was uneven, which resulted in much lower seed yields. In the discussed region the most unfavourable for faba bean yielding were the years of 1989, 1990, 1994 and 2000 when the produced yields were much below the average – 3.95 Mg · ha^{-1} whereas the lowest – 2.11 Mg · ha^{-1} was obtained in 1990 (Fig. 2).

Due to negative correlations between plant density and the numbers of pods and seeds per plant, the detailed analysis of the weather factors effect on individual yield structure elements was abandoned and replaced by a synthetic indicator, ie the number

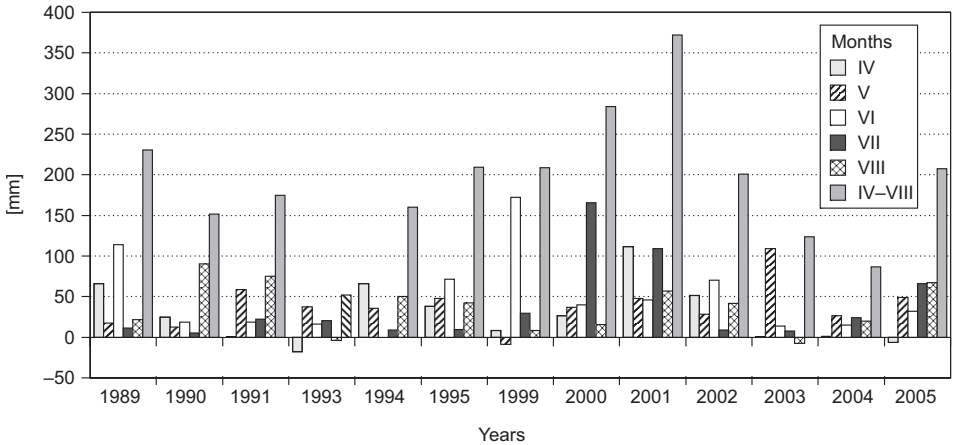

Fig 1. Excess and deficit of precipitation in relationship to faba bean rainfall requirements

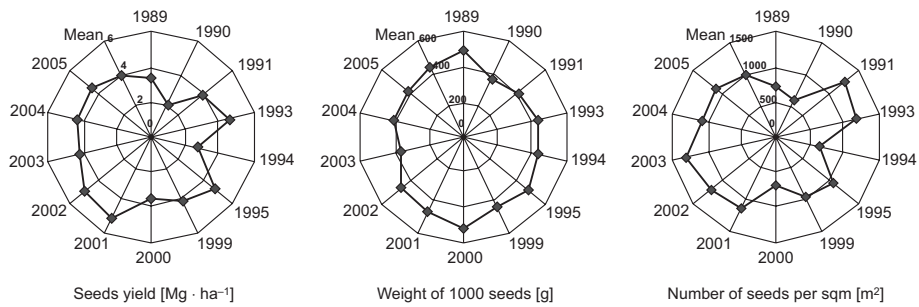

Fig 2. Yield of seeds, weight of 1000 seeds and number of seds per sqm in the respective yers of study

of seeds per 1 m^2 of the plantation area. As demonstrated by Nachi and Guen [11] in 15 analyzed genotypes this feature was significantly correlated with seed yield (r = 0.85). Low seed yield in 1990 was the consequence of both small number of seeds per 1 m^2 and 1000 seed weight. As has been demonstrated by the results for the whole period of investigations, the number of seeds per 1 m^2 is significantly positively correlated with the amount of faba bean seed yield (Table 2). Faba bean plant development was unfavourably influenced in 1990 by a considerable amount of rainfall in August, however no significant correlation was found between the values of meteorological factors in the individual months and the seed yield. 1000 seed weight revealed the highest correlation with the deviations of rainfall total from rainfall requirements in the April–August period and average monthly temperature in April, whereas the number of seeds per m^2 with rainfall total deviations from rainfall requirements in May and average monthly temperature in April (Table 1). 1000 seeds weight was to a greater degree correlated with the values of Sielianinov hydrothermal coefficient than the number of seeds per 1 m^2.

Table 2

Simple correlation coefficients between investigated traits

Months	Seeds yield	Weigth of 1000 seeds	Number of seeds per sqm (x_1)
Weigth of 1000 seeds	0.28	—	
Number of seeds per sqm (x_1)	0.83***	–0.14	—
Mean air temperature			
IV	–0.22	0.59**	–0.54*
V	0.26	0.35	0.11
VI	0.08	–0.16	0.30
VII	0.24	0.08	0.27
VIII	0.24	0.30	0.23
IV–VIII	0.26	0.49*	0.14
Sum of precipitation			
IV	0.06	0.44	–0.15
V	0.20	–0.38	0.46*
VI	0.15	0.44	–0.07
VII	0.11	0.49*	–0.23
VIII	–0.34	–0.29	–0.22
IV–VIII	0.13	0.54*	–0.19
Sielianinow coefficient			
IV	0.12	0.34	–0.04
V	0.12	–0.43	0.43
VI	0.13	0.46	–0.11
VII	0.04	0.48*	–0.28
VIII	–0.36	–0.33	–0.23
V–VIII	0.06	0.46	–0.22
Excess and deficit of precipitation			
IV	0.03	0.54	–0.31
V	0.28	–0.29	0.56*
VI	0.16	0.43	–0.10
VII	0.15	0.52	–0.16
VIII	–0.32	–0.25	–0.23
V–VIII	0.17	0.61**	–0.18

Using stepwise regression a multiple regression equation first class quality soil was set up for estimating 1000 seed weight of faba bean, Nadwislanski c.v. for the analyzed period at the Experimental Station in Prusy, which assumed the following form. Introduction of new variables to the model from x_1 to x_2 increased the determination coefficient from 0.37 to 0.69.

$$Y = 447.8 + 0.658\ x_1 - 0.478\ x_2 - 0.339\ x_3;$$
$$F(3.9) = 6.6592;\ p < 0.0116;\ R^2 = 0.69$$

where: Y – weight of 1000 seeds,
x_1 – total deviation of rainfall from the rainfall requirements in the period April–August,
x_2 – Sielianinow coefficient in August,
x_3 – sum of precipitation in May.

Conclusions

1. Over the13-year period of experiments on degraded chernozem the seeds yield of faba bean Nadwislanski cv. ranged from 2.11 to 5.20 Mg · ha^{-1}. It was significantly positively correlated with the number of seeds per 1 m^2.

2. 1000 seeds weight revealed the highest correlation with the rainfall total deviations from the water requirements during the April–August period and average monthly temperature in April, whereas the number of seeds per 1 m^2 with the deviations of rainfall total from rainfall needs in May and average monthly temperature in April. No significant correlation was found between the values of meteorological factors in the individual months and seed yield.

3. Using stepwise regression a multiple regression equation was set up for estimating 1000 seeds weight on the basis of the following variables: a total of rainfall deviations from rainfall requirements in the April–August period, value of Sielianinov coefficient in August and rainfall total in May.

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WP£YW WARUNKÓW POGODOWYCH NA PLONOWANIE BOBIKU

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Abstrakt: Celem pracy by³o okre lenie zale¿no ci pomiêdzy plonem nasion, liczb¹ nasion na m^2 oraz mas¹ 1000 nasion bobiku (Nadwi lañski) uprawianego na czarnoziemie zdegradowanym a czynnikami pogodowy-

mi (œredni¹ miesiêczn¹ temperatur¹ powietrza, miesiêczn¹ sum¹ opadów, wspó³czynnikiem hydrotermicznym oraz odchyleniami sumy opadów od potrzeb opadowych bobiku). W pracy wykorzystano wyniki badañ polowych przeprowadzonych w Stacji Doœwiadczalnej w Prusach (Uniwersytet Rolniczy w Krakowie) w latach 1989–1991, 1993–1995 i 1999–2003. Bobik plonowa³ w granicach 2,11–5,20 Mg · ha^{-1}. Plon nasion wykazywa³ wysok¹ istotn¹ korelacjê z liczb¹ nasion na 1 m^2, a znacznie mniejsz¹ i nieistotn¹ z mas¹ 1000 nasion. Nie stwierdzono istotnej korelacji pomiêdzy wielkoœciami czynników meteorologicznych w poszczególnych miesi¹cach a plonem nasion. Masa 1000 nasion wykazywa³a najwiêksz¹ korelacjê z odchyleniami sumy opadów od potrzeb opadowych w okresie kwiecieñ–sierpieñ oraz œredni¹ miesiêczn¹ temperatur¹ kwietnia, natomiast liczba nasion na m^2 z odchyleniami sumy opadów od potrzeb opadowych w maju oraz œredni¹ miesiêczn¹ temperatur¹ kwietnia

S³owa kluczowe: bobik, opady, temperatura, wspó³czynnik Sielianinowa

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Vol. 18, No. 8 2011

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IMPACT OF WEATHER CONDITIONS ON CHEMICAL COMPOSITION OF THE SEEDS OF THREE SOYBEAN CULTIVARS

WP£YW WARUNKÓW POGODOWYCH NA SK£AD CHEMICZNY TRZECH ODMIAN SOI

Abstract: The paper presents the results of a three-year field experiment aimed at an assessment of the effect of the weather conditions on the yield and the contents of macroelements (P, Mg, Ca, Na and K) and microelements (Fe, Mn, Cu and Zn) in seeds of three soybean cultivars (2 large seed Aldana and Gaj and 1 small seed Nawiko). A significant effect of meteorological conditions on the yield and chemical composition of the analyzed soybean seeds was demonstrated. In the year 2002 when the course of thermal and moisture conditions was the most advantageous, the largest seed yields were obtained (3.1 Mg · ha^{-1}) with the highest mineral content. Among the analyzed varieties in the subsequent years the small seed one accumulated in its seeds greater amounts of Mg, Ca, K, Mn and Zn in comparison with the large seed cultivars.

Keywords: soybean, yield, microelements, macroelements

Versatile applications of soybean *Glycine max* (L.) raise great interest in this plant not only abroad but also in Poland. Its seeds are a rich source of protein containing all crucial amino acids, particularly great amounts of exogenic amino acids, which are not synthetized by living organism [1, 2]. Soybean also supplies about 20 % of fat which contains considerable quantities of linolic acid and enzymes counteracting rancidity. In comparison with other plant oils, soybean oil is abundant in omega-3 and omega-6 fatty acids. Soybean seeds are also a reserve of nutritious and non-nutritious substances important for the organism, vitamins (from B, PP and R group) and mineral salts. They also contain a considerable amount of macro- and microelements, particularly phosphorus, potassium, calcium, magnesium, iron, zinc, copper and sodium [3, 4]. Because of growing consumption of soybean seeds and soybean derived products there is a

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necessity to conduct investigations on the effect of habitat conditions on the chemical composition of the seeds.

The investigations aimed at comparing the effect of habitat conditions on the content of macroelements (P, K, Ca, Na and K) and microelements (Fe, Mn, Cu and Zn) in the seeds of three national soybean cultivars: two large seed Aldana and Gaj and one small seed Nawiko variety.

Material and methods

The investigations were conducted in the years 2002–2004 at the Experimental Station of the Crop Production Department in Prusy near Krakow on a degraded chernozem well abundant in phosphorus and potassium. The content of basic nutrient available forms in soil, as determined by the analyses carried out by the Chemical--Agricultural Station in Krakow, was as follows: 25.0 mg P_2O_5, 23.0 mg K_2O and 14.1 Mg 100 g^{-1} of air-dried soil, and abundance in microelements: 264 mg Mn, 6.6 mg Cu, 43.5 mg Zn and 148 mg Fe · kg^{-1} of soil. The soil contained 0.127 % of nitrogen and its pH was 6.2.

A one-factor field experiment was set up in a split-plot design in four replications. The analyzed factor were three soybean cultivars: two large seed Aldana and Gaj and one small seed Nawiko.

In the subsequent years of the experiment, cultivation measures did not differ from those commonly applied in large seed legume cultivation. Following the forecrop (spring cereals) harvesting first ploughing and harrowing were conducted. Phosphorus and potassium fertilizers: 70 kg P_2O_5 · ha^{-1} as triple superphosphate and 120 kg K_2O as 57 % potassium salt were sown in autumn and then pre-winter ploughing was done. Nitrogen fertilization, dosed 30 kg N/ha as ammonium nitrate, was applied as pre-sowing in spring. Prior to sowing the seeds were treated with Zaprawa Nasienna T (seed dressing) and inoculated with *Bradyrhizobium japonicum* bacteria. The quantity of sown seeds for individual varieties were calculated for the density of 80 pieces per m^2. The seeds were sown in the first days of May using Bratek plot seeder in rows 25 cm apart. After sowing the field was sprayed with Gesagard 50WP and during vegetation with Basagran 600SL. The seeds were collected at a single-phase by means of a plot combine harvester in the first days of September.

Dried and crushed seeds were mineralized in a muffle furnace (in two replications, at 450 ^oC for 5 hours), the remains were dissolved in a diluted nitric acid 1:2 (v/v) [5]. The contents of calcium, sodium and potassium in the solutions were determined using flame photometry (FES), magnesium by means of atomic absorption spectrometry (AAS) and concentrations of phosphorus and trace elements (Cr, Zn, Pb, Cu, Cd, Ni, Fe and Mn) were assessed using ICP-AES method. A reference sample of plant material (NCS DC733448 China National Analysis Center for Iron & Steel) was added to the analysed series and the result was regarded reliable if the *relative standard deviation* (RSD) did not exceed 5 %.

Results and discussion

Soybean yielding was significantly influenced by the climatic conditions during the conducted experiments (Table 1).

Table 1

Characteristics of climatic conditions in years 2002–2004 at Prusy

Year	Months						Mean
	IV	V	VI	VII	VIII	IX	
Temperature [^{o}C]							
2002	10.4	18.4	18.7	21.3	21.6	14.0	17.4
2003	7.8	16.5	19.1	19.5	19.9	14.2	16.2
2004	9.5	12.7	16.8	18.3	18.9	13.7	15.0
1984–1994	8.8	13.8	16.4	18.5	18.4	13.6	14.9
Rainfall [mm]							Sum
2002	85.2	49.3	102.1	42.9	62.7	50.7	392.9
2003	34.3	125.4	35.4	126.2	23.6	30.4	375.3
2004	32.4	42.6	56.4	97.4	77.2	36.1	342.1
1984–1994	44.4	72.0	79.3	56.6	67.2	57.5	377.0

The year 2002 proved the most advantageous for soybean growth and development as it was the year of relatively long vegetation (ca 240 days). Average air temperature in this period was 17.4 ^{o}C and precipitation total 392 mm. The years 2003 and 2004 were characterized by lower air temperatures and a small amount of precipitation, which created hardly favourable conditions for growth and development of soybean and negatively affected the obtained seed yields (Table 2).

Table 2

Seed yield of soybean [Mg · ha^{-1}]

Years	Cultivar			Mean for years	$LSD_{\alpha=0.05}$ for years
	Aldana	Gaj	Nawiko		
2002	2.75	3.17	3.10	3.10	
2003	1.32	1.76	1.38	1.38	0.89
2004	1.30	1.20	1.73	1.73	
Mean for cultivars	1.79	2.04	2.32		
$LSD_{\alpha=0.05}$ for cultivars	n.s.				

The highest seed yields from the analyzed soybean cultivars were obtained from the small seed Nawiko c.v. in 2002 (3.2 Mg · ha^{-1}). In the subsequent years the yields, although smaller, were always higher than those obtained from the other varieties, which on average were lower by 22 % for Aldana c.v. and by 12 % for Gaj c.v. Great differences in the yields of the studied varieties during the period of the experiment confirmed soybean sensitivity to the course of weather conditions during plant

vegetation. Bobrecka-Jamro and Pizlo as well as Michalek and Borowski obtained similar results in their research [6, 7].

The mineral content in soybean seeds depends on many factors, among which the most important are: the soil concentrations of these elements, appropriate fertilization, the course of vegetative conditions and the cultivar variety [8, 9]. In the discussed experiment the course of meteorological conditions in the subsequent years of the investigations had the greatest influence on the content of macro and microelements in soybean seeds (Tables 3, 4).

Table 3

Content of P, Mg, Ca, Na, K in seeds of soybean cultivars under analysis [%]

Years	Cultivar			Mean for years	$LSD_{\alpha=0.05}$ for years
	Aldana	Gaj	Nawiko		
P					
2002	0.78	0.81	0.90	0.83	
2003	0.69	0.70	0.71	0.70	0.04
2004	0.93	1.01	1.10	1.01	
Mean for cultivars	0.80	0.84	0.90		
$LSD_{\alpha=0.05}$ for cultivars	n.s.				
Mg					
2002	0.110	0.107	0.120	0.112	
2003	0.102	0.102	0.112	0.105	0.004
2004	0.107	0.107	0.117	0.110	
Mean for cultivars	0.106	0.105	0.116		
$LSD_{\alpha=0.05}$ for cultivars	0.004				
Ca					
2002	0.17	0.17	0.18	0.17	
2003	0.17	0.17	0.20	0.18	0.01
2004	0.21	0.20	0.23	0.21	
Mean for cultivars	0.18	0.18	0.20		
$LSD_{\alpha=0.05}$ for cultivars	0.01				
Na					
2002	0.020	0.017	0.020	0.019	
2003	0.010	0.010	0.010	0.010	0.002
2004	0.012	0.010	0.010	0.010	
Mean for cultivars	0.014	0.012	0.013		
$LSD_{\alpha=0.05}$for cultivars	n.s.				
K					
2002	2.11	2.00	2.14	2.08	
2003	1.41	1.52	1.49	1.47	0.03
2004	1.62	1.61	1.63	1.62	
Mean for cultivars	1.71	1.71	1.75		
$LSD_{\alpha=0.05}$ for cultivars	0.02				

Table 4

Content of Fe, Mn, Cu, Zn in seeds of soybean cultivars under analysis [mg · kg^{-1} d.m.]

Years	Cultivar			Mean for years	$LSD_{\alpha=0.05}$ for years
	Aldana	Gaj	Nawiko		
Fe					
2002	81.90	82.10	81.94	81.98	n.s.
2003	64.56	73.03	69.99	69.19	
2004	63.31	64.54	66.19	64.68	
Mean for cultivars	69.92	73.23	72.70		
$LSD_{\alpha=0.05}$ for cultivars	n.s..				
Mn					
2002	14.00	13.87	14.35	14.07	n.s.
2003	13.98	13.72	14.89	14.20	
2004	14.29	13.29	13.83	13.81	
Mean for cultivars	14.09	13.63	14.36		
$LSD_{\alpha=0.05}$ for cultivars	0.57				
Cu					
2002	12.51	14.46	14.68	13.88	0.90
2003	6.11	6.27	6.32	6.23	
2004	11.21	12.41	13.78	12.46	
Mean for cultivars	9.94	11.05	11.59		
$LSD_{\alpha=0.05}$ for cultivars	0.59				
Zn					
2002	49.18	46.01	46.76	47.32	1.66
2003	45.40	42.82	44.32	44.18	
2004	47.87	44.56	46.48	46.30	
Mean for cultivars	47.48	44.46	45.85		
$LSD_{\alpha=0.05}$ for cultivars	1.38				

The concentrations of macroelements: phosphorus, magnesium, sodium and potassium in the seeds were the highest in 2002 in comparison with the other years. Optimal conditions for absorption and accumulation of these elements in seeds occurred that year. Only for calcium, the highest content was registered in 2004. Warm spring in 2002 also affected higher cumulation of Cu and Zn in seeds. Gorlach [10] obtained similar results concerning the influence of meteorological conditions on heavy metal accumulation in seeds. On the other hand, no effect of atmospheric conditions was noted on Fe or Mn content.

Statistically significant differences were registered among the investigated soybean varieties concerning concentrations of Mg, Ca and K. Nawiko c.v. accumulated the highest Mg amounts (by 0.116 %), whereas the other cultivars had on average 10 % less Mg. Also the contents of calcium and potassium were the highest in Nawiko c.v. seeds (0.20 and 1.75 %). Aldana and Gaj c.vs. contained respectively 10 and 2 % less of these elements.

Among the analyzed microelements greater amounts of Fe and Zn were found in the compared soybean cultivars. Accumulation of the analyzed heavy metals in the seeds of studied soybean varieties did not exceed the value critical for the plant growth [9]. High concentration of iron in seeds improves their value; therefore they may be used in human nutrition for people with anemia symptoms. No differences between the studied soybean cultivars were registered for Fe content. Large seed Aldana c.v. had the highest Zn concentrations in its seeds (47.48 mg · kg^{-1}), whereas the smallest quantities of this element were also noted in large seed Gaj c.v. (44.46 mg · kg^{-1}). A similar dependence occurred for zinc content. Aldana c.v. seeds had the greatest amounts of Zn (47.48 mg · kg^{-1}) whereas Gaj c.v. had the smallest (44.46 mg · kg^{-1}).

Conclusions

1. The yields of the researched soybean cultivars were significantly diversified depending on the variety and the course of weather conditions in the years of the experiment. Considering the three compared soybean cultivars, the highest yields were obtained from the small seed Nawiko c.v. The yields from this variety were on average 12 % and 23 % higher in comparison with Aldana and Gaj large seed varieties.

2. Cumulation of macro and microelements in the seeds of the investigated soybean cultivars was diversified and depended on the soybean cultivar variety and the course of meteorological conditions. The contents of individual elements may be put in the following order: macroelements [%] Na < P < Mg < Ca < K and microelements (mg · kg^{-1} d.m.) Cu < Mn < Zn < Fe.

3. Small seed Nawiko c.v. accumulated more macroelements in its seeds in comparison with the analyzed large seed Aldana and Gaj c.vs.

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WP£YW WARUNKÓW POGODOWYCH NA SK£AD CHEMICZNY TRZECH ODMIAN SOI

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Abstrakt: Przedstawiono wyniki trzyletniego doœwiadczenia polowego, maj¹cego na celu ocenê wp³ywu warunków pogodowych na plon i zawartoœæ makroelementów (P, Mg, Ca, Ma, K) i mikroelementów (Fe, MN, Cu, Zn) w nasionach trzech odmian soi (dwóch odmian grubonasiennych Aldana i Gaj oraz jednej drobnonasiennej Nawiko). W doœwiadczeniu stwierdzono istotny wp³yw warunków meteorologicznych na plon i sk³ad chemiczny nasion badanych odmian soi. W roku 2002 o najkorzystniejszym przebiegu warunków termiczno-wilgotnoœciowych uzyskano najwiêksze plony nasion (3,1 Mg · ha^{-1}) o najwiêkszej zawartoœci sk³adników mineralnych. Z badanych odmian soi odmiana drobnonasienna w kolejnych latach uprawy gromadzi³a w nasionach wiêcej Mg, Ca, K, Mn, Zn w porównaniu z odmianami grubonasiennymi.

S³owa kluczowe: soja, plon, mikroelementy, makroelementy

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SPATIAL VARIABILITY OF TOTAL MERCURY CONTENT IN SURFACE HORIZON OF SOILS OF GNIEZNIENSKIE LAKELAND AREA

ZMIENOŒÆ PRZESTRZENNA CA£KOWITEJ ZAWARTOŒCI RTÊCI W POZIOMIE POWIERZCHNIOWYM GLEB OBSZARU POJEZIERZA GNIE NIEÑSKIEGO

Abstract: The paper presents the results of total mercury content in terms of pollution of agricultural soils of Gniezno Lakeland by this element, as well as spatial characteristics of this metal. Statistical relationship between content of mercury in arable-humous horizon and active, exchangeable and hydrolytic acidity, organic carbon and total nitrogen was also demonstrated. For the analysis 213 bulk samples from surface horizons were collected. The total mercury content in selected soil samples were determined by mercury analyzer AMA 254. The precision of the method and obtained results was confirmed with reference material TILL-3 (Certificate of Analysis 1995) and S-VM (Certificate Czechoslovak Reference Material) for Hg determination. Physical and chemical properties of soil samples were determined following standard procedures. Statistical analysis of results was evaluated on the basis of computer programme "STATISTICA" 6.0 PL.

Studies of the total mercury content showed relatively small differences in the concentrations of the element in analyzed area. The total mercury content ranged from 0.0081 to 0.1620 mg $\cdot$ kg^{-1}, and only in the one case exceeded the average natural concentration of this element. On the basis of statistical analysis between the total mercury content and selected physicochemical properties of soil such as pH in H_2O, pH in KCl, hydrolytic acidity, organic carbon and total nitrogen was found that there are average correlations by Pearson ($0.3 \leq r_{xy} < 0.5$).

Keywords: mercury, soil, surface horizon, spatial variability

Soil is one of the most important factors in heavy metals cycle. Among various heavy metals mercury is worthy of interest. It is a trace element which is an environmental problem because of its toxicity to all living organisms, especially at elevated concentrations [1]. The compounds of mercury are involved in many kinds of natural cycles. Biogeochemical Hg cycle depends on both concentration and reactions

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influenced forms of its occurrence. This metal is hazard contaminant on account of high geochemical and biochemical activity and also easy conversion from liquid to volatile state [2].

In consideration of source and geochemical properties, there are three types of origin of mercury: lithogenic, pedogenic and anthropogenic [3]. Hg gets into the soil largely in form of Hg^0, with dusts (Hg^{2+}) and with the rainfall [4, 5]. The increased concentration of mercury can occur in soils under the influence of geological factors (migration of ore) and as a result of diverse human activities (use of mercury fungicides, uncontrolled emission of industrial dust, improper storage of waste, the use of outdated processes). Circulation of volatile mercury in the soil air and its transition into the atmosphere to some extent reduces the content of this element in soil. Mercury vapor sorption by organic matter and clay minerals results in an increased accumulation of mercury and reduce migration, despite of its volatility. Therefore, a gradual increase in content of this metal, especially in the surface layers of soil, is noticed [2].

Mercury in soil undergoes various transformations, whose direction depends on factors such as cation exchange capacity and the type of sorbents, soil pH, redox conditions, microbial activity, the content of sulfur, iron, manganese, aluminum and chlorine compounds, the amount and form of input mercury and sunlight, moisture and specific surface, of which the most important is organic matter and soil pH [6–8].

The aim of this study was to evaluate the total mercury content and the possibility of accumulation of this element in some of the Gniezno Lakeland arable soils. Statistical relationship between Hg content and the various physicochemical properties of some soils was also evaluated.

Material and methods

As a research material soil samples from Gniezno Lakeland were gathered. The object of the study was soils derived from the four sampling sites located in the Jeziora Wielkie district. Soil samples were taken from surface (0–20 cm) horizon, using drill loop. The individual samples, collected from each hectare, were combined into bulk samples. The number of 213 samples were analyzed.

Soil samples were dried and sieved with 1 mm mesh. Such prepared samples were subjected to physicochemical analysis. pH in H_2O and in 1 mol $\cdot$ dm^{-3} KCl solution [9], hydrolytical acidity (Hh) using Kappen's method [10], the total content of organic carbon using Turin's method [9] and the total content of nitrogen by Kjeldahl method [10].

The total mercury content was determined in solid samples with atomic spectrometry method, using a mercury analyzer AMA 254. The accuracy of the method and obtained results was confirmed with reference material TILL-3 (Certificate of Analysis 1995) and S-VM (Certifikate Czechoslovak Reference Material) for Hg determination. The analyses were triplicated.

To determine relationships among mercury content and physicochemical properties of soils Pearson's correlation coefficient were evaluated by statistical calculations using Statistica 6.0 programme.

Results and discussion

Soil acidity increases the solubility of potentially toxic elements, and thus, increases their accumulation in plants [11]. The higher *cation exchange capacity* (CEC) and pH value, the soil is more resistant to degradation, due to the immobilization of most heavy metals [12, 13]. Depending on the sampling site, pH in H_2O varied within the range of 5.79–7.09, while the lowest value was found in Wola Ko¿uszkowa, and the highest in Siedlimowo (Table 1).

Table 1

Minimum, maximum and average values of pH and hydrolytic acidity of analyzed soils

Sampling site	pH in H_2O			pH in 1 M KCl			Hh [mmol(+) · kg^{-1}]		
	minimum	maximum	mean	minimum	maximum	mean	minimum	maximum	mean
Kozuszkowo	6.04	6.99	6.43	5.77	6.36	5.97	74.6	127.3	100.5
Siedlimowo	5.96	7.09	6.35	5.72	6.45	5.94	66.6	146.9	109.8
Wola Kozuszkowa	5.79	7.01	6.31	5.27	6.37	5.89	64.9	144.7	110.1
Wojcin	5.88	7.01	6.32	5.21	6.80	5.87	61.1	179.4	105.6

The pH value in KCl was in the range from 5.21 to 6.80. The lowest and the highest values were found in samples taken from the site located in Wojcin. Considering the division of Polish soils depending on the pH value [14], both the active and exchangeable acidity, analyzed soils were placed in the acidic to neutral soils. Mean soils pH values of the four tested sites indicated that it was slightly acidic.

Hydrolytic acidity of soils ranged from 61.1 to 179.4 mmol · kg^{-1}. The lowest and the highest values of Hh were recorded in Wojcin. The results analysis of the active, exchangeable and hydrolytic acidity of soil samples showed no significant differences in acidification status of arable-humous horizon of the studied area. Such results may be affected by systematic soil manure fertilization, which reduces the acidification of soils and increased the content of organic matter and also liming, which decreases the value of hydrolytic acidity [15].

Humus content increases cation exchange capacity, stabilizes pH, takes part in the creation of aggregate structure, and also improves the ratio between water and air in soils [16]. The results of the analysis showed that organic carbon content in studied soils of Gniezno Lakeland ranged from 10.8 to 52.4 g · kg^{-1} (Table 2). Both the lowest and the highest content were found in soils of Wojcin.

The average carbon content of various sites was in the range between 13.0 and 17.7 g · kg^{-1}. These values are lower than the average levels of humus content in the arable-humous horizons of Polish soils. The total nitrogen content in the analyzed soil samples was in the range of 0.9 to 3.3 g · kg^{-1}. The average nitrogen content in the soils of four tested sites ranged from 1.1 to 1.5 g · kg^{-1} and was in the typical range for Polish soils.

Table 2

Content of organic carbon, total nitrogen and C/N ratio of studied soils

Sampling site	Org. C [g · kg^{-1}]			N [g · kg^{-1}]			C/N		
	minimum	maximum	mean	minimum	maximum	mean	minimum	maximum	mean
Kozuszkowo	11.5	15.6	13.5	0.9	1.5	1.2	9.83	14.12	11.48
Siedlimowo	10.6	17.5	13.6	0.9	1.6	1.1	8.61	16.76	12.14
Wola Kozuszkowa	11.0	15.3	13.0	0.9	1.6	1.1	7.98	17.06	11.96
Wojcin	10.8	52.4	17.7	0.9	3.3	1.5	6.00	18.61	12.06

The C/N ratio determines the intensity of the transformation of soil organic matter. Fluctuations in the value of C/N are usually only on an annual basis and may be due to the value of C/N of used fertilisers. In the analyzed soils carbon to nitrogen ratio was in the range of 6:1 to 18.61:1. Extreme values were recorded at Wojcin area. Mean ratio of carbon to nitrogen corresponded to the typical values in Polish arable soils and ranged from 11.48:1 to 12.14:1.

Mercury content in the analyzed soils ranged between 0.0081 and 0.1620 mg · kg^{-1} (Fig. 1).

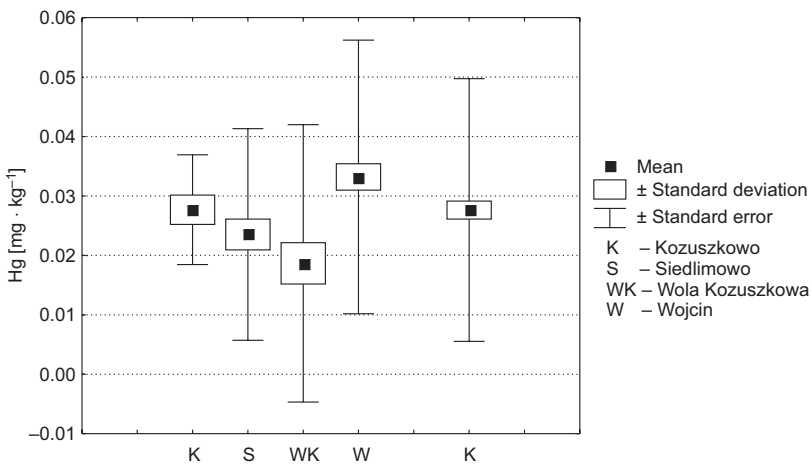

Fig. 1. Range, mean and standard deviation of mercury content in individual sampling sites and total

In Wola Kozuszkowa soils there was reported both the lowest and the highest content of this metal. The average mercury content in the analyzed soils developed range of 0.0187 mg · kg^{-1} in Wola Kozuszkowa to 0.0332 mg · kg^{-1} in Wojcin. It is assumed that the natural level of total mercury content in Polish soils is within the limits 0.05–0.3 mg · kg^{-1} [2], so determined contents of this element in the studied soils were at levels found to be natural. Among all studied samples only in the one case the natural average content of mercury in soils was exceed, with a value of 0.1 mg · kg^{-1} [2], and its also

lower than permissible level for agricultural areas [17]. That is why the analyzed soils were qualified as non-contaminated by this metal.

Statistical analysis of results showed on average no significant correlation between the mercury and selected physicochemical properties of soils.

Conclusions

1. Total mercury contents were within the range considered to be natural. The content of Hg did not exceed 2 mg · kg^{-1}, and allows to qualify the soils as non-contaminated.

2. Statistical analysis of the results showed no significant relationship between the total mercury content and selected physicochemical properties of arable-humous horizon.

3. The studies of the total mercury content in Gnieznienskie Lakeland soils show relatively low variation in the contents of Hg, which ranges between 0.0081 and 0.1620 mg · kg^{-1}.

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ZMIENNOŒÆ PRZESTRZENNA CA£KOWITEJ ZAWARTOŒCI RTÊCI W POZIOMIE POWIERZCHNIOWYM GLEB OBSZARU POJEZIERZA GNIE NIEÑSKIEGO

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Abstrakt: W pracy przedstawiono wyniki badañ ca³kowitej zawartoœci rtêci w aspekcie zanieczyszczenia gleb uprawnych Pojezierza Gnieŭnieñskiego tym pierwiastkiem, a tak¿e specyfiki rozmieszczenia prze-

strzennego tego metalu. Oceniono równie¿ statystyczne zale¿no¶ci pomiêdzy zawarto¶ci¹ rtêci a kwasowo¶ci¹ czynn¹, wymienn¹ i hydrolityczn¹, zawarto¶ci¹ wêgla organicznego oraz ogóln¹ zawarto¶ci¹ azotu. Do analiz pobrano 213 zbiorczych próbek z powierzchniowych poziomów ornopróchnicznych. Ca³kowit¹ zawarto¶æ rtêci w wybranych do analiz próbkach glebowych oznaczono w analizatorze rtêci AMA 254. Dok³adno¶æ zastosowanej procedury analitycznej oznaczania Hg potwierdzono na podstawie analizy certyfikowanego materia³u odniesienia TILL-3 oraz S-VM. W³a¶ciwo¶ci fizykochemiczne próbek glebowych oznaczono metodami analitycznymi ogólnie przyjêtymi w gleboznawstwie. Analizê statystyczn¹ wyników badañ przeprowadzono za pomoc¹ programu komputerowego „STATISTICA” 6.0 PL.

Badania ca³kowitej zawarto¶ci rtêci wykaza³y stosunkowo niewielkie zró¿nicowanie stê¿enia tego pierwiastka na analizowanym obszarze. Ca³kowita zawarto¶æ rtêci waha³a siê w zakresie od 0,0081 do 0,1620 $mg \cdot kg^{-1}$, i tylko w jednym przypadku przekroczy³a naturalne ¶rednie stê¿enie tego pierwiastka. Na podstawie przeprowadzonych obliczeñ zale¿no¶ci statystycznych pomiêdzy ca³kowit¹ zawarto¶ci¹ rtêci i wybranymi w³a¶ciwo¶ciami fizykochemicznymi ornopróchniczych poziomów gleb uprawnych, tj. kwasowo¶ci¹ czynn¹, wymienn¹ i hydrolityczn¹, zawarto¶ci¹ wêgla organicznego oraz ogóln¹ zawarto¶ci¹ azotu stwierdzono, ¿e pomiêdzy badanymi zmiennymi wystêpuj¹ przeciêtne statystycznie korelacje wg Pearsona ($0,3 \le r_{xy} < 0,5$).

S³owa kluczowe: rtêæ, gleba, poziom powierzchniowy, zmienno¶æ przestrzenna

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 8 2011

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CONTENT OF ASSIMILATION DYES AND WATER BALANCE IN COMMON DANDELION FOUND NEARLY CHEMICAL WORKS "POLICE"

ZAWARTOŒÆ BARWNIKÓW ASYMILACYJNYCH ORAZ BILANS WODNY U MNISZKA POSPOLITEGO WYSTÊPUJ¥CEGO BLISKO ZA£ADÓW CHEMICZNYCH „POLICE"

Abstract: The aim of the present studies was to assess air pollution around Chemical Works "Police" by means of selected physiological parameters (chlorophyll *a*, *b*, carotenoids and water balance) of common dandelion. Accumulation of sulfur in leaves of common dandelion was clearly larger at the distance of up to 0.7 km from the emitter than its content in control plants. On the basis of the coefficient of correlation, it was observed that the amount of sulfur in leaves had a significant effect on the content of assimilation dyes in common dandelion. Concentration of chlorophyll *a*+*b* and carotenoids in the assimilation apparatus and the value of the relative water content index of the plants gathered in the area of the Chemical Works "Police" was respectively lower by 31 %, 23 % and 14 % in comparison with these parameters in control leaves.

Keywords: water balance, assimilation dyes, sulfur, *Taraxacum officinale*

Chemical Works "Police" belong to the world leading manufacturers of complex fertilizers. Moreover, they are the only producer of titanium white [1, 2]. The high production is bound to produce the emission of sulfur dioxide, sulfur trioxide and mists of sulfuric acid, fluorine, ammonia and dust. The use of indicator plants plays a significant role in monitoring and evaluating the quantity and range of gaseous and dust impurities.

Common dandelion (*Taraxacum officinale* Weber) [3] is widely regarded and used as an indicator plant showing the environment contamination with heavy metals and sulfur [4–9]. The reaction of plants to the environment pollution is manifested, among other things, by changes occurring in the assimilation apparatus and in physiological processes. Physiological processes caused by biochemical reactions in the presence of

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sulfur compounds can result in disturbances of the work of stomatal apparatuses, the photosynthesis and the decrease in the level of assimilation dyes and the water balance in plants [10–13]. The studies showed that the process of photosynthesis as well as the chlorophyll content in the assimilation apparatus of plants constitute good indicators of the air pollution rate [14, 15].

The aim of the present investigation was the evaluation of air pollution around Chemical Works "Police" by means of selected physiological parameters of common dandelion.

Material and methods

The studies were carried out in 2007 at 7 research points set within the distance of 0.5 to 25 km from Chemical Works "Police". Within the area of the Works five sites were fixed: No. 1 – 0.6 km southwards in front of the company management building, No. 2 – westwards, 0.5 km from the emitter at gate 3; No. 3 – westwards, 0.6 km from the emitter at gate 4; No. 4 –eastwards, 0.6 km from the emitter in the vicinity of the belt conveyor flight; No. 5 – northwards, 0.8 km from the emitter. The two remaining sites were set farther from the emitter of impurities: No. 6 – about 4 km from the emitter, in the centre of Police commune; No. 7 – about 25 km from the emitter, on Pucka Island (the control site). From the fixed research sites, leaves of common dandelion were taken for laboratory analysis, three times during the vegetation (June, July, August) The content of assimilation dyes (chlorophyll *a*, *b*, total and carotenoids) in the leaves was determined by means of Lichtenthaler and Welburn method [16]. The water balance was defined on the basis of two indices: RWC (*relative water content*) and WSD (*water saturation deficit*) [17]. The general content of sulfur in leaves was determined using an elementary analyzer manufactured by Costech (CHNS).

Results and discussion

The average content of sulfur in leaves of common dandelion gathered from the vicinity of Chemical Works "Police" varied from 0.29 to 0.49 %. The largest amount of this element was found in plants from the site situated eastwards near the belt conveyor flight – 0.49 % and southwards at the company management building – 0.47 %. On the control site this species was characterised by a low amount of sulfur – 0.19 % (Fig. 1).

The amounts of this element in leaves of common dandelion range within the values reported by Kabata-Pendias et al [18] and Litynski and Jurkowska [19]. The studies carried out by Kabata-Pendias and Motowicka-Terelak [20] and Motowicka-Terelak [5] show that the leaves of this plant growing in west Pomeranian province contained sulfur within the range of natural background – below 0.3 %. Such values were obtained in leaves of control plants. Referring to the threshold values of sulfur for common dandelion reported by Motowicka-Terelak and Terelak [7], it was observed that the content of this element was exceeded on four research sites. The obtained values of sulfur concentration in leaves of common dandelion growing in the direct neigh-

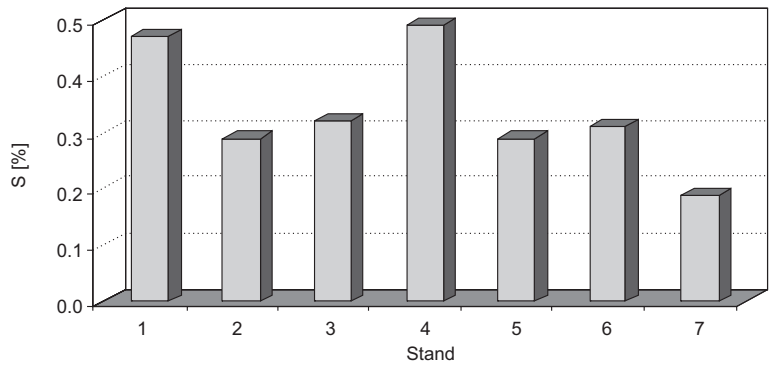

Fig. 1. The average amount of sulfur [%] in leaves of common dandelion found in the area of Chemical Works "Police"

bourhood of Chemical Works "Police" were also higher by 0.19 % southwards, than the amounts of this element in leaves of dandelion from industrial regions of Poland reported by Motowicka-Terelak and Terelak [7]. On the basis of the values of the correlation coefficient, a significant negative correlation relationship was observed between the content of assimilation dyes (chlorophyll *a*, *b*, total and carotenoids) and the concentration of sulfur in leaves of common dandelion (Fig. 2A–D).

The analysis of the two factor variance showed the significance of the influence of the site and time of studies on the content of assimilation dyes in leaves of the examined plant (Table 1).

The lowest content of total chlorophyll was characteristic of common dandelion gathered from the site near the company management building – on average 1.41 mg · g^{-1} of fresh matter (Table 1). The average amount of chlorophyll *a* and *b* in leaves from this site was about 48 % and 51 %, respectively, of its average content in control plants. The average content of chlorophyll *a*+*b* in leaves of the studied species in Police commune amounted to 2.04 mg · g^{-1} of fresh matter and this value was lower by 0.80 mg · g^{-1} of fresh matter than the concentration of these dyes in control leaves (Table 1).

The amount of carotenoids in the assimilation apparatus of common dandelion growing in the distance of 0.5–0.8 km from the emitter was slightly differentiated (0.46–0.76 mg · g^{-1} of fresh matter). Leaves gathered from the neighbourhood of Chemical Works contained by about 24 % less of this dye than the control plants (Table 1). A lower amount of chlorophyll in plants growing in the area of contaminated air was obtained by Brej et al [14]. The authors showed that the decrease in the concentration of this dye depended on the species of plant and on the source of emission. Sawicka-Kapusta [10] shows that already low concentration of SO_2 results in the formation of necrosis, which is accompanied by a decrease in the concentration of chlorophyll. Zimny, Zukowska-Wieszczek [15] stated that under the influence of air contamination, the relation of chlorophyll *a* and *b* to pheopytin changes. Wozny [13] thinks that sulfur dioxide causes disturbances in biosynthesis of chlorophyll and decay of carotenoids.

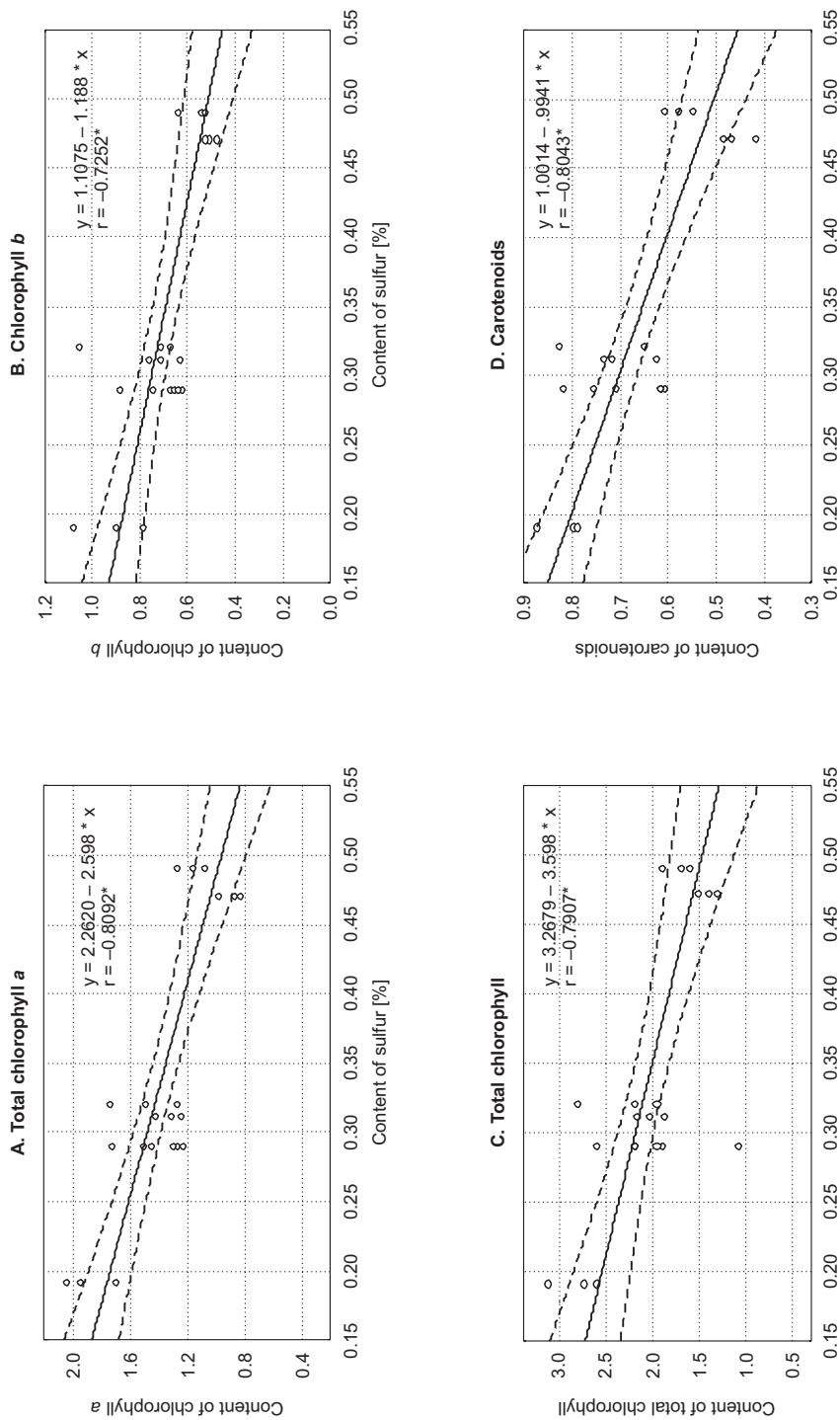

Fig. 2. Correlation between the content of assimilation dyes [mg · g^{-1} of fresh matter] and sulfur in leaves of common dandelion found in the area of Chemical Works "Police"

Table 1

The content of assimilation dyes [mg · g^{-1} of fresh matter] in leaves of common dandelion found in the area of Chemical Works "Police"

Date – factor II	Stand – factor I							
	1	2	3	4	5	6	7	The average of factor II
Content of chlorophyll *a* [mg · g^{-1} f.m.]								
VI	0.89	1.52	1.50	1.17	1.28	1.33	1.96	1.38
VII	0.84	1.46	1.29	1.09	1.24	1.25	1.71	1.27
VIII	0.99	1.74	1.76	1.28	1.32	1.43	2.06	1.51
The average of factor I	0.91	1.57	1.52	1.18	1.28	1.34	1.91	
$LSD_{0.05}$ for factor I – 0.39; factor II – 0.20; interaction I × II – 0.52; interaction II × I – 0.67								
Content of chlorophyll *b* [mg · g^{-1} f.m.]								
VI	0.51	0.68	0.71	0.55	0.63	0.71	0.79	0.65
VII	0.48	0.75	0.68	0.53	0.66	0.64	0.91	0.66
VIII	0.53	0.89	1.06	0.65	0.65	0.76	1.08	0.80
The average of factor I	0.51	0.77	0.82	0.58	0.65	0.70	0.93	
$LSD_{0.05}$ for factor I – 0.33; factor II – 0.17; interaction I × II – 0.45; interaction II × I – 0.58								
Content of total chlorophyll [mg · g^{-1} f.m.]								
VI	1.40	2.20	2.21	1.72	1.91	2.04	2.75	2.03
VII	1.32	2.21	1.97	1.62	1.09	1.89	2.62	1.93
VIII	1.52	2.63	2.82	1.93	1.97	2.19	3.14	2.31
The average of factor I	1.41	2.35	2.33	1.76	1.93	2.04	2.84	
$LSD_{0.05}$ for factor I – 0.47; factor II – 0. 24 interaction I × II – 0.63 interaction II × I – 0.81								
Content of carotenoids [mg · g^{-1} f.m.]								
VI	0.47	0.71	0.69	0.58	0.61	0.74	0.80	0.66
VII	0.42	0.76	0.65	0.55	0.62	0.63	0.88	0.64
VIII	0.49	0.82	0.83	0.61	0.62	0.72	0.79	0.70
The average of factor I	0.46	0.76	0.72	0.58	0.62	0.70	0.82	
$LSD_{0.05}$ for factor I – 0.72; factor II – 0.31; interaction I × II – 0.97; interaction II × I – 1.25								

A significant factor deciding about gaseous pollution of air is the wind. In the west Pomeranian province west winds dominate from March to September [21]. The present studies showed that leaves of common dandelion growing leeward of the source of impurities contained significantly higher amounts of sulfur and a lower content of total chlorophyll than control plants and plants growing windward of the source of the

emission of impurities. The average amount of total chlorophyll in dandelion gathered leeward constituted 75 % of its concentration in plants growing windward and 62 % in relation to control plants. In each term of studies the observed content of chlorophyll was lower in leaves of the examined plant growing leeward.

The studies showed distinct diversity of water relations in dandelion found in the area of Chemical Works "Police". The index of the relative water content (RWC) in tissues of this plant varied from 70 to 93 %. The value of this index of the species gathered at the distance of 0.5–0.8 km from the source of the emission was lower by about 14 % in relation to control plants (Fig. 3).

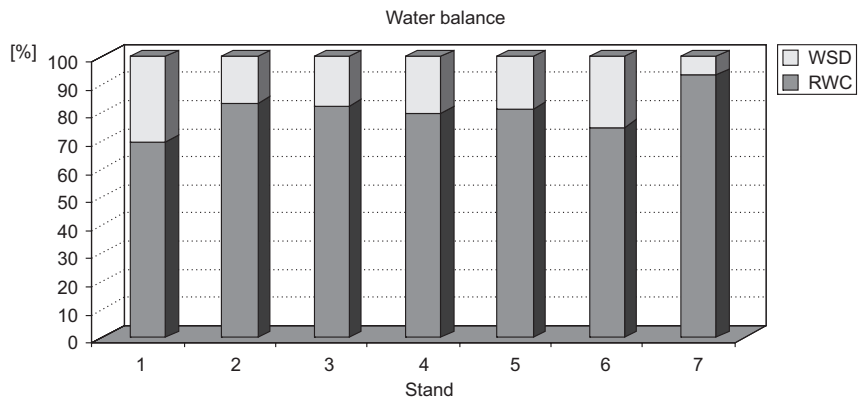

Fig. 3. Water indices [%] of common dandelion found in the area of Chemical Works "Police"

Conclusions

1. Accumulation of sulfur in leaves of common dandelion was clearly larger at the distance of up to 0.7 km from the emitter than its content in control plants.

2. On the basis of the coefficient of correlation, it was observed that the amount of sulfur in leaves had a significant effect on the content of assimilation dyes in common dandelion.

3. The amount of chlorophyll *a+b* and carotenoids in the assimilation apparatus of the examined plant gathered in the area of Chemical Works "Police" was respectively lower by 31 % and 23 % in relation to their concentration in control leaves.

4. Common dandelion gathered in the direct neighbourhood of Chemical Works "Police" was characterised by a lower index of the relative water content by 14 % as compared with control plants.

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ZAWARTOŒÆ BARWNIKÓW ASYMILACYJNYCH ORAZ BILANS WODNY U MNISZKA POSPOLITEGO WYSTÊPUJ¥CEGO BLISKO ZA£ADÓW CHEMICZNYCH „POLICE"

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Abstrakt: Celem badañ by³a ocena zanieczyszczenia powietrza wokó³ Zak³adów Chemicznych „Police" za pomoc¹ wybranych parametrów fizjologicznych (chlorofilu *a*, *b*, ca³kowitego, karotenoidów, bilansu wodnego) mniszka pospolitego. Przeprowadzone badania wykaza³y, ¿e akumulacja siarki w liœciach badanego gatunku by³a wyraŸnie wiêksza w odleg³oœci do 0,7 km od emitora od jej zawartoœci w roœlinach kontrolnych. Na podstawie wspó³czynnika korelacji stwierdzono, ¿e iloœæ siarki w liœciach mia³a istotny wp³yw na zawartoœæ barwników asymilacyjnych u mniszka pospolitego. Koncentracja chlorofilu *a+b* i karotenoidów w aparacie asymilacyjnym oraz wartoœæ wskaŸnika wzglêdnej zawartoœci wody roœliny zebranej w obrêbie ZCh „Police" by³a odpowiednio ni¿sza o 31 %, 23 % i 14 % w porównaniu do tych parametrów w liœciach kontrolnych.

S³owa kluczowe: bilans wodny, barwniki asymilacyjne, siarka, *Taraxacum officinale*

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INVASIVENESS OF THE ENTOMOPATHOGENIC NEMATODES *Steinernema feltiae* (Filipjev 1934) ISOLATED FROM VARIOUS HABITATS IN POLAND

INWAZYJNOŒÆ NICIENI ENTOMOPATOGENNYCH *Steinernema feltiae* (Filipjev 1934) WYIZOLOWANYCH Z RÓ¯NYCH ŒRODOWISK W POLSCE

Abstract: The paper presents results of a study on the effect of environmental lead pollution on the invasiveness of nematodes obtained from the field. The nematode *Steinernema feltiae* and larvae of the last growth stage of the greater wax moth *Galleria mellonella* L. were the study material. Nematodes were isolated from soil under laboratory conditions using Bedding and Akhurst's (1975) method. Physical and chemical analyses of soil samples were made in the Analytical Centre of the Warsaw Agricultural University. Total lead content was analysed with the flame atomic absorption spectrophotometry and soil pH with the potentiometric method acc. to the pB procedure (2 ed. of 21. June 2005). *Steinernema feltiae* were determined based on keys for entomopathogenic nematodes. The invasiveness of nematodes originating from various regions of Poland was evaluated.

Keywords: entomopathogenic nematodes, *Steinernema feltiae*, *Galleria mellonella*, heavy metals, lead ions

Bioinsecticides based on entomopathogenic nematodes are one of the biological plant protection means. Nematodes are characterised by a high reproductive potential, broad food spectrum and an ability to produce dormant stages in a form of invasive larvae. The larvae possess an ability to actively search for host-insect and to penetrate its body [1–5].

These traits are affected by both biotic and abiotic factors like heavy metals in the soil and soil pH.

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In the soils contaminated by heavy metals we may observe changes in the invasiveness and disturbed reproduction [6–9]. Heavy metals (including lead) get to the atmosphere from industrial emissions from various smelters, cement mills, steel works and intense transport. Then the metals fall and accumulate in the upper soil layers where they remain and affect various soil organisms for many years. Lead contamination is the most important threat to light acidic soils [10–12].

Total lead content and soil pH of analysed soil habitats was determined in this study.

Soils in Poland are usually acidic as a result of the presence of carbon dioxide in the air. The gas dissolved in water produces weak carbonic acid which falls onto soil with atmospheric precipitation. Soil pH is the key element decisive for many biological and physicochemical processes that take place in soil. Soil acidification largely affects mobility and bioavailability of heavy metals and ionic organic pollutants. Heavy metal activity increases with the increase of soil habitat acidity. Optimum range of soil pH for metabolic processes of most plant species and soil organisms varies from 5.5 to 7.2 [13]. A decrease in soil pH decreases pathogenicity and survival of entomopathogenic nematodes [14, 15].

New sites and new nematode species are still a matter of studies worldwide [16–19]. Few faunistic studies are carried out in Poland. The sites of entomopathogenic nematodes in the country were studied by Bednarek in the years 1985–1988. Now, results of these studies are outdated since soil properties and composition could alter during several years. Such changes may in turn affect the density and species composition of entomopathogenic nematodes.

This paper presents new sites of entomopathogenic nematodes in Poland. Performed studies were aimed at evaluating the invasiveness of entomopathogenic nematodes from various sites in Poland. At the same time lead content and acidity of these sites were determined.

Materials and methods

To find out the presence and to isolate entomopathogenic nematodes, 50 soil samples were collected from each of the 8 selected study areas representing possibly similar ecosystems in various zoogeographic regions of the country. Samples were collected with the Egner's sampler (diameter 2.5 cm) to the soil depth of 25 cm in an even grid on uniform surfaces. The method guarantees complete and even sampling from a given area which allows to estimating actual spatial distribution of nematode density and lead pollution of the soil habitat.

Soil samples were analysed for the presence of entomopathogenic nematodes.

Nematodes were isolated from soil samples in the laboratory with the Bedding and Akhurst's (1975) method [20]. Well mixed soil samples were placed in plastic boxes of a capacity of 250 cm^3 together with two trap insects (caterpillars of *Galleria mellonella* L.). Samples were placed in Sanyo incubator for 16 days at a temperature of 25 ^oC. Every two days dead insects were removed from boxes to estimate the reason of their death and transferred to the White's traps [21] to obtain invasive larvae. Dead insects were replaced by new live caterpillars.

Nematode larvae obtained in that way were used to infect (at a rate of 50 larvae per insect) next larvae of *Galleria mellonella* (of a mean body mass 0.165 g) to study insects mortality and the extensity and intensity of infection. Soil samples were also analysed for their physical and chemical properties in the Analytical Centre of the Warsaw University of Agriculture. Lead content in soil was estimated with the flame atomic absorption spectrophotometry (FAAS) and soil pH – with potentiometric method (acc. to PB 14 procedure 2nd edition 21. 06.2005) [22]. Analyses were performed at 25 ^{o}C which is the optimum temperature for the growth of *S. feltiae* [23]. All experiments were duplicated.

Results and discussion

Analyses of soil samples (Table 1) showed almost natural lead content (from 5.942 to 34.4 mg/kg) and strongly to slightly acidic reaction (pH from 4.74 to 6.7). Isolated nematodes were estimated with keys for species determination as *Steinernema feltiae*.

Table 1

Results of the analyses of studied soil samples

Site / Habitat	Lubin		Krasnik		Slupsk		Wielun	
	field	meadow	field	meadow	field	meadow	field	meadow
Pb [mg/kg]	17.05	11.25	34.4	22.3	11.88	5.942	7.21	6.86
pH [-]	6.15	4.74	6.64	6.7	6.14	6.64	5.35	5.62
Insect mortality [%]	100	100	100	97	100	100	100	100
Extensity of infection [%]	100	100	100	97	97	100	100	100
Intensity of infection [ind.]	11 (A)	13	13 (B)	10 (C)	22 (A,B)	12	17 (A)	15 (C)

A, B, C – highly significant differences between groups.

Analysis of variance was used to test the significance of differences between sites and habitats. No differences were found in the extensity of infection between particular groups.

Highly significant differences were found in the intensity of infection among the following sites and habitats: Lubin field – Slupsk field – Wielun field, Krasnik field – Slupsk field, Krasnik meadow – Wielun meadow. This result may indicate that populations from various regions of the country may be characterised by different degrees of invasiveness.

Acknowledgement

The paper was financed as a research project from public funds in the years 2007–2011.

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INWAZYJNOŒÆ NICIENI ENTOMOPATOGENNYCH ***Steinernema feltiae*** (Filipjev 1934) WYIZOLOWANYCH Z RÓ¯NYCH ŒRODOWISK W POLSCE

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Abstrakt: W pracy przedstawiono wyniki badañ nad wp³ywem zanieczyszczenia œrodowiska o³owiem na inwazyjnoœæ nicieni pozyskanych z terenu. Materia³ do badañ stanowi³y nicienie *Steinernema feltiae* oraz larwy ostatniego stadium barciaka wiêkszego (*Galleria mellonella* L.). Nicienie wyizolowano z próbek glebowych warunkach laboratoryjnych, metod¹ Beddinga i Akhursta (1975). Próbki glebowe zosta³y zbadane pod wzglêdem fizykochemicznym w Centrum Analitycznym SGGW. Oznaczono ca³kowit¹ zawartoœæ o³owiu w glebie metod¹ p³omieniowej absorpcyjnej spektrometrii atomowej FAAS oraz odczyn gleby metod¹ potencjometryczn¹ (wg procedury pB 14, wyd. 2 z 21.06.2005 r.). *Steinernema feltiae* oznaczono na podstawie kluczy do oznaczania gatunku nicieni entomopatogennych. Oceniono inwazyjnoœæ nicieni pochodz¹cych z ró¿nych rejonów Polski.

S³owa kluczowe: nicienie entomopatogeniczne, *Steinernema feltiae*, *Galleria mellonella*, metale ciê¿kie, jony o³owiu

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EFFECT OF AN INCREASED CONCENTRATION OF SODIUM CHLORIDE ON SOME PHYSIOLOGICAL FEATURES OF LETTUCE (*Lactuca sativa* var. *capitata*)

WP£YW PODWY¯SZONEGO STÊ¯ENIA CHLORKU SODU NA NIEKTÓRE CECHY FIZJOLOGICZNE SA£ATY MAS£OWEJ (*Lactuca sativa* var. *capitata*)

Abstract: Too high concentration of salt in the environment is one of the most important stressful factors causing serious morphological and physiological changes in plants. The aim of the present studies was to evaluate the effect of an increased concentration of sodium chloride in the medium under the condition of diverse fertilization with potassium on selected physiological features of head lettuce var. 'Justyna'. The content of assimilation dyes in leaves, the dry matter of the plant's aboveground part and root system and parameters of water balance were determined – the indices of relative water content and water saturation deficit of tissues.

Keywords: *Lactuca sativa* var. *capitata*, sodium chloride, water balance, assimilation pigments, dry matter

Excessive salinity of environment, caused mainly by large concentration of NaCl is one of the most important stressogenic factors limiting the growth and productivity of plants [1–3]. Metabolic disturbances of plants under the conditions of salt stress have a complex character and include dysfunctions resulting from both unbalanced water relations in tissues and limited uptake and long-distance transport of many substances indispensable to plants [4, 5]. The knowledge of plant mechanisms of tolerance towards excessive salinity is not satisfactory, which makes it difficult to work out effective methods of softening its influence. The results of some research works show that an increase in the content of mineral components in the medium can prevent negative consequences of environment salinity. Most of the data refer to the use of micro-elements, mainly potassium and calcium [6–8], and some refer to the effect of chemical elements belonging to other groups [9].

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The present study shows the results of experiments the aim of which was to determine how an increased concentration of NaCl in the medium, at a diversified level of fertilizing plants with potassium, affects the content of assimilation dyes in leaves, the production of biomass and the parameters of water balance of head lettuce var. 'Justyna'.

Material and methods

In 2007 in the laboratory of the Department of Plants Physiology of West Pomeranian University of Technology in Szczecin, a vegetative experiment was carried out using the method of water cultures. A two-factor system of complete randomization in five replications was used. The first experimental factor was concentration of sodium chloride in the medium: the control, 0.068 and 0.136 mol NaCl · dm^{-3}, whereas the second factor was the level of fertilization with potassium: + 20 % K, the control (without additional fertilization with potassium). The control was a full Hoagland's medium (pH 5.5). The experiment was carried out in two series (repetitions in time) in June and July.

The biological material of the research was head lettuce var. 'Justyna'. The lettuce seedlings were produced in our greenhouse from seeds. About 16 days after the time of sowing, when the plants reached the height of about 10 cm, they were carried to water cultures (glass flasks of 70 dm^3 capacity, filled with a full Hoagland's medium), where they grew for three successive days. Then the composition of the media was diversified in respect of NaCl and potassium concentration. The volume of the medium was systematically completed and it was aerated.

14 days after placing the plants in hydroponics, the content of assimilation pigments (chlorophyll *a*, *b* an total) in leaves, the total dry matter of the aboveground part and root system and the parameters of water balance were determined in three replications. In order to determine the content of assimilation pigments the method of Lichtenthaler and Wellburn [10] was used. The amount of chlorophyll and carotenoids was calculated according to Arnon et al [11]. Indexes of *relative water content* (RWC) and *water saturation deficit* (WSD) were defined according to Barry and Weatherly [12]. The dry matter of the investigated items were determined using the dryer method, after the plants had been dried up to the stable weight at the temperature of 105 ^oC in a dryer.

The obtained results concerning the content of assimilation dyes and dry matter of lettuce were worked out using the two-factor analysis of variance. The significance of differences between averages were determined by means of the Duncan test at the level of significance of $\alpha = 0.05$. Due to homogeneity of the variance of error the synthesis of results of two series of experiments [13] were carried out.

Results and discussion

Lettuce, like most usable plants, is regarded as the one belonging to typical glycophytes of relatively large sensitivity to too large salinity of environment. In the present studies it was shown that the applied concentration of 0.068 mol NaCl · dm^{-3}

did not cause any increase in the content of assimilation pigments in leaves of lettuce. In the case of plants growing in the medium of the 0.136 mol NaCl · dm^{-3} concentration, a decrease in concentration of chlorophyll *a*, *b* and total and carotenoids by about 30 % in relation to the control was observed, although the differences were not statistically significant. It proves, then, that the application of higher concentration of salt has a phytotoxic influence on the plants of the studied species. Similar results of studies on the effect of salt stress on the content of photosynthetic pigments in cucumber were reported by Hawrylak [9].

Introduction of potassium to saline environment did not cause any increase in the content of assimilation pigments. Only in the case of lettuce growing in the medium of the 0.068 mol NaCl · dm^{-3} concentration, a slight increase in the content of total chlorophyll (by about 5 %) after an additional supply of potassium to plants was observed (Table1).

Table 1

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

Dose of NaCl [mol · dm^{-3}]	Dose of K		Mean
	Full Hoagland's medium	Full Hoagland's medium + 20 % K	
Chlorophyll *a*			
Control	0.456 a	0.451 a	0.453 a
0.068	0.443 a	0.460 a	0.451 a
0.136	0.320 a	0.307 a	0.314 a
Mean	0.406 a	0.406 a	
Chlorophyll *b*			
Control	0.229 a	0.219 a	0.224 a
0.068	0.218 a	0.242 a	0.230 a
0.136	0.167 a	0.160 a	0.164 a
Mean	0.205 a	0.207 a	
Chlorophyll *a+b*			
Control	0685 a	0.670 a	0.677 a
0.068	0.662 a	0.701 a	0.681 a
0.136	0.488 a	0.467 a	0.477 a
Mean	0.611 a	0.613 a	
Carotenoids			
Control	0.237 a	0.239 a	0.238 a
0.068	0.237 a	0.253 a	0.245 a
0.136	0.169 a	0.167 a	0.168 a
Mean	0.214 a	0.219 a	

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

Under the conditions of salt stress a distinct limitation of water availability to plants was noticed and this in turn leads to disturbances of almost all vital processes [14]. In the present studies the largest index of the relative content of water was characteristic of the plants growing in the control medium with addition of 20 % K (RWC – 90.6 %) and in the control medium (RWC – 85.9 %) – Fig. 1. The increase in salinity of the environment resulted in the distinct worsening of water balance parameters of lettuce, which was illustrated by an increase in the index of water saturation deficit in tissues (in plants growing in the media of the concentrations of 0.068 and 0.136 mol NaCl · dm^{-3} it was respectively larger by 6.8 and 15.5 % than that of the control) – Fig. 2. The

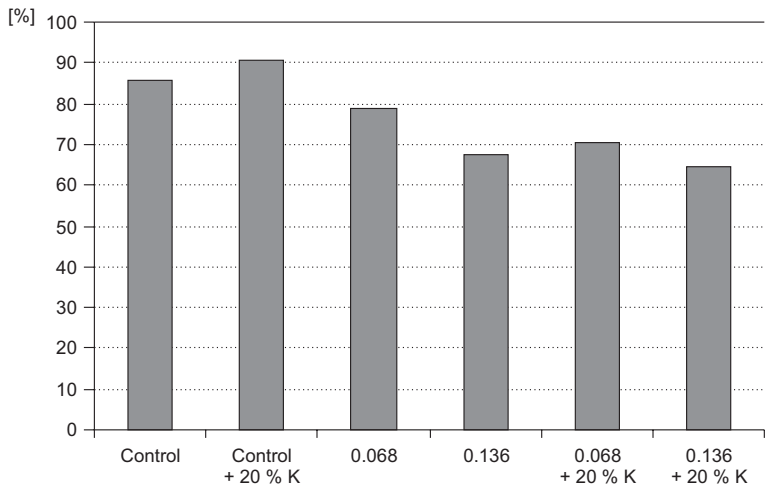

Fig. 1. Index of relative water content (RWC) in leaves of *Lactuca sativa* var. *capitata*

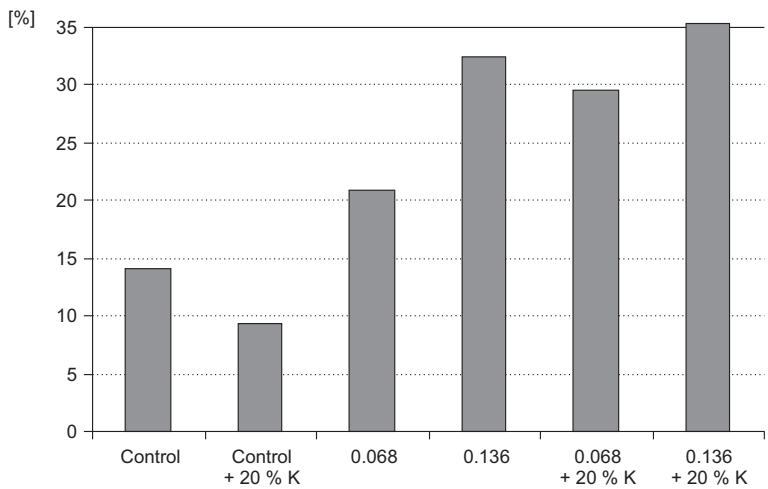

Fig. 2. Index of water saturation deficit (WSD) in leaves of *Lactuca sativa* var. *capitata*

enrichment of water cultures with potassium did not lower the unfavourable influence of the NaCl excess on water relations of the examined plant.

In most plants under the conditions of saline environment the inhibition of growth, the decrease in size and number of leaves and roots are observed, and at the same time the inhibition of growth of aboveground organs is usually larger than the limitation of root growth [15, 16]. Biomass production usually decreases as a result of an unfavourable impact of salt stress on parameters connected with gaseous exchange of plants. In the present studies, the dry matter of the aboveground parts of lettuce decreased significantly under the effect of the growing concentration of NaCl in the medium. In the plants growing in cultures of the concentrations 0.068 and 0.136 mol NaCl · dm^{-3} it was smaller by 40 and 60 %, respectively, in relation to the control. The largest dry matter of the root system was observed in lettuce growing in cultures of the concentration 0.136 mol NaCl · dm^{-3}. The increase in salinity of the environment up to the level of 0.136 mol NaCl · dm^{-3} caused a significant decrease in dry matter of roots of the studied plant (Table 2).

Table 2

Dry matter of *Lactuca sativa* var. *capitata* [g · $plant^{-1}$]

Dose of NaCl [mol · dm^{-3}]	Above-ground part			Roots		
	Full Hoagland's medium	Full Hoagland's medium + 20 % K	Mean	Full Hoagland's medium	Full Hoagland's medium + 20 % K	Mean
0.068	0.062 b	0.045 b	0.054 b	0.015 c	0.011 b	0.013 c
0.136	0.031 a	0.031 a	0.035 a	0.008 ab	0.009 ab	0.008 b
Control	0.110 c	0.070 c	0.090 c	0.005 a	0.006 a	0.0055 a
Mean	0.068 b	0.048 a		0.009 a	0.008 a	

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

Differentiated changes in cumulating the biomass in the response to salinity were also noticed by Khan et al [17] in species *Atriplex griffithii* var. *stocksii*, Ashraf et al [18] in *Brassica* and Wrochna et al [16] in ornamental amaranth. The addition of potassium to saline environment did not significantly influence the size of dry matter of both the aboveground parts and the root system of lettuce.

Conclusions

1. The 0.068 mol NaCl · dm^{-3} concentration did not cause a decrease in the content of assimilation dyes in leaves of lettuce.

2. In plants growing in the medium of the concentration 0.136 mol NaCl · dm^{-3} a decrease in the concentration of both chlorophyll and carotenoinds in leaves by about 30 % was observed.

3. The increase in salinity of environment resulted in the distinct worsening of water balance parameters of lettuce, which was illustrated by an increase in the index of water saturation deficit in tissues.

4. The dry matter of the aboveground parts of lettuce decreased significantly under the influence of the growing concentration of sodium chloride in the medium.

5. The effect of a diverse concentration of sodium chloride in the media on the yield of dry matter of root system was equivocal.

6. The introduction of potassium to saline environment was not proved to influence the increase in the content of assimilation pigments, the improvement of water balance parameters and the production of plant dry matter.

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WP£YW PODWY¯SZONEGO STÊ¯ENIA CHLORKU SODU NA NIEKTÓRE CECHY FIZJOLOGICZNE SA£ATY MAS£OWEJ (***Lactuca sativa*** var. ***capitata***)

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Abstrakt: Zbyt du¿e stê¿enie soli w œrodowisku to jeden z wa¿niejszych czynników stresowych wywo³uj¹cych powa¿ne zmiany morfologiczne i fizjologiczne u roœlin. Celem przeprowadzonych badañ by³a ocena wp³ywu podwy¿szonego stê¿enia chlorku sodu w po¿ywce, w warunkach zró¿nicowanego ¿ywienia roœlin potasem na wybrane cechy fizjologiczne sa³aty g³owiastej mas³owej odmiany 'Justyna'. Okreœlono zawartoœæ barwników asymilacyjnych w liœciach, such¹ masê czêœci nadziemnej i systemu korzeniowego oraz parametry bilansu wodnego – wskaŸniki wzglêdnej zawartoœci wody oraz deficytu wysycenia tkanek wod¹.

S³owa kluczowe: *Lactuca sativa* var. *capitata*, chlorek sodu, bilans wodny, barwniki asymilacyjne, sucha masa

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EFFECT OF MICROELEMENT FERTILIZATION ON THE QUALITY AND NUTRITIONAL VALUE OF THE MEADOW SWARD HAY PART I. THE CONTENT OF ORGANIC COMPONENTS AND NUTRITIONAL VALUE

WP£YW NAWO¯ENIA MIKROELEMENTAMI NA JAKOŒÆ I WARTOŒÆ POKARMOW¥ SIANA RUNI £¥KOWEJ CZ. I. ZAWARTOŒÆ SK£ADNIKÓW ORGANICZNYCH I WARTOŒÆ POKARMOWA

Abstract: The aim of the conducted study was the determination of the quality and nutritional value of the meadow sward as affected by the fertilization with microelements. Monocomponent copper, zinc and manganese foliar fertilizers and multicomponent Plonvit P preparation, containing chelated elements, were applied during the experiment. The prepared plant material was subjected to the analyses of: elemental chemical composition by the standard method, NDF, ADF and ADL contents were determined using ANKOM Fiber Analyser apparatus according to the method described by Goering and Van Soest. The nutritional value was expressed in the units of INRA system (1988) using Winwar 1.6 software (DJG). The evaluation was performed on the basis of tabular coefficients of forage distribution in the rumen and intestines. Significant increase of the mean total protein, raw ash, ADF, ADL, NDF, BTJN and BTJE content was stated as the effect of foliar application of multicomponent preparation as well as copper, zinc and manganese fertilizers when compared with the non-fertilized object. However, slight decrese of the determined values was found in the case of raw fat and N-free extracts.

Keywords: meadow sward, microelement fertilization, the quality and nutritional value

The balanced mineral fertilization, both with micro- and macroelements, is required for the proper growth and development of cultivated plants. Microelements, however, absorbed by plants in very small amounts, significantly affect the yielding of cultivated plants as well as influence the qualitative forage characteristic [1, 2]. As the

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microelements are constituents or activators of the most enzymes, their deficiency leads to sigificant perturbations of biochemical and physiological processes proceeding in plants [3, 4]. Microelements are provided from the soil simultaneously with the other mineral and organic fertilizers, but their amounts usually do not cover the fertilization requirements of plants, so there is a need to supplement their level in the form of microfertilizers. Therefore, the aim of the present study was to compare the effect of different microelements foliar fertilizers applied in the form of single, crystal chelates or as the multicomponent Plonvit P preparation, containing microelements in the chelated form, on the content of organic components as well as on the feeding value of the meadow sward.

Materials and methods

The field study was conducted in the years 2006–2008 in an individual agricultural farm in Solca, in the Pilica administrative disctrict, in Zawiercie county, in Silesia province, at the altitude of 320 m. The one-factor experiment was designed by the method of random block sampling, in four replicants, in the brown, acid soil (pH_{KCl} = 5.2) classified to the V quality class. The soil contained medium levels of assimilable potassium, manganese and zinc and were poor in assimilable phosphorus and copper.

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

The kind of microelement fertilization was the determining factor of the experiment. Single microelements: zinc, manganese, copper and multicomponent Plonvit P preparation, containing elements in the form of chelate complexes, were applied. The following forms and doses of microelements were used:

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

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

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

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

Plonvit P is a multicomponent, concentrated microelement fertilizer containing chelated elements. The doses of single microelements and Plonvit P were adjusted in such proportions to achieve the equal levels of the respective microelements in the single fertilizers and in the multicomponent preparation. Plonvit P fertilizer contains a single dose of 2 dm^3, recommended by the producer: 100 g Zn, 100 g Mn and 60 g Cu in the form of chelates. The sprying solutions were prepared by dissolution of the proper amounts of chelates containing microelements in such water volume as to obtain the volume of working liquid corresponding to 300 dm^3 · ha^{-1}. The tap water of the medium hardness degree was used for that purpose. The application of fertilizers was done as follows: the first sprying – after the beginning of the spring vegetation, the following –

after the harvesting at the stage of the initial sward regrowth but not later than 3 weeks before the next mowing. During the investigated years the basic mineral fertilization was also applied: under I regrowth – 80 kg N · ha^{-1} and under II and III regrowths – 60 kg N · ha^{-1} for each regrowth in the form of ammonium saltpetre; phosphorus – once in the spring, in the amount of 120 kg P_2O_5 · ha^{-1} as a triple superphosphate and potassium – under the first and third regrowths in a dosage of 60 kg K_2O · ha^{-1} (for each regrowth) as 57 % potassium salt.

The area of each field amounted to 10 m^2. In the collected plant material the chemical composition of the forage was determined. The content of the basic nutrients was evaluated by the Weenden method [5]. The feeding value was expressed in the INRA (Institut National de la Recherche Agronomique) system units using 1.6 Winwar software (DJG). The estimation of hay was performed using the tabular coefficients of forage distribution in rumen and intestines. Results of the field experiments were subjected to statistical analysis of variance and the significance of differences between average values was estimated on the basis of the confidence interval according to Tuckey at the significance level of $\alpha = 0.05$.

Results and discussion

On the basis of the obtained results the significant influence of the applied fertilization on the content of organic components and the feeding value of the meadow sward was stated (Table 1). As a result of foliar sprying with microelements a significant increase of crude protein content in the meadow sward was observed. The fertilization with the multicomponent Plonvit P preparation as well as with single microelements ie: zinc, copper and manganese contributed to the considerable growth of this component in relation to the control object – differences amounted to: 33, 33, 32 and 26 %, respectively. Foliar application of manganese, multicomponent fertilizer and copper influenced a significant drop in the crude fat content in the meadow sward (56, 41 and 24 %, respectively) in comparison with the control field. The treatment with copper, Plonvit P and manganese fertilizers affected higher concentration of crude fiber, which increased by on average 11.1; 6.2; and 5.8 % in comparison with its content in the meadow sward collected from the non-fertilized object. Our investigations revealed that the foliar application of copper, zinc, multicomponent and manganese fertilizers caused the decreased level of nitrogen-free extract. The respective values were 16.8; 14.8; 12.5 and 8.2 % lower than in the case of the control field. As the fiber fractions are taken into consideration it was found that ADF, ADL and NDF concentrations were positively affected by the foliar fertilization with microelements. The mean differences between the respective values for the fertilized objects and the control field reached the level of 7, 44 and 4 %.

As regards the energetic value, the examined fodders were characterized with comparable UFL and UFV levels. On the contrary, the protein value was much more diversified and fluctuated in the range of 49–73 for PDIN and 71–81 g · kg^{-1} d.m. for PDIE. The average difference between the values stated for the fields fertilized with microelements and the non-fertilized field was equal to 30 % for PDIN and 11 % for PDIE.

Table 1

The weighted mean content of organic components and the feeding value of the meadow sward as affected by the kind of microelement fertilization (mean for three years)

Parameter	Fertilized object					Mean	$NIR_{0.05}$
	Control	Multicomponent fertilizer	Cu	Zn	Mn		
	[g · kg^{-1} d.m.]						
Crude protein	80.6	120.3	118.7	120.1	109.2	109.8	9.0
Crude fat	29.9	21.2	24.1	29.3	19.2	24.7	3.5
Crude fiber	279.0	297.5	313.9	288.5	296.1	295.0	6.4
Nitrogen-free extract	542.4	482.0	464.2	472.5	501.3	492.5	4.3
Raw ash	68.1	79.1	79.1	89.6	74.2	78.0	6.5
ADF	326.8	345.5	369.5	331.2	359.3	346.5	6.4
ADL	27.1	50.8	58.5	35.5	50.1	44.4	9.3
NDF	531.8	566.6	590.4	516.5	537.1	548.5	5.8
UFL	0.75	0.74	0.74	0.75	0.73	0.74	11.32
UFV	0.66	0.66	0.66	0.66	0.64	0.66	30.19
PDIN	49.0	73.0	68.0	73.0	68.0	66.2	19.1
PDIE	71.0	81.0	78.0	81.0	78.0	77.8	6.3

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

The obtained results, as regards the feeding value of the examined plants, did not vary from the results reported by other authors [6]. It is worth emphasizing that under the conditions of foliar fertilization with microelements the forage quality of the meadow sward was significantly improved. The highest enhancement of the protein value was observed after the application of multicomponent preparation and zinc fertilizer, which is the cofactor of many enzymes and whose deficiency affects perturbations in the carbohydrates metabolism as well as auxins, DNA and RNA synthesis [2]. The function of zinc as a regulator of the level of saccharides and energetic balance was also emphasized by Grzywnowicz-Gazda [7]. The obtained results are partly consistent with the data found in literature concerning other cultivated plant species [8, 9].

Conclusions

1. The fertilization with multicomponent Plonvit P preparation as well as with single microelements ie: zinc, copper and manganese contributed to the considerable increase of the crude protein content in relation to the control object – by 33, 33, 32 and 26 %, respectively.

2. The treatment with copper, multicomponent and manganese fertilizers affected higher concentration of crude fiber, which increased by on average 11.1; 6.2; and 5.8 % in comparison with the non-fertilized object.

3. Foliar application of manganese, multicomponent fertilizer and copper influenced 56, 41 and 24 % (respectively) drop in the crude fat content in the meadow sward in comparison with the control field.

4. The fertilization with microelements resulted in 30 % and 11 % (on average) higher level of the protein value expressed as PDIN and PDIE (respectively).

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WP£YW NAWO¯ENIA MIKROELEMENTAMI NA JAKOŒÆ I WARTOŒÆ POKARMOW¥ SIANA RUNI £¥KOWEJ CZ. I. ZAWARTOŒÆ SK£ADNIKÓW ORGANICZNYCH I WARTOŒÆ POKARMOWA

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Abstrakt: Celem podjêtych badañ by³o okreœlenie jakoœci i wartoœci pokarmowej runi ³¹kowej w zale¿noœci od zastosowanego nawo¿enia mikroelementowego. Stosowano dolistnie pojedyncze mikroelementy miedzi, cynku i manganu oraz wielosk³adnikowy nawóz Plonvit P, zawieraj¹cy pierwiastki w formie schelatowanej. W przygotowanym materiale roœlinnym oznaczono podstawowy sk³ad chemiczny metod¹ standardow¹, analizy na zawartoœæ NDF, ADF i ADL przeprowadzono za pomoc¹ aparatu ANKOM Fiber Analyser, wed³ug metody zaproponowanej przez Goeringa i Van Soesta. Wartoœæ pokarmow¹ wyceniono w jednostkach systemu INRA 1988 za pomoc¹ programu komputerowego Winwar, wersja 1.6. firmy DJG. Do wyceny pos³u¿ono siê tabelarycznymi wspó³czynnikami rozk³adu pasz w ¿waczu oraz jelitach. W wyniku dolistnego stosowania wielosk³adnikowego nawozu oraz miedzi, cynku i manganu stwierdzono znaczny wzrost œredniej zawartoœci bia³ka ogólnego, popio³u surowego, ADF, ADL, NDF, BTJN oraz BTJE w porównaniu z obiektem nienawo¿onym. Natomiast w przypadku t³uszczu surowego oraz wyci¹gów niezawieraj¹cych azotu i JPM odnotowano nieznaczne obni¿enie siê ich zawartoœci.

S³owa kluczowe: ruñ ³¹kowa, nawo¿enie mikroelementami, jakoœæ i wartoœæ pokarmowa

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BIODIVERSITY OF SOIL FAUNA DEPENDING ON VEGETAL COVER AND FERTILIZATION

BIORÓ¯NORODNOŒÆ FAUNY GLEBOWEJ W ZALE¯NOŒCI OD OKRYWY ROŒLINNEJ I NAWO¯ENIA

Abstract: The investigations were conducted in the years 2006–2007 in Czarny Potok village at the foot of Jaworzyna Krynicka Mt. Three experimental areas were established on the investigated terrain (differing in the soil vegetal cover): grassland, arable land and forest. The soil samples were collected three times: in autumn 2006, in spring 2007 and in summer 2007. The numbers of soil fauna differed depending on the vegetal cover and the land use. The soil fauna was most numerously trapped in the mountain meadow soil used for hay production and fertilized with farmyard manure. Less numerous meso- and macrofauna was observed on the arable land.

Keywords: mesofauna, macrofauna, vegetal cover, fertilization

Soil organisms play a crucial role in processes of organic matter decomposition. Microorganisms such as fungi, Actinomycetales and bacteria participate in this process, whereas organisms classified as mesofauna and macrofauna also play an important role. Soil animals also affect the soil structure [1]. Mesofauna significantly participates in the restoration of the proper structure in compacted soils [2]. Species composition and numbers of these organisms depend on many environmental factors, such as soil physico-chemical properties or environment pollution with heavy metals [3]. Also the way and methods of soil cultivation affect the living environment of soil organisms [4, 5]. Mesofauna is often used for the environment monitoring.

The investigations aimed at identification of the impact of various vegetal cover types and land use on the occurrence of soil fauna.

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Material and methods

The research was conducted in the years 2006–2007 in Czarny Potok village at the foot of the Jaworzyna Krynicka Mt. (650 m a.s.l., 20°55′34″ E, 49°24′35″ N). Brown soil with granulometric structure of loamy sand cover the experimental field. The soil is acid, medium abundant in potassium and greatly phosphorus-deficient, its chemical composition was shown in Table 1.

Table 1

Chemical composition of soil (collected 20.09.2006)

Vegetal cover	Variant	pH_{H_2O}	pH_{KCl} [1 mol · dm^3]	Organic matter [%]	N_{total} [%]	Available [mg · kg^{-1} d.m.]		
						P	K	Mg
Grassland	0 – control	5.37	4.29	4.87	0.27	10.5	34.9	133
	fallow	5.16	4.18	4.52	0.22	11.0	47.2	123.2
	$P_{18}K_{50}$	5.42	4.53	4.7	0.25	12.6	47.3	137
	$P_{18}K_{50}N_{100}$	5.53	4.84	4.18	0.26	13.1	39.0	150.6
	manure 10 Mg · ha^{-1}	5.46	4.51	5.47	0.27	14.0	55.6	149
	manure 10 Mg + P_4N_{31}	5.26	4.2	4.77	0.17	8.7	34.9	11.2
	sheep pen	4.95	4	4.98	0.24	13.5	97.1	58
Arable land	cereals	4.95	4.17	4.44	0.15	12.0	64.2	112.7
Forest	mixed forest	3.89	2.95	11.22	0.29	7.3	31.5	46.8

The length of the vegetation period in the presented region fluctuates from 150 to 180 days and the snow cover usually remains for about 150 days.

Three parts (differing with their vegetal cover) were identified in the experimental area: grassland, arable land and forest.

The following variants were applied on the grassland:

– non-fertilized and uncut meadow (fallow),
– unfertilized but cut meadow (0 – control),
– fertilized and cut meadow ($P_{25}K_{50}N_{100}$),
– fertilized and cut meadow ($P_{25}K_{50}$),
– meadow fertilized with sheep manure and cut (manure 10 Mg · ha^{-1}),
– meadow fertilized with sheep manure plus mineral fertilizers and cut (manure 10 Mg + P_4N_{31}),
– meadow fertilized by penning and cut (sheep pen).

The following crop succession with $P_{25}K_{50}N_{100}$ fertilization is applied on the arable land:

1) Root crops, cereals, cereals with undersowing, legumes:
   – a rye mixture with vetch was sown in 2006,
   – oat was sown in 2007.
2) In the forest area:
   – mixed forest (beech and spruce).

Unfertilized and uncut meadow is an object where no cultivational measure are applied. The unfertilized and cut meadow was considered as the control site. On the fertilized and cut meadow mineral fertilization was applied each year: phosphorus – once in spring as a 46 % triple superphosphate, potassium as 56 % potassium salt in equal part under the I and II regrowth; nitrogen as 34 % ammonium nitrate was divided into two parts applied under the I and II regrowth, respectively 60 % and 40 %, sheep manure was used early in spring. The manure chemical composition was as follows: total N – 0.69; P – 0.14; K – 0.60; Ca – 0.25; Mg – 0.08; Na – 0.06 % in the fresh mass. On the manure-mineral treatment phosphorus and nitrogen doses were supplemented with mineral fertilizers to the amounts used on the treatment receiving mineral fertilizers ($P_{25}K_{50}N_{100}$). The sheep pen was established in the early spring 2005. There was one sheep per 1 m^2 of the meadow. The sheep spent two nights (2 × 7 hrs) in the pen. During 7 hours spent in the pen a single sheep produced 479 g of excrement and 371g of urea. The sheep staying in the pen left: 184 kg total N, 27.8 kg P and 294.2 kg $K \cdot ha^{-1}$. The sward was cut twice a year, the first regrowth was harvested at the turn of the second and third decade of June and the second in the third decade of August. The following doses of fertilizers were applied pre-sowing on the arable land: 25 kg ha^{-1} phosphorus as 45 % triple superphosphate, 50 kg ha^{-1} potassium as 56 % potassium salt and 120 kg ha^{-1}nitrogen as 34 % ammonium nitrate. Nitrogen was divided into two doses, proportionately 60 and 40 % of which the first was applied pre-sowing and the second as a topdressing.

The soil was sampled three times: in the autumn 2006, in the spring 2007 and in the summer 2007. Three spoil samples were collected from each experimental area using a 15 cm long cylinder with 10 cm diameter. The soil samples were taken to a laboratory to isolate and determine the soil fauna in them. The soil samples were first sifted through sieves to isolate the macrofauna and subsequently the sifted soil was placed in the Tullgren apparatus to extract smaller organisms into the vials containing 75 % alcohol. The collected material was counted and labelled [6, 7].

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

Results and discussion

Depending on the vegetal cover and meadow use the number of nematodes isolated from the soil of the analysed object differed considerably (Table 2). Nematodes were most numerous in the soil of the meadow fertilized with sheep manure. A smaller number of nematodes in comparison with the control was noted in the soil of the minerally fertilized meadow, whereas the least was found in the forest ground and from the arable land. Soil mites are an important group of soil organisms since they play a crucial role in matter transformation processes in the soil environment [8]. In the analyzed soil environment, soil mites were the most numerous in the meadow fertilized through penning. Much fewer mites were observed in the arable soil and on minerally fertilized meadow. The greatest number of captured soil mesofauna specimens belonged

to the *Collembola*. The *Collembola* were particularly numerous in the mountain meadow soil fertilized with sheep manure and in the pen soil. In the arable soil on average 30 % less *Collembola* were trapped than in the mountain meadow soil. Numerous cultivational measures, such as ploughing, negatively affect *Collembola* numbers in soil [7], which may be the cause of a smaller number of this organism group in the arable soil under analysis.

Table 2

Occurrence of soil mesofauna – average number of specimens captured on three dates: autumn, spring and summer

Vegetal cover	Variant	Number of specimens [pcs.]		
		Nematoda nematodes	*Acari* mites	*Collembola* springtails
Grassland	0 – control	11.1	5.1	59.3
	fallow	8.6	5.4	52.3
	$P_{18}K_{50}$	8.4	3.6	50.2
	$P_{18}K_{50}N_{100}$	7.8	3.7	46.2
	manure 10 Mg · ha^{-1}	15.7	4.1	66.8
	manure 10 Mg + P_4N_{31}	10.8	5.6	60.3
	sheep pen	11.8	6.1	66.3
Arable land	cereals	9.7	3.1	42.6
Forest	mixed forest	7.1	5.6	50.4
$LSD_{< 0.05}$		4.24	1.19	8.56

In the observed macrofauna the greatest number of insects belonging to the beetle order were captured (Table 3). On the other hand, representatives of beetles were scarce in the soil samples collected from the fallow, whereas in the samples taken from the meadow fertilized with manure, beetles were particularly numerously captured. In this soil also representatives of myriapods and earthworms occurred in a great number. Earthworms were not spotted in the soil samples collected from the minerally fertilized meadow, there were also only few of them in the forest soil.

Soil mesofauna is often used as an indicator in ecological research, among others due to the sensitivity to environmental factors and common occurrence [9]. The present research shows that mineral fertilization may lead to a decrease in the numbers of this organism group. On the other hand, organic fertilization favours a more numerous occurrence of nematodes and *Collembola*. Also fertilization through penning caused an increase in the mesofauna numbers in the mountain meadow soil. While comparing the effect of vegetal cover on soil fauna one may notice its more numerous presence in meadow soils in comparison with arable lands. Permanent vegetal cover on a meadow favours greater soil biodiversity. Lesser numbers of nematodes in the forest soil might have been due to its stronger acidification as compared with the meadow and the arable soil.

Table 3

Occurrence of soil macrofauna – average number of specimens captured on three dates: autumn, spring and summer

Vegetal cover	Variant	Number of specimens [pcs]						
		Araneae	*Myriapoda*	*Coleoptera*	*Diptera*	*Hymenoptera*	*Lepidoptera*	*Lumbricidae*
Grassland	0 – control	0.9	1.3	5.7	0.3	0.8	0.3	0.3
	fallow	1.3	0.9	2.9	1.3	1.1	1.6	0.4
	$P_{18}K_{50}$	1.0	0.4	4.1	0.8	1.1	0.3	0.0
	$P_{18}K_{50}N_{100}$	0.7	0.6	4.4	0.7	1.8	0.3	0.0
	manure 10 Mg · ha^{-1}	1.2	2.2	4.9	1.0	1.0	0.4	2.1
	manure 10 Mg + P_4N_{31}	1.2	1.6	4.1	0.7	0.8	0.4	1.0
	sheep pen	1.6	1.9	4.3	1.4	1.0	0.2	0.9
Arable land	cereals	1.4	1.0	4.1	0.9	0.7	0.2	0.4
Forest	mixed forest	1.7	2.4	4.4	0.2	0.3	0.1	0.1
$LSD_{< 0.05}$		n.s.	1.60	1.47	0.97	1.09	0.78	0.69

n.s. – non-significant differences.

Conclusions

1. Manure fertilization benefits more numerous occurrence of nematodes in a mountain meadow soil.

2. Mineral fertilization caused a decrease in the numbers of nematodes and soil mites.

3. The meadow soil environment was settled by a more numerous mesofauna in comparison with the arable land.

4. Manure fertilization favours earthworm presence.

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BIORÓ¯NORODNOŒÆ FAUNY GLEBOWEJ W ZALE¯NOŒCI OD OKRYWY ROŒLINNEJ I NAWO¯ENIA

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Abstrakt: Badania przeprowadzone zosta³y w latach 2006–2007 w Czarnym Potoku u podnó¿a Jaworzyny Krynickiej. Na terenie doœwiadczalnym wydzielono trzy obszary doœwiadczalne (ró¿ni¹ce siê okryw¹ roœlinn¹ gleby): u¿ytek zielony, grunt orny i las. Próbki gleby pobrano trzykrotnie: jesieni¹ 2006 r., wiosn¹ 2007 r. i w lecie 2007 r. Liczebnoœæ fauny glebowej ró¿ni³a siê w zale¿noœci od okrywy roœlinnej i sposobu u¿ytkowania. Fauna glebowa najliczniej by³a od³awiana w glebie ³¹ki górskiej u¿ytkowanej koœnie i nawo¿onej obornikiem. Mniej liczn¹ mezo- i makrofaunê glebow¹ zaobserwowano na gruncie ornym.

S³owa kluczowe: mezofauna, makrofauna, okrywa roœlinna, nawo¿enie

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NITRATES(V) CONTENT IN POTATO TUBERS CULTIVATED UNDER VARIOUS FERTILIZATION SYSTEMS

ZAWARTOŒÆ AZOTANÓW(V) W BULWACH ZIEMNIAKA UPRAWIANEGO W RÓ¯NYCH SYSTEMACH NAWO¯ENIA

Abstract: In a static field experiment established on brown soils in 1973, two types of natural fertilizers, farmyard manure (FYM) and liquid manure, mineral fertilizers (NPK) and combined organic and mineral fertilization were applied. Liquid manure was applied in two doses: rate I balanced with FYM and mineral fertilizers in the amount of nitrogen and rate II balanced in the amount of carbon. The experiment ran in two series: with and without liming. During the experiment, a 7-year plants rotation system was carried out.

It has been found that each fertilization system contributed to an increase in the amount of nitrate(V) nitrogen in potato tubers. The highest concentration of nitrates(V), exceeding from 2.1-fold (limed soil series) up to 2.3-fold (soil not limed) the permissible amount of these compounds, was determined in potato tubers fertilized with liquid manure in rate II and rate II + PK. Among the fertilizers with which equal amounts of nitrogen were introduced to soil, higher excess of the permissible concentration of nitrates(V) in potato tubers was caused by the application of mineral fertilizers and liquid manure in rate I than FYM. Supplementation of liquid manure with mineral fertilizers PK caused a further increase in the concentration of nitrates, whereas in the case of FYM the effect of PK was opposite. Soil liming differentiated the content of nitrates(V) in potato tubers but the effect of this treatment was ambiguous.

Keywords: fertilization, potato tubers, content of nitrates(V)

Nitrogen in plants appears primarily in the form of organic compounds and just small amounts of this element remain in the mineral form. Under increased content of mineral nitrogen in soil, where the process of reduction of nitrate(V) ions does not keep pace with their uptake, the mineral form of nitrogen may accumulate in plants. Accumulation of nitrates in plants is a complex process and depends on a number of factors, such as: genetic traits, soil and climatic conditions, fertilization and period of crop cultivation [1–5]. Genetic control of the content of nitrates may be a species – or even cultivar – specific trait but in many cases the ability to accumulate nitrates is relatively poorly inheritable [6]. The agronomic factor which mostly affects the accumulation of nitrates in plants is fertilization. High content of these compounds occurs mainly after

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unidirectional, excessive fertilization with nitrogen, introduced to soil with mineral or natural fertilizers [7–11].

Potato belongs to crops which do not tend to accumulate nitrates. Notwithstanding this, potato tubers grown experimentally or commercially are found to contain varied and often excessive amounts of nitrates. This is a disturbing finding because potato tubers make up a large share of foodstuffs in human diet [5, 7, 12].

The aim of this study has been to determine the effect of long-term application of three fertilization systems (organic, mineral and mixed organic and mineral) on the content of nitrates(V) in potato tubers.

Material and methods

In a static field experiment set up in 1973 on brown acid soil formed from slightly loamy sand class IVb, two types of natural fertilizers, FYM and liquid manure, mineral fertilizers (NPK) and combined mineral and organic fertilization were applied*. Rate I of liquid manure, FYM and mineral fertilization were balanced every year with an appropriate amount of nitrogen while rate II of liquid manure was balanced with a dose of FYM by introducing an appropriate dose of organic carbon. The annual average quantities of nutrients (NPK) introduced to soil with natural and mineral fertilizers during the 37 years of the experiment were the following (per 1 ha):

Liquid manure rate I	133 kg N	56 kg P	84 kg K,
Liquid manure rate II	393 kg N	168 kg P	240 kg K,
FYM	133 kg N	45 kg P	108 kg K,
Mineral fertilizers NPK	133 kg N	43 kg P	124 kg K.

Against the background of natural fertilizers, supplementary mineral PK fertilization was carried out, equal 1/2 of the rate of these fertilizers introduced in the treatment with mineral fertilization (NPK). Phosphorus and potassium fertilizers were applied before sowing in the form of 46 % superphosphate and 60 % of potassium salt, respectively.

The amounts of total nitrogen and organic carbon in the soil sampled from the treatment which for 37 years had not been fertilized were, respectively, 53 mg · kg^{-1} and 428 mg · kg^{-1}. As a result of the annual fertilization, the total nitrogen content rose from 5.7 % in soil fertilized with rate I of liquid manure to 32.1 % in soil enriched with rate II of liquid manure + PK, whereas the content of organic carbon increased from 14 % in soil treated with mineral fertilizers to 80.6 % in soil fertilized with FYM + PK. The value of soil reaction measured in KCl solution of the concentration equal 1 mol · dm^{-3} ranged from 4.40 to 5.45.

The experiment was run in two series – with and without liming. Soil liming was carried out in 2006, ie 34 years after the experiment had been set up. A seven-year crop rotation system was maintained during the study period. The experimental plots were sown with crops in the following rotation sequence: potatoes, spring barley + red clover with grasses sown between barley, red clover with grasses, winter oilseed rape, winter wheat + aftercrop rye, maize grown for green mass, spring barley, winter wheat. Clover

* Long-term field experiment set up according to schema processed by professor Teofil Mazur.

with grasses was grown only in the first sequence of the rotation. This paper presents the results of our studies obtained during the sixth rotation. In 2009, edible potato, 'Cekin' cv., was grown. Potato tubers were harvested after 123 days of the growing season.

The total and protein nitrogen content in potato tubers was determined using Kjeldahl's method. Solution of trichloroacetic acid of the final concentration of 12 % was used to precipitate protein substances from the plant material.

The content of nitrates(V) in potato tubers was determined in fresh mass, according to the standard analytical method elaborated by Orion [13], using an Ionanlyzer® Orion Model 407 potentiometer and a Thermo Orion model 9307™ ionselective nitrogen electrode. The results of the analyses underwent statistical processing with STATISTICA version 9 software programme [14], at the level of significance $\alpha = 0.05$ using a two-factor analysis of variance ANOVA.

Results and discussion

Potato tubers, apart from nutrients, may also contain compounds harmful to health, including some forms of nitrogen. The total content of this element and the forms in which it appears in potato tubers depends on the genetic traits of this plant and on some environmental conditions. High rates of fertilizers, particularly nitrogen, tend to raise the content of nitrates(V) and (III) and depress the nutritive value of this crop [4, 9, 15–17].

In the present experiment, the total content of nitrogen in potato tubers ranged from 0.91 to 1.42 % (Fig. 1), whereas that of protein nitrogen was between 0.58 to 0.74 % (Fig. 2).

Each fertilization system contributed to increasing the concentration of both forms of nitrogen compared with the control. In unlimed soil, the highest increase in the content of total nitrogen appeared in tubers of potato plants fertilized with rate II of liquid manure, with which nearly three-fold more nitrogen was introduced to soil than with the other fertilizers. This observation is confirmed by the data presented in papers by other

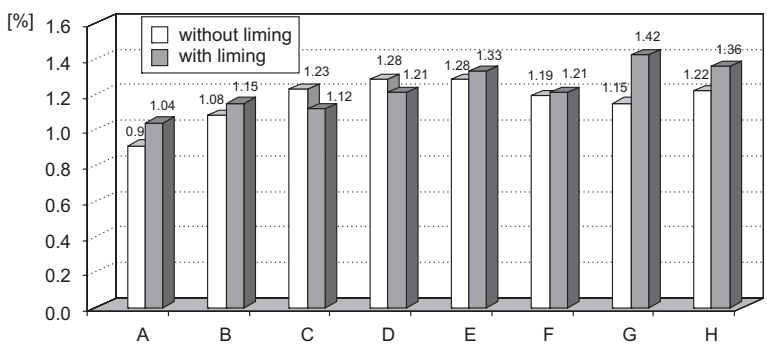

Fig. 1. Total nitrogen content in potato tubers: A – without fertilization; B – liquid manure rate I; C – liquid manure rate I + PK; D – liquid manure rate II; E – liquid manure rate II + PK; F – FYM; G – FYM+ PK; H – NPK; LSD: fertilization – 0.03; liming – 0.02; interaction – 0.05

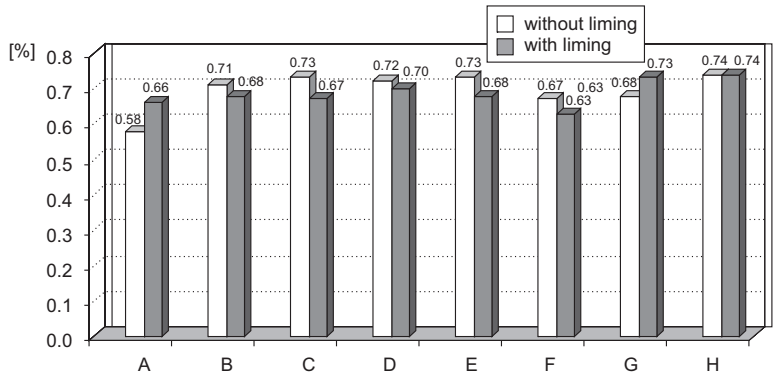

Fig. 2. Protein nitrogen content in potato tubers: A – without fertilization; B – liquid manure rate I; C – liquid manure rate I + PK; D – liquid manure rate II; E – liquid manure rate II + PK; F – FYM; G – FYM + PK; H – NPK; LSD: fertilization – 0.05; liming – 0. 01; interaction – 0.02

authors [4, 9, 11, 15], who report that increasing rates of nitrogen caused a significant increase in the content of this element in potato tubers, although the recorded increments were varied. Among the treatments where nitrogen balanced fertilizer rates were applied, higher concentrations of nitrogen were found in tubers of potatoes nourished with mineral fertilizers and FYM than the ones fertilized with liquid manure.

In the series with unlimed soil, the type of fertilizers or the rate of liquid manure did not have significant effect on the content of protein nitrogen in plants (Fig. 2). However, they significantly modified its share in the total nitrogen content (Table 1).

Table 1

Share of protein nitrogen in total nitrogen content [%]

Fertilization	Unlimed soil	Limed soil
Without fertilization	63.7	63.5
Liquid manure rate I	65.7	59.1
Liquid manure rate I + PK	59.3	59.8
Liquid manure rate II	56.3	57.9
Liquid manure rate II + PK	57.0	51.1
FYM	56.3	52.1
FYM + PK	59.1	51.4
NPK	60.7	54.4
$LSD_{0.05}$ fertilization (I)	2.1	
liming (II)	1.0	
interaction (I · II)	2.9	

In most of the fertilized treatments, the share of protein nitrogen in the total amount of this element was depressed, with the decline being larger after an application of FYM and liquid manure in rate II than after soil enrichment with mineral fertilizers. There was just one exception, namely potato tubers harvested from treatments fertilized with

rate I of liquid manure or rate I of liquid manure + PK, where the contribution of protein nitrogen to total nitrogen was 2 % higher than in the control.

No unambiguous effect was observed of the phosphorus-potassium fertilization applied in conjunction with natural fertilizers on the total nitrogen and protein nitrogen content in potato tubers. In most treatments, however, a tendency appeared for an increase in both forms of this element compared with the treatments receiving exclusively organic fertilization.

The soil liming treatment applied in the experiment added to a significant increase in the total nitrogen content in potato tubers except the treatments which were enriched with liquid manure in rate I + PK or liquid manure in rate II, where the content of nitrogen was higher compared to the unlimed series. With respect to protein nitrogen, liming did not have an unambiguous effect on the value of this parameter. In most treatments, there was a decreasing tendency regarding protein nitrogen in potato tubers compared with unlimed treatments.

Many authors [1, 4, 7, 16, 18] claim that as the amount of nitrogen introduced to soil under potatoes increases, so does – proportionately – the content of nitrates in potato tubers. Excessive accumulation of this form of nitrogen can be alleviated by more favourable weather conditions during the growth of potatoes [5]. According to Wojciechowska [6], under good insolation conditions it is possible to obtain a low content of nitrates in the crop yield, whereas low intensity of sunlight (autumn–winter) may raise their concentration several fold.

In the present research, the content of nitrate(V) nitrogen in potato tubers oscillated within 154.4–466.6 mg · kg^{-1} fresh mass in the unlimed series and between 158.7 and 421.2 mg · kg^{-1} fresh mass in the series where soil was limed (Fig. 3). In all the treatments, fertilization contributed to a significant increase in this form of nitrogen, up to the values exceeding their permissible quantities as established by the Ordinance of the Minister for Health of 13 January 2003 [19]. This threshold level was exceeded by 44.0 to 266.6 mg NO_3^- · kg^{-1} fresh mass.

Among the treatments where natural and mineral fertilizers were applied in rates balanced with respect to nitrogen introduced to soil, in both experimental series (limed and unlimed), a smaller increase in nitrates(V) in potato tubers occurred after

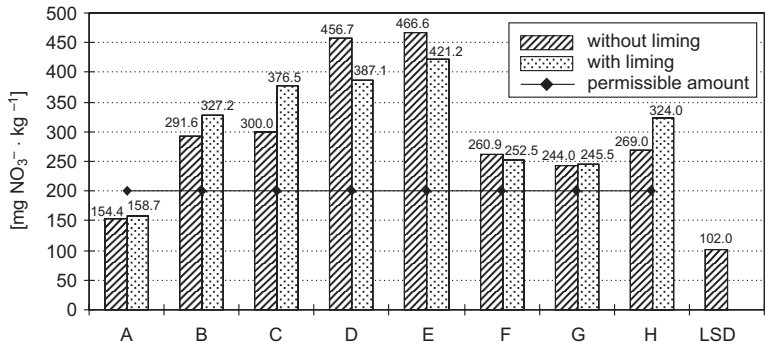

Fig. 3. Content of nitrates in potato tubers; A–H: key as in Figs. 1 and 2

fertilization with FYM and mineral fertilizers than after an application of liquid manure. An exceptionally high increase in the content of nitrates(V) was caused by fertilization with liquid manure rate II, balanced with FYM in respect of the amount of organic carbon introduced to soil. Three-fold more nitrogen was introduced with this fertilization system than with liquid manure rate I, FYM and mineral fertilizers.

The content of nitrates(V) was also affected by phosphorus-potassium fertilization applied in combination with natural fertilizers. When these fertilizers were used together with liquid manure, nitrate(V) nitrogen in potato tubers tended to increase but when the same fertilizers were applied alongside FYM, a non-significant decrease in this form of nitrogen in tubers appeared. Rogozinska et al [20], who emphasized the fact that potassium fertilization increased the content of nitrates in potato tubers, added that magnesium fertilization caused a reduction in the concentration of these compounds.

Soil liming did not have any significant effect on the increase in the content of nitrates(V) in potato tubers except the treatments with the second rate of liquid manure and FYM, where the effect of soil liming appeared as a significant increase in the concentration of the analysed form of nitrogen.

The results reveal large differentiation in the level of nitrates(V) in tubers of potatoes grown on soil fertilized with FYM versus the other types of fertilizers. The low content of nitrates(V) in tubers of potatoes fertilized with FYM is in accord with the data reported by other authors [4, 5, 8, 20, 21] but does not confirm the results of the author's previous experiment on grey-brown podsolic soil [22].

Numerous authors [12, 21, 23, 24] suggest that potatoes grown at organic farms, where FYM is mainly applied as a fertilizer, contain less nitrates compared with potatoes grown at conventional farms. In a study conducted by Murawa et al [12], organic potatoes contained two-fold less nitrates(V) and in an experiment completed by Wawrzyniak et al [24], they had four-fold less of these compounds versus potatoes grown conventionally. Higher amounts of nitrates in potatoes from conventional plantations compared with organic farms are attributed to the fact that natural and organic fertilizers applied in organic farming add to soil organic substance, which becomes a substrate for edaphon, the main link in the organic matter metabolism that transforms organic compounds into plant available forms. Presence of edaphon is a condition for decomposition of organic compounds. It also accelerates release of mineral components. Under such conditions, the nitrogen taken up by plants is completely used up for the formation of protein. In contrast, mineral fertilizers and pesticides, applied in conventional agriculture, either damage or destroy living organisms, which may lead to excessive or uncontrollable uptake of nutrients, including nitrogen, by plants [9, 21, 23, 24].

The authors' own research is supported by reports written by other researchers on the effect of the type of soil on accumulation of nitrates in plants [17, 25]. Growing potatoes on more compact soil leads to weaker accumulation of NO_3^- in potato tubers compared with lighter soil, which may be explained by more intensive leaching of these compounds from lighter than from more compact soil [18, 22]. Jarych-Szyszka [7] as well as Poberezny [26] demonstrated that small amounts of rainfall and higher temperatures stimulated more intensive accumulation of nitrates in potato tubers. The

growing season during which this study was performed was characterized by alternate periods of droughts and heavy rainfall. Under such conditions, nitrification process can be activated, which may improve the availability of a nitrate nitrogen pool and, as a result, may lead to a higher concentration of nitrates in plants. Another external factor which may have affected the level of nitrates in potato tubers could have been the ambient temperature [27]. Higher temperatures occurring in 2009 during the growth of potatoes may have favoured the uptake of nitrates by plants.

Conclusions

1. Under the influence of fertilization, an increase in the total and protein nitrogen in potato tubers appeared, reaching on average 0.29 % and 0.13 % in the series without soil liming and 0.22 % and 0.03 % in the soil limed series versus the control. The share of protein nitrogen in the total nitrogen content ranged from 51.1 % to 65.7 % and was the highest in tubers of potato plants fertilized with rate I of liquid manure.

2. Each fertilization system caused an increase in the content of nitrates(V) in potato tubers above the permissible level. Among the treatments where the same amount of nitrogen was introduced with fertilizers, in both experimental series (with and without liming), smaller excess of the permissible concentration of nitrates(V) was determined in potato tubers fertilized with farmyard manure, whereas a higher one appeared in tubers of potatoes fertilized with mineral fertilizers and rate I of liquid manure.

3. The highest content of nitrates(V) exceeding from 2.1–fold (limed soil) to 2.3–fold (unlimed soil) their allowable amount, was found in tubers of potatoes fertilized with liquid manure rate II, balanced with FYM in respect of organic carbon introduced to soil. By adding PK fertilizers to liquid manure, a further increase in the content of nitrates was observed. In the case of FYM, the effect of PK fertilizers was opposite.

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ZAWARTOŒÆ AZOTANÓW(V) W BULWACH ZIEMNIAKA UPRAWIANEGO W RÓ¯NYCH SYSTEMACH NAWO¯ENIA

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Abstrakt: W statycznym doœwiadczeniu polowym za³o¿onym w 1973 r. na glebie brunatnej stosowano dwa rodzaje nawozów naturalnych – obornik i gnojowicê, nawozy mineralne (NPK) oraz ³cznie nawo¿enie organiczno-mineralne. Gnojowicê stosowano w dwóch dawkach: I zrównowa¿onej z obornikiem i nawozami mineralnymi iloœci¹ azotu oraz II zrównowa¿onej z obornikiem iloœci¹ wêgla organicznego. Doœwiadczenie przeprowadzono w dwóch seriach – bez wapnowania i z wapnowaniem. W okresie badawczym stosowano 7-letnie zmianowanie roœlin.

Stwierdzono, ¿e ka¿dy rodzaj nawo¿enia przyczyni³ siê do wzrostu zawartoœci azotu azotanowego(V) w bulwach ziemniaka. Najwiêksz¹ zawartoœæ azotanów(V), przekraczaj¹c¹ od 2,1 (seria z wapnowaniem) do 2,3 razy (seria bez wapnowania) dopuszczaln¹ ich iloœæ, zawiera³y bulwy ziemniaka nawo¿onego gnojowic¹ w dawce II oraz dawce II + PK. Spoœród nawozów, z którymi wprowadzono do gleby jednakow¹ iloœæ azotu, wiêksze przekroczenie dopuszczalnej zawartoœci azotanów(V) w bulwach ziemniaka nast¹pi³o w wyniku stosowania nawozów mineralnych i gnojowicy w I dawce ni¿ obornika. Uzupe³nienie gnojowicy o mineralne nawozy PK powodowa³o dalszy wzrost zawartoœci azotanów, natomiast w przypadku obornika ich dzia³anie by³o odwrotne. Przeprowadzony w doœwiadczeniu zabieg wapnowania gleby ró¿nicowa³ zawartoœæ azotanów(V) w bulwach ziemniaków, jednak jego dzia³anie nie by³o jednoznaczne.

S³owa kluczowe: nawo¿enie, bulwy ziemniaka, zawartoœæ azotanów(V)

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Barbara SKWARY£O-BEDNARZ[1]

CONTENTS OF FORMS OF LEAD IN THE SOILS OF THE PROTECTED ZONE IN THE ROZTOCZE NATIONAL PARK AND ADJACENT PRODUCTION AREAS

ZAWARTOŒÆ FORM O£OWIU W GLEBACH OTULINY ROZTOCZAÑSKIEGO PARKU NARODOWEGO I TERENÓW PRODUKCYJNYCH DO NIEJ PRZYLEG£YCH

Abstract: The aim of the study was to determine the total contents of acid soluble and water soluble Pb in light soil profiles that belong to rusty soils in the protected zone in the Roztocze National Park and adjacent industrial areas. The investigated soils had a natural content of total Pb. In spite of that, the results revealed accumulation of the analysed metals in surface levels (Ap), as compared with parent rock levels (C). Higher concentration [%] of lead, zinc and copper in Ap levels was found in the soils of the production areas than in the soils of the protected zone. It was observed that the contents of total forms of Pb, acid soluble forms of Pb and water soluble forms of Pb in the analysed soils were significantly correlated with chemical properties, soil reaction, contents of organic carbon, and soil absorbing capacity.

Keywords: forms of Pb, light soils, protected zone, industrial areas, Roztocze National Park

National Parks are created in order to protect the areas with extraordinary natural values and unique landscapes. The parks allow the nature to preserve its original, undisturbed cycle [1]. The network of existing national parks in Poland covers, although not evenly, the whole territory of the country [2]. The Roztocze National Park is one of these parks. It was founded in 1974 in the Middle Roztocze and includes within its boundaries the most precious natural values of the region. The protected zone is situated in the areas adjacent to the park. Its aim is to counteract any harmful effects of external factors. The protected zone is a buffer zone between the pristine ecosystems and production areas with intensive agricultural activity.

The area of the Middle Roztocze where the Roztocze National Park is located has different types of soils; however, the majority of soils belong to light soils [3]. Light

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soils are usually acidic and not resistant to chemical degradation, including excessive accumulation of heavy metals [4].

The aim of the study was to determine the total contents of Pb and its forms soluble in hydrochloric acid and water, in light soils in the protected zone of the Roztocze National Park and its adjacent production areas.

Material and methods

The study was carried out on rusty soils deriving from loose sands and slightly loamy sands situated in southern and south-western parts of the protected zone of the Roztocze National Park (which is located in south-eastern part of Poland) and its adjacent production areas. The samples for the analysis were taken from whole soil profiles (from levels Ap, Bv, and C) – five samples from the arable fields situated in the protected zone and five from the areas adjacent to the protected zone. Field studies were carried out on arable lands where potatoes were cultivated.

The soil samples were analysed with methods commonly used in soil science.

The following were marked: granulometrical composition with Cassagrande's method in Proszynski's modification, contents of C organic with Tiurin's method in Simakow's modification, pH in H_2O and in 1 mol · dm^{-3} KCl potentiometrically, total absorptive capacity of soil (T) according to the formuls T = Hh + S, total contents of Pb with the atomic absorptive spectrometry method (AAS), Pb soluble in 1 mol · dm^{-3} HCl and water-soluble with Hornburg and Brummer's method, after Niemyska-Lukaszuk [5], which determines the percentage of forms of metals that can be absorbed, as compared with the total contents of these metals.

Results and discussion

The investigated soils in the protected zone of the Roztocze National Park had strongly acidic or acidic pH in the whole soil profile (Table 1). The values of pH_{KCl} varied: in Ap levels from 4.2 to 5.4, in Bv levels from 4.6 to 5.0, and in C levels from 4.4 to 5.0. Generally, Ap soil levels in the protected zone had lower values of pH_{KCl} than the soils situated in the production areas. Genetic levels of these soils had slightly acidic or acidic pH (Table 1). Ap levels of the soils in the production areas were about 56 % richer in C organic than the soils in the protected zone (Table 1). Ap levels of the soils in the production areas had about 40 % higher absorptive capacity than the soils in the protected zone (Table 1). Values of absorptive capacity of all the investigated soils decreased alongside with the increase in depth of the analysed soil profiles.

Contents of heavy metals in soil depends on chemical composition of soil and pollution emitted into the atmosphere by industry, cars, and agriculture. Trace elements (such as: Pb, Cr, Cd, Hg, Ni, Cu, Zn and other) that accumulate in soil in excessive amounts constitute the source of the most troublesome and long-enduring soil pollution. This is mainly due to slow migration of heavy metals.

Table 1

Basic chemical properties of investigated soils – range and mean values

Horizon	pH_{H_2O}	pH_{KCl}	Organic C [%]	Soil absorbing capacity (T) [cmol(+) · kg^{-1}]
Soils in the protected zone				
Ap	5.5[a] 4.4–5.8[c]	5.2[a] 4.2–5.4[c]	1.45[b] 0.85–1.52[c]	5.38[b] 4.25–5.85[c]
Bv	5.2[a] 4.9–5.7[c]	4.9[a] 4.6–5.0[c]	0.15[b] 0.12–0.16[c]	3.55[b] 3.47–3.76[c]
C	5.0[a] 4.8–5.4[c]	4.8[a] 4.4–5.0[c]		2.15[b] 2.02–2.18[c]
Soils in the production areas				
Ap	6.1[a] 5.9 –6.4[c]	5.8[a] 5.6–6.1[c]	2.26[b] 1.97–2.35[c]	7.48[b] 6.92–7.75[c]
Bv	5.7[a] 5.2–5.9[c]	5.2[a] 5.0–5.7[c]	0.21[b] 0.16–0.27[c]	3.92[b] 3.65–4.10[c]
C	5.2[a] 5.0–5.4[c]	4.8[a] 4.6–5.2[c]		2.66[b] 2.45–3.21[c]

[a] – value with logarithm; [b] – mean values; [c] – range of changes.

Heavy metals, including lead, are accumulated in the surface level of soil, also referred to as the humus level. It is due to chemical relation of heavy metals with organic matter present in the humus level [6, 7]. Lead is a low-mobility element because it undergoes strong absorption not only by organic matter but also by clay minerals, iron hydroxides and aluminium hydroxides [8]. Because of low migration of Pb, its natural distribution in a soil profile reflects its contents in the mother rock, and is treated as an indicator for geochemical prospection [9].

From vertical distribution of Pb in the investigated soil profiles located in the area of the protected zone in the Park and beyond the boundaries of the protected zone, it can be observed that Pb is accumulated in the surface levels. In the case of the soils located in the closest vicinity of the Park, enrichment of the Ap levels in Pb when compared with C levels was 553.8 %, and in the case of the soils from production areas – 600 % (Table 2). In spite of such a large enrichment of the humus levels in total lead, as compared with the mother rock levels, the investigated soils did not have the content of total lead that would exceed the norms, which means that the content of lead was natural. The results obtained by other authors confirm this. The natural content of lead in the surface levels of different arable soils in Poland ranges from 20 to 60 mg · kg^{-1} [10, 11]. Other authors suggest that when natural content of heavy metals in soil is estimated, type of soil and chemical properties of soil should be taken into account [12]. Natural content of lead in light soils with acidic pH and strongly acidic pH are 30 mg · kg^{-1}, and in the soils with neutral pH natural contents of Pb is 50 mg · kg^{-1}. On the basis of this criterion, it can be presumed that the investigated soils in the protected area of the Park and its adjacent production areas have natural content of lead. According to

Kabata-Pendias and Pendias [9], concentration of lead in given levels of soil is, to a large extent, connected with the influence of anthropogenic factors, and is usually higher than the natural content. Even soils situated beyond the reach of industrial emission and car fumes show higher content of lead in surface levels.

Table 2

Forms of Pb in investigated soils – mean values

Genetic level	Total Pb in soils [mg · kg^{-1}]	Enrichment of genetic levels as compared to mother rock [%]	Acid-soluble forms of Pb [mg · kg^{-1}]	Mobility index [%]	Water-soluble forms of Pb [mg · kg^{-1}]	Mobility index [%]
Soils in protected zone of RPN						
Ap	28.8	553.8	18.9	65.6	2.3	8.0
Bv	18.4	353.8	12.7	69.0	1.5	8.2
C	5.2	100.0	4.1	78.8	0.4	7.7
Soils in production areas						
Ap	26.4	600.0	15.7	59.5	1.9	7.2
Bv	15.8	359.1	10.0	63.3	1.2	7.6
C	4.4	100.0	3.6	81.8	0.4	9.1

The statistical analysis that was carried out revealed many significant, positive correlations between the amount of total lead and pH, content of $C_{org.}$, and absorptive capacity, no matter where the soils were situated (Table 3).

Table 3

Correlation coefficients between Pb and basic chemical properties of soils (correlation significant at p = 0.01)

Listing	pH_{H_2O}	pH_{KCl}	$C_{org.}$	T
pH_{KCl}	1. 0.999 2. 0.998	—		
Organic C	1. 0.994 2. 0.928	1. 0.994 2. 0.946	—	
T	1. 0.911 2. 0.966	1. 0.911 2. 0.978	1. 0.951 2. 0.993	—
Total content of Pb	1. 0.830 2. 0.998	1. 0.830 2. 0.993	1. 0.886 2. 0.902	1. 0.986 2. 0.947
Acid-soluble forms of Pb	1. 0.816 2. 0.997	1. 0.816 2. 0.992	1. 0.874 2. 0.896	1. 0.982 2. 0.943
Water-soluble forms of Pb	1. 0.817 2. 0.997	1. 0.817 2. 0.991	1. 0.875 2. 0.894	1. 0.982 2. 0.941

1. – soils in the protected zone; 2. – soils in the production areas.

Ap levels of the soils in the protected zone of the Park had higher content of lead soluble in hydrochloric acid than Ap levels of the soils in the production areas (Table 2). The mean content of lead in the surface levels of the soils in the protected zone of RPN was 18.9 mg · kg^{-1}, and 15.7 mg · kg^{-1} in the soils in the production areas (Table 2). The mean mobility index [5] for the surface levels of the soils in the protected zone was 65.6 % of the total content of lead, and 59.5 % in the soils situated beyond the boundaries of the protected zone. The value of the mobility index increased systematically with the depth of soil profiles. Soil acidity helps in creation of phytosociological components because heavy metals, such as lead, occur in their soluble forms, which is more accessible for plants [7]. Badora [13] observed that when soil pH is acidic, solubility of most toxic elements for plants (including lead) increases, and solubility of main nutrients decreases. Mobile forms of lead in acidic soils occur mainly as cations Pb^{2+} and $PbHCO_3^+$, and organic complexes. Alkaline soils are dominated by PbOH and $Pb(CO_3)_2^{2-}$ [9].

The statistical analysis that was carried out revealed that forms of lead soluble in 1 mol · dm^{-3} HCl in the investigated soils positively correlated with pH (Table 3). The correlation coefficient for the soils in the protected zone of the park was r = +0.816, and for the soils in the production areas r = +0.997. Moreover, significant, positive correlations were observed between the amount of lead soluble in 1 mol · dm^{-3} HCl and the contents of total C and absorptive capacity of the investigated soils, no matter where these soils were situated.

Many authors emphasize the fact that the majority of lead compounds does not dissolve in water easily [14]. Compared with other metals, the mobility of lead is as follows: $Cd^{2+} > Zn^{2+} > Pb^{2+} > Cu^{2+}$ [8]. In the humus levels in the protected zone slightly higher content of water-soluble forms of lead was observed than in the same levels of the soils in the production areas (Table 2). For Ap levels of the soils situated in the protected zone, the mean value of water-soluble form of copper was 2.3 mg · kg^{-1}, with the mobility index 8.0 %, and for Ap levels of the production areas – 1.9 mg · kg^{-1}, with the mobility index 7.2 %. Regardless of where the soils were situated, the content of the investigated form of lead tended to decrease. The statistical analysis that was carried out revealed significant, positive correlations between the content of water-soluble forms of lead and pH of the soils in the protected zone of the park (r = +0.817), and the soils in the production areas (r = +0.991) (Table 3). Significant, positive correlations were obtained between the content of water-soluble forms of lead and the content of $C_{org.}$, and the absorptive capacity (Table 3).

A significant influence of the content of C organic on total content of lead and its soluble forms was observed in this study. This was proved by high, positive correlation coefficients (Table 3). Badora [13] noted that organic matter and mineral matter in soil largely contribute to changes in amounts of given forms of elements in soil, because they undergo various processes on surfaces. Moreover, a correlation between the negative value of the absorptive capacity and the content of the investigated forms of lead was observed.

Conclusions

1. The distribution of the content of total forms of lead throughout the soil profiles in the protected zone of the Roztocze National Park, and the production areas situated beyond its boundaries, is within the common norms for rusty soils.

2. Total forms of lead accumulated in the humus levels of the investigated soils in the amounts found in unpolluted soils.

3. Ap levels of the protected zone had higher content of acid-soluble and water-soluble forms than the corresponding levels of soils in the production areas.

4. The content of the investigated form of copper tended to decrease alongside with the increase in depth of the soil profiles; at the same time the mobility index increased.

5. Higher content of total forms of copper in Ap levels of the investigated soils points to their anthropogenic origin.

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ZAWARTOŒÆ FORM O£OWIU W GLEBACH OTULINY ROZTOCZAÑSKIEGO PARKU NARODOWEGO I TERENÓW PRODUKCYJNYCH DO NIEJ PRZYLEG£YCH

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Abstrakt: Celem pracy by³o okreœlenie zawartoœci ogólnej Pb i jego form rozpuszczalnych w kwasie solnym i w wodzie w profilach gleb lekkich nale¿¹cych do klasy gleb rdzawych po³o¿onych na terenie otuliny Roztoczañskiego Parku Narodowego i terenów produkcyjnych do niej przyleg³ych. Badane gleby charak-

teryzowa³y siê naturaln¹ zawartoœci¹ Pb ogólnego. Pomimo tego otrzymane wyniki badañ œwiadcz¹ o nagromadzeniu analizowanych metali w poziomach wierzchnich (Ap) w odniesieniu do poziomów ska³ macierzystych (C). Wiêksz¹ wzglêdn¹ koncentracj¹ [%] o³owiu w poziomach Ap charakteryzowa³y siê gleby terenów produkcyjnych w porównaniu do gleb otuliny. Stwierdzono, i¿ zawartoœæ ogólnych form o³owiu i jego form rozpuszczalnych w kwasie solnym i wodzie w analizowanych glebach by³a istotnie skorelowana z w³aœciwoœciami chemicznymi, w tym odczynem, zawartoœci¹ wêgla organicznego i pojemnoœci¹ sorpcyjn¹.

S³owa kluczowe: formy o³owiu, gleby lekkie, otulina parku, tereny produkcyjne, Roztoczañski Park Narodowy

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 8 2011

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EFFECT OF DIFFERENT FACTORS ON THE NEMATODE *Heterorhabditis megidis* (POINAR, JACKSON AND KLEIN, 1987) – MUTUALISTIC BACTERIA *Photorhabdus luminescens* (THOMAS AND POINAR, 1979) *IN VITRO* CULTURES

WP£YW RÓ¯NYCH CZYNNIKÓW NA UK£AD NICIEŃ *Heterorhabditis megidis* (POINAR, JACKSON I KLEIN, 1987) – BAKTERIA MUTUALISTYCZNA *Photorhabdus luminescens* (THOMAS I POINAR, 1979) W HODOWLI *IN VITRO*

Abstract: Entomopathogenic nematodes (EPN) of the families *Heterorhabditidae* and *Steinernematidae* mutualistically connected with bacteria of the genera *Photorhabdus* and *Xenorhabdus* have long been used in biological plant protection.

The aim of this study was to optimise the conditions of liquid culture of the nematode *Heterorhabditis megidis*. The performed studies were aimed at estimating the effect of biotic and abiotic parameters (temperature, aeration, the amount of the initial nematode dose) on the number of larvae undergoing further growth (larval recovery) and on the final yield of invasive larvae. It was found that the biotic parameter that directly and significantly affects initiation of further larval growth is the dose of nematodes introduced to the culture. Final productivity was significantly affected by aeration. The optimum set of parameters is: temperature of 25 ^{o}C, aeration of 121 rpm and the initial dose of 1370 L3/cm^3.

Keywords: *Heterorhabditis megidis*, *Photorhabdus luminescens*, liquid *in vitro* cultures, optimisation of parameters

Dynamic development of methods of mass culture in artificial media that started in the 1980s was a key factor in commercial use of entomopathogenic nematodes. Now, biological means containing nematodes of the genera *Steinernema* and *Heterorhabditis* are produced by many firms worldwide [1–3]. From among many species only 6 (*S. carpocapsae* (Weiser, 1955), *S. feltiae* (Filipjev, 1934), *S. riobravis* (Cabanillas et al, 1994), *S. scapterisci* (Nguyen i Smart, 1990), *H. bacteriophora* (Poinar, 1976),

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H. megidis (Poinar, Jackson, Klein, 1979) found commercial application [4]. Essential precondition for succeeding in the production biopreparations of nematodes is the ability of their mass multiplication. Nematode cultures have been carried out for more than 70 years [5] and now nematodes are produced on a large scale using 3 methods: *in vivo*, *in vitro* on solid substratum and *in vitro* in liquid media [6, 7]. Each of these methods has its pros and contras resulting from: production costs, total financial input, required know-how, economy, product quality and, moreover, each of these methods might potentially be developed [8]. Optimisation of culture conditions in the case of nematodes of the family *Heterorhabditidae* should concentrate on the productivity of hermaphroditic larvae of the first generation. Though the next, second generation is amphimictic, males and females are not able to copulate in liquid media [9]. Maximising hermaphroditic individuals in liquid medium is inextricably linked with the larval recovery [10]. The percentage of recovered larvae *in vivo* is nearly 100 % [11] while in liquid media the phenomenon of departure from the invasive stage is unstable and varies from 0 to 86 % [10–13]. Differences in the number of recovered larvae are often found in experiments even if culture conditions remain the same. Hence, gaps should be filled in our knowledge of various aspects of nematode and bacterial physiology, of their mutual relationships and of a possibility of their reproduction under stress conditions prevailing in fermentors [14]. The aim of this study was to determine the effect of abiotic (temperature, aeration) and biotic (initial dose) factors on the number of recovered larvae and on the final yield of invasive larvae of the entomopathogenic nematode *H. megidis*.

Material and methods

Study material: a strain of entomopathogenic nematodes – monoxenic cultures of the nematode *Heterorhabditis megidis* (strain KV – 136) [15] were obtained from the firm Koppert Biological Systems B.V. (Netherlands); bacteria *Photorhabdus luminescens* were isolated from NBTA substratum and identified as pure phase I; liquid medium for nematode culture – 3 g of specific culture substratum (composition reserved by Koppert Biological Systems B.V., Netherlands), 3 cm^3 of maize oil, 75 cm^3 distilled water/300 cm^3 in Erlenmayer flasks.

Microbial substrata: liquid medium YSE – 0.5 g of yeasts extract, 0.5 g $NH_4H_2PO_4$, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot (7H_2O)$, 5.0 g NaCl, 1.0 g lecitine, 5.0 g of maize oil, 1000 cm^3 distilled water; agar substratum NBTA – 37 g Nutrient agar, 25 mg *bromothymol blue* (BTB), 1000 cm^3 distilled water, 4.0 cm^3 1 % 2,3,5-triphenyltetrazoliumchloride; Wouts agar – 19 g Bacto Nutrient Broth, 12 g Bacto Agar, 5 g of maize oil, 1000 cm^3 distilled water.

Culture parameters: temperature – 20^{o}C, 25 ^{o}C; aeration (expressed as rotations of the rotary shaker) – 121 rpm, 160 rpm and 200 rpm; initial dose of larvae L3/cm^3 – 1370 L3/cm^3; 2340 L3/cm^3; 4440 L3/cm^3.

In total 18 combinations of parameters were used. Each experiment lasted 26 days.

Population dynamics of *H. megidis* in the studied cultures were analysed by estimating their density and growth stages every third and second day, consecutively

(on days 3, 5, 8, 10, 13, 15, 18, 21, 23 and 26). Based on the obtained data, the following indices of population dynamics were calculated:

1) recovery percentage $R = (H/a) \cdot 100\ \%$ [11],

where: R – larval recovery,
H – the number of hermaphroditic individuals,
a – initial dose of nematodes introduced to the culture on day 0.

2) final yield $W_k = l.\ l._{L3} \cdot V$,

where: W_k – final yield,
$l.\ l._{L3}$ – the number of invasive (L3) larvae,
V – volume of Erlenmeyer flask.

Result and discussion

The effect of physical parameters on population dynamics of nematodes in liquid media

The effect of physical parameters on recovery (H/cm^3)

Initial doses (1370 L3/cm^3, 2340 L3/cm^3, 4400 L3/cm^3) exerted a significant effect ($p < 0.05$) on recovery (Fig. 1). Statistically significant differences were found between

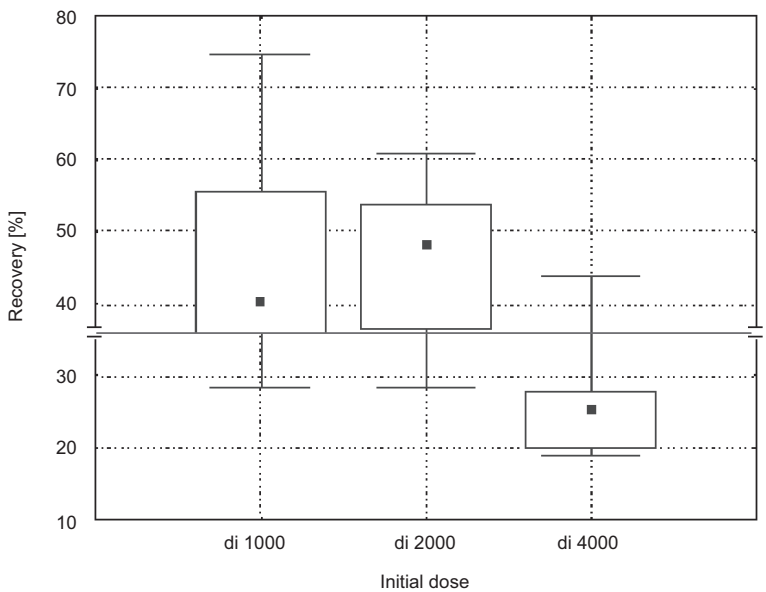

Fig. 1. The effect of initial dose (L3/cm^3) on recovery: di 1000 – initial dose 1370 L3/cm^3, di 2000 – initial dose 2340 L3/cm^3; di 4000 – initial dose 4400 L3/cm^3

doses 1370 L3/cm^3 and 4400 L3/cm^3 and between 2340 L3/cm^3 and 4400 L3/cm^3. The number of recovered larvae at the doses of 1370 L3/cm^3 and 2340 L3/cm^3 remained at the same level. The highest percentage of recovered larvae R = 74.8 % and R = 61.1 % were obtained at initial doses of 1370 L3/cm^3 and 2340 L3/cm^3, respectively. At the initial dose of 4400 L3/cm^3 the percentage of recovered larvae did not exceed 50 %, being equal to R = 43.9 %.

The effect of physical parameters on final productivity (L3/cm^3)

It was found that aeration had a significant impact ($p < 0.05$) on the final productivity of invasive larvae in culture (Fig. 2). Statistically significant differences were found between aeration of 121 rpm and 200 rpm and between 160 rpm and 200 rpm. The highest final yield of invasive larvae (190560.0 L3/cm^3) was obtained at aeration of 160 rpm. Similarly high yields (174760.0 L3/cm^3, 175260.0 L3/cm^3, and 189700.0 L3/cm^3) were obtained at 121 rpm. When applied aeration was 200 rpm the productivity of invasive larvae was very low and equalled 72800 L3/cm^3.

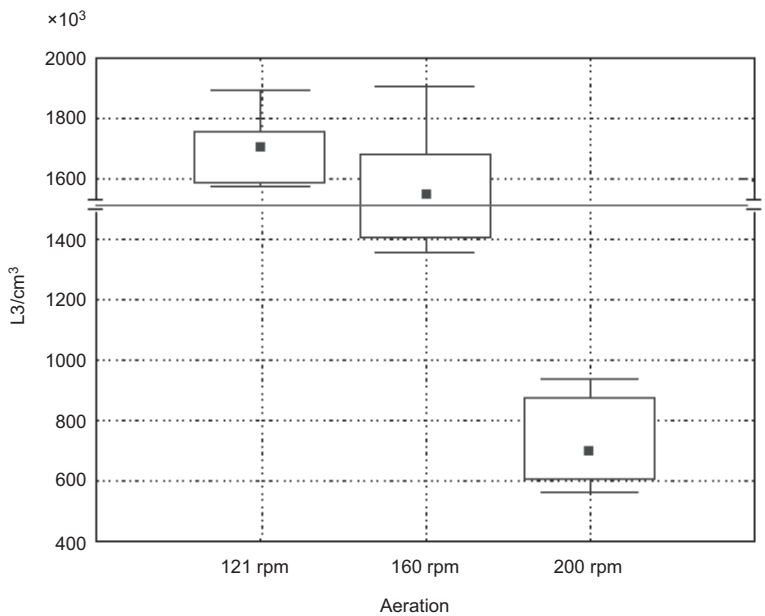

Fig. 2. The effect of aeration on final productivity of invasive larvae, L3/cm^3

Correlation between larval recovery (H/cm^3) and final productivity (L3/cm^3) of invasive larvae

Correlation between the recovered larvae (H/cm^3) and final yield (L3/cm^3) showed that the number of hermaphroditic individuals significantly ($p < 0.05$) affected final productivity (Fig. 3). In almost all cases high numbers of hermaphroditic individuals

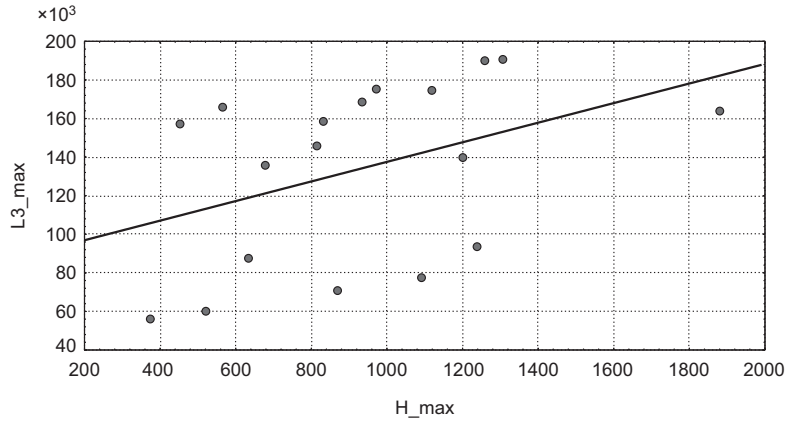

Fig. 3. Correlation between recovery (H/cm^3), and final productivity (L3/cm^3) of invasive larvae

(1306.4 H/cm^3, 1259.6 H/cm^3, and 1120.0 H/cm^3) resulted in the highest final yields (190560.0 L3/cm^3, 189700.0 L3/cm^3, and 174750 L3/cm^3, respectively). The only difference could be observed at aeration of 200 rpm at which high number of hermaphroditic individuals (1000 H/cm^3) gave low yields of invasive larvae (irrespectively of the initial dose and temperature).

Literature data on the effect of environmental factors on the size and stability of larval recovery indicate that the *in vitro* recovery is highly variable ranging from 18 to 90 % [16]. The performed studies demonstrated that the initial dose of nematodes introduced to the culture is the factor directly and significantly affecting recovery percentage. It was shown that the lowest initial dose (1370 L3/cm^3) gave best results and that in these cultures the highest recovery percentage was recorded. Johnigh et al [17] studying the effect of the initial dose on recovery also found that increasing the initial dose was accompanied by the decrease of recovered larvae. Statistical analysis of the effects of temperature and aeration on recovery percentage did not show a significant impact of these parameters. Aeration was found to be the only parameter significantly affecting the final yield of invasive larvae. Strauch and Ehlers [18] in their study also pointed to the effect of this parameter on the final yield. The maximum final yield (190560 L3/cm^3) was obtained in performed tests at aeration of 160 rpm. Aeration at 121 rpm gave slightly lower yields. It, however, provided the highest stability of final yield that remained at a level of 157320–189700 L3/cm^3. Aeration at 200 rpm gave the lowest yields.

Considering profitability ie taking into account the ratio of introduced to finally obtained larvae the use of the lowest dose of 1370 L3/cm^3 allows for obtaining highest efficiency between 11220.7 % and 13560.5 % of hermaphroditic larvae of the I generation.

The maximum final yield obtained in the performed cultures (190560 L3/cm^3) was relatively high as compared with the literature data (138000 L3/cm^3 [11]).

Conclusion

1. The physical parameter directly and significantly affecting:

– larval recovery is the number of nematodes introduced to the culture. The best results were obtained at the lowest dose of 1370 L3/cm^3.

– final yield of invasive larvae is strongly dependent on aeration. Application of 160 rpm aeration allowed for obtaining the highest final yields of invasive larvae.

2. The final yield of invasive larvae significantly depends on the number of hermaphroditic individuals.

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WP£YW RÓ¯NYCH CZYNNIKÓW NA UK£AD NICIEÑ *Heterorhabditis megidis* (POINAR, JACKSON I KLEIN, 1987) – BAKTERIA MUTUALISTYCZNA *Photorhabdus luminescens* (THOMAS I POINAR, 1979) W HODOWLI *IN VITRO*

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Abstract: Nicienie entomopatogenne (EPN) z rodziny *Heterorhabditidae* i *Steinernematidae* zwi¹zane mutualistycznie z bakteriami nale¿¹cymi do rodzaju *Photorhabdus* i *Xenorhabdus* od wielu lat s¹ stosowane w biologicznej ochronie roœlin. Celem badañ by³a optymalizacja warunków hodowli nicieni *Heterorhabditis megidis* w ciek³ym œrodowisku. Prowadzone badania zmierza³y do okreœlenia wp³ywu parametrów biotycznych i abiotycznych (temperatury, napowietrzania oraz dawki inicjalnej nicieni) na liczbê larw przechodz¹cych dalszy rozwój i na wydajnoœæ koñcow¹ larw inwazyjnych. Okreœlono równie¿ korelacjê miêdzy rozwojem nicieni *H. megidis* a ich bakteriami mutualistycznymi *P. luminescens* w warunkach *in vitro*.

W wyniku przeprowadzonych badañ stwierdzono m.in., ¿e parametrem biotycznym, który bezpoœrednio i istotnie wp³ywa na inicjowanie przechodzenia larw inwazyjnych w dalszy rozwój jest dawka nicieni wprowadzonych do hodowli. Na wydajnoœæ koñcow¹ hodowli istotnie wp³ywa napowietrzanie. Optymalnym uk³adem parametrów jest: temperatura 25 ^{o}C, napowietrzanie 121 rpm, dawka inicjalna 1370 L3/cm^{3}.

S³owa kluczowe: *Heterorhabditis megidis*, *Photorhabdus luminescens*, plynne hodowle *in vitro*, optymalizacja parametrów

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 8 2011

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KINETICS OF RELEASING HERBICIDE METAZACHLOR FROM HYDROGEL MICROCAPSULES TO AQUATIC ENVIRONMENTS

KINETYKA UWALNIANIA HERBICYDU METAZAOCHLOR Z HYDRO¯ELOWYCH MIKROKAPSU£EK DO ŒRODOWISKA WODNEGO

Abstract: Formulation of metazachlor in the form of hydrogel capsules was carried out in the Packaging and Biopolymers Center at the West Pomeranian University of Technology in Szczecin. To produce the hydrogel matrix sodium alginate was used in 1.5 % solution. Kinetics of releasing metazachlor from the matrix was carried out for various aquatic environments (distilled water Wd and two natural waters W1 and W2) at 4 ± 1 °C and 20 ± 1 °C under laboratory condition. The release data were fitted to the generalized model proposed by Ritger and Peppas. For all the combinations in study, the high values of the correlation coefficient R (from 0.9254 to 0.9997) show a very good adjustment between the experimental data and the model applied. It was found that amount of metazachlor released from the capsules to water increased exponentially in time and depended on type of the aquatic environment and temperature of storage. For metazachlor, the highest values of time T_{50}, were recorded for the distilled water. For the natural waters, time T_{50} is significantly shorter, and, comparing with the distilled water, it was decreased by 83–90 % at 20 °C and by 69–96 % at 4 °C.

Keywords: metazachlor, release, hydrogel microcapsules, alginate, water environment

Pesticides make for a group of pollutants quite frequent in surface and underground waters. Their highest concentration is observed during the runoff of thaw water and on application of agricultural chemistry procedures [1–4]. Due to the necessity to protect the natural environment, there is an objective set forth to reduce the amount of pesticides by application of micro-doses or adjuvants, or by modification of the usable forms [5, 6].

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Formulas based on technologies of controlled release help to maintain the active substance on a constant, pre-defined level. This way it is possible to reduce the amount of the necessary pesticides and, at the same time, to minimize such negative environmental phenomena, as surface runoff, leaching or evaporation [7–10].

Parameters having an impact on quality of *controlled release* formulation (CR formulation) include structure and characteristics of the used polymers. Polymers, which are most frequently used in agriculture for CR formulation, are the natural polymers, ie starch, ethyl cellulose, lignin, bentonite, kaolin, chitosan or alginate, which, contrarily to the synthetic ones, feature the lack of toxicity, low price, availability, and, first of all, biodegradability [11, 12].

To obtain matrixes for controlled release of soil-applied herbicides the encapsulation method is used, as based on setting some emulsion containing the active substance through polymer cross-linking. This consists in binding the matrix components with a cross-linking polymer, at presence of multivalent metal cations (eg: Ca^{2+}). These conditions are met by the algae-originated biopolymer belonging to the polysaccharides – the biodegradable sodium alginate. This compound is used both for production of medicine controlled release carriers and for pesticides. Thanks to its specific chemical structure and the spatial configuration of its monomers, ie D-mannuric acid and L-guluronic acid, it has strong gelling qualities in aquatic environment. Additionally, application of such CR modifiers as natural clays, active carbon, humic acids, linseed acid for production of alginate formulations has an influence on the immobilization process, as well as ensures a better control of the process of releasing the active substance from the matrix [13–17].

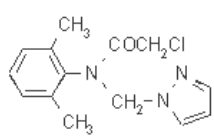
Therefore, our research was conducted in order to determine the kinetics of releasing herbicide metazachlor from hydrogel capsules to aquatic environments.

Material and methods

In the course of research, metazachlor herbicide of the chloroacetanilid group were used. Metazachlor is a selective herbicide, a sprout inhibitor, penetrating the roots and hypocotyl. It is applied to such plants as winter or spring rape. The active substance was made available by company FEINCHEMIE SCHWEBDA GMBH of Germany. Selected physical and chemical characteristics are presented in Table 1.

Table 1

Selected physical and chemical characteristics for metazachlor [17]

Molecular structure	Chemical name	2-chloro-*N*-(pyrazol-1-ylmethyl)acet--2',6'-xylidide
	Form	Yellowish crystals; (tech., beige solid)
	Molecular formula:	$C_{14}H_{16}ClN_3O$
	Molecular mass:	277.8 g/mol
	Solubility in water:	430 mg/dm^3 (20 ^{o}C).
	Octanol / water partition coefficient:	log P_{ow} = 2.49

Production of Metazachlor Hydrogel Capsules

Formulation of metazachlor in the form of hydrogel capsules was carried out in the Packaging and Biopolymers Center at the West Pomeranian University of Technology in Szczecin. To produce the hydrogel matrix sodium alginate was used in 1.5 % solution. To form microcapsules containing some immobilized active substance emulsion with concentration of the active substance equal to 18.55 % (0.5 C_{max}, where C_{max} = 37.1 %) was prepared in a mix of solvents: methyl acetate / vegetable oil (ratio 1:1). Metazachlor emulsion was prepared at the ambient temperature (23 ^{o}C ± 1), with application of a homogenizer (Heidolph, Germany), at the constant speed of 12 thousand rpm. After 3-minute homogenizing, the metazachlor emulsion was dropped into the cross-linking agent – calcium chloride solution – where spherical hydrogel capsules with diam. 0.30–0.35 mm were obtained. Time of the cross-linking reaction in the calcium chloride for creation of an internally stable miscrocapsule membrane was about 15 minutes.

Preparations process

Kinetics of releasing metazachlor from the matrix was carried out for various aquatic environments (distilled water Wd and two natural waters W1 and W2) at 4 ± 1 ^{o}C and 20 ± 1 ^{o}C. Table 2 presents selected physical and chemical qualities of the waters used in the experiment. Weighed amounts (5 g each) of metazachlor in the form of hydrogel capsules were placed in 200 cm^3 of appropriate water in polished-surface-closed Erlenmeyer flasks with capacity of 500 cm^3 and mixed with a magnetic agitator at 100 rpm (for each water three cycles were made). Solution samples of 5 cm^3 were collected after 60, 120, 240, 480 minutes and after 24 and 48 hours. In order to avoid saturation effect, the collected amount was re-filled with the appropriate water. The collected water samples were made subject to extraction process with dichloromethane, according to the general methods proposed by Ambrus at al [19].

Table 2

Chemical indicators of waters

Water	pH	$N\text{-}NH_4$ [mgN · dm^{-3}]	$N\text{-}NO_2$ [mgN · dm^{-3}]	$N\text{-}NO_3$ [mgN · dm^{-3}]	$P\text{-}PO_4$ [mgP · dm^{-3}]
W1	7.93	0.72	0.001	0.21	0.09
W2	7.61	0.58	0.004	0.17	0.02

Analysis of metazachlor in the microcapsules

With laboratory scales, in five cycles, 0.1 g portions of the microcapsules were weighed, containing immobilized metazachlor. These were transferred to conical flasks of capacity 100 cm^3 and underwent extraction process with acetone (50 cm^3). All the flasks were agitated by a rotary shaker for 8 hours. After 24 hours spent in ambient

temperature, all samples were percolated, separating the alginate matrix from the acetone layer, which contained the determined active substance. The acetone was evaporated dry with a vacuum evaporator made by Büchii, and dichloromethane and waterless Na_2SO_4 were added to the remnant. After the drying, all samples were filtered again and concentrated up to the volume of 2 cm^3 with the vacuum evaporator. In the samples thus prepared, concentration of metazachlor was determined with the GC/MS method.

GC/MS analysyis

Determination of metazachlor released from the hydrogel matrix to the water was carried out by gaseous chromatography. In order to do so, a gaseous chromatograph provided with an MS detector made by Perlan Technologis was used, making use of an Elite 5MS column (30 m × 0.25 mm × 0.5 μm). Helium was used as the carrying gas at a flow of 1.0 cm^3/min. For the analysis, a programmed temperature of the column was applied: 30 ^oC – 1 min, 25 ^oC/min to 290 ^oC – 10 min, detector temperature 320 ^oC. To determine metazachlor in the tests, the method of electronic ionization type El+ was used. Quantitative analysis was carried out according to the surface area.

Results and discussion

Results of tests on kinetics of releasing the metazachlor herbicide from hydrogel capsules at 4 ^oC and 20 ^oC are presented on Fig. 1. It was found that amount of metazachlor released from the capsules to water increased exponentially in time and depended on type of the aquatic environment and temperature of storage. After 48 hours, the highest concentrations of the herbicide were recorded for the natural waters at 20 ^oC, where 84 % of metazachlor got released from the hydrogel matrix. In the case of the distilled water, the release kinetics of the substance in question proceeded very efficiently too. After 48 hours, 78.7 % of metazachlor got released. Storage of the metazachlor microcapsules at low temperatures affected its release kinetics strongly. At 4 ^oC, its lowest concentration was observed also in the distilled water, where within 48 hours 56.4 % of metazachlor immobilized in the gel matrix got released. At the same time, at low temperatures, for the natural waters, impact of their physical and chemical qualities on the process in study was recorded. Within 48 hours, between 56.8 % (W2) and 66 % (W1) of the active substance were released.

Kinetics of releasing metazachlor to aquatic environments from the hydrogel capsules was studied based on a mathematical model proposed by Ritger and Peppas [19–21].

$$\frac{M_t}{M_0} = \mathrm{K} \cdot t^n$$

where: M_t – represents quantities of the active substance released in time t,
M_0 – total amount of the active substance in the carrier,

M_t/M_0 – refers to percentage of the active substance released in time t,
K – a constant describing qualities of the component being released and qualities of the matrix that forms the carrier,
n – a diffusion parameter helping to describe the transport mechanism.

For all the combinations in study, the high values of the correlation coefficient R (from 0.9254 to 0.9997) show a very good adjustment between the experimental data and the model applied. The constant values K and n were calculated with the Levenberg-Marquardt non-linear estimation method. For all the water combinations, low values of constant n were obtained, as below 0.5 ($n < 0.5$), which shows that the process of diffusion and release of metazachlor from the hydrogel matrix proceeds according to the Fick's laws of diffusion [12, 14, 21]. Regardless the temperature of the process, the highest values of n occurred for the distilled water and were as follows: for 20 ^{o}C = 0.1292, for 4 ^{o}C = 0.1382. At the same time it was established that the parameter in study, ie the process of release of the active substance from the matrix, was strongly affected by physical and chemical qualities of the natural waters and by the temperature. For the natural waters, n underwent a substantial decrease by 46–51 % at 20 ^{o}C and by 24–30 % at 4 ^{o}C.

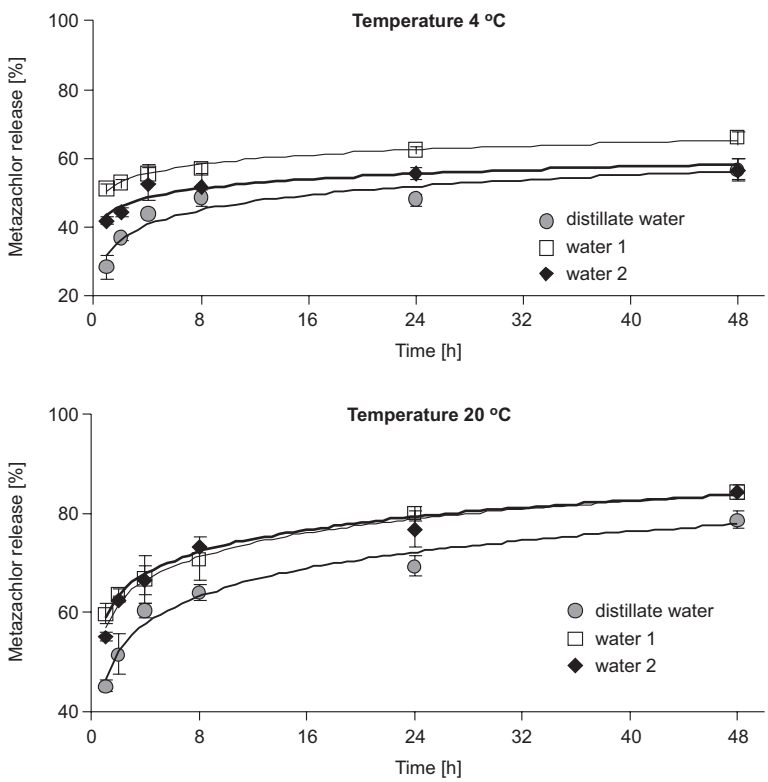


Fig. 1. Kinetics of releasing herbicide metazachlor from hydrogel microcapsules to water

Based on the constant K and *n*, for all the combinations, the time was calculated during which 50 % of the active substance was released from the hydrogel matrix (T_{50}) (Table 3). Obtained times T_{50} make it possible to state that the speed at which metazachlor is released depends both on the process temperature and the physical and chemical qualities of the water. For metazachlor, the highest values of time T_{50}, were recorded for the distilled water. For the natural waters, time T_{50} is significantly shorter, and, comparing with the distilled water, it was decreased by 83–90 % at 20 ^{o}C and by 69–96 % at 4 ^{o}C.

Table 3

Release parameters for metazachlor from hydrogel microcapsules

Metazachlor	Temperature 20 ^{o}C				Temperature 4 ^{o}C			
	R	K [h^{-n}]	*n*	Time T_{50}	R	K [h^{-n}]	*n*	Time T_{50}
Distillate water	0.9967	0.4760	0.1292	1.46	0.9843	0.3310	0.1382	19.78
Water 1	0.9997	0.5936	0.0906	0.15	0.9934	0.5045	0.0673	0.88
Water 2	0.9982	0.5748	0.0986	0.24	0.9254	0.4363	0.0747	6.20

Regardless the fact which water was analyzed, for the temperature of 4 ^{o}C higher values of metazachlor times T_{50} were observed. This dependence can be attributed to an increase of the diffusion barrier because of the growth of density of the oil used in the metazachlor microcapsules production process, as well as a decreased solubility of metazachlor.

Release of the herbicide from the matrix in an aquatic environment is a function of physical and chemical characteristics of the herbicide, as well as of contents of the controlled release matrix. The herbicyde outflow rate is also correlated with its solubility in water and the higher solubility, the quicker release. This is confirmed by such studies as those by Cespedes at al [16], who, when studying kinetics of release of chloridazon (solubility in water 340 mg · dm^{-3}) and metribuzin (1050 mg · dm^{-3}) from alginate capsules, obtained much longer times T_{50} for substances with lower solubility. The experiments show that release of active substances depends strongly on the matrix porosity, and thus on its components. Addition of such modifiers as minerals, eg montmorillonite, bentonite, or linseed or soya bean oils slows the outflow of active substances from the matrix dramatically down. An additional parameter that impacts the pace of the active substance release is the size of the microcapsules. According to Yongsong and others' studies [11], the smaller the diameter of the CR granules is, the shorter the times T_{50} are. The time T_{50} values obtained for acetamipirid ranged from 1.65 week for the smallest granules to 3.16 week for large granules.

The available literature does not provide any information on formulation of metazachlor controlled release. According to our own research, a sufficient control on the release process was not achieved, which results from the short matazachlor T_{50} times. This might be attributed to the fact that for testing the release of the active substance from the hydrogel matrix wet capsules were used, and thus the capsule swelling process (absorption of water), which makes the release process slow down,

was overlooked. Simultaneously, studies on kinetics of the metazachlor release from hydrogel capsules were conducted on small-diameter capsules (0.25–0.3 mm).

Conclusions

1. Concentration of metazachlor released from the hydrogel capsules to the aquatic environment grows exponentially in time and depends on type and temperature of the aquatic environment.

2. After 48 hours, the highest concentrations of the herbicide were recorded for the natural waters at 20 ^{o}C, where 84 % of metazachlor underwent the release from the hydrogel matrix.

3. The mathematical model, as proposed by Ritger and Peppas, describes the process of metazachlor release from the hydrogel matrix very precisely.

4. The lowest values time T_{50} for metazachlor were obtained for the distilled water.

Acknowledgements

Project financed by the Ministry of Science and Higher Education. The contract no. 1514/B/P01/2007/33.

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KINETYKA UWALNIANIA HERBICYDU METAZACHLOR Z HYDRO¯ELOWYCH MIKROKAPSU£EK DO ŒRODOWISKA WODNEGO

Zak³ad Chemii Ogólnej i Ekologicznej, Wydzia³ Kszta³towania i Rolnictwa
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Abstrakt: W badaniach wykorzystano hydro¿elowe mikrokapsu³ki herbicydu metazachlor otrzymane w Zak³adzie Opakowalnictwa i Biopolimerów Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. Badania kinetyki uwalniania przeprowadzono w warunkach laboratoryjnych, w ró¿nych œrodowiskach wodnych (woda destylowana Wd, dwie wody powierzchniowe W1, W2) w temperaturze 4 ^{o}C ± 1 i 20 ^{o}C ± 2. Kinetykê uwalniania metazachloru do œrodowiska wodnego z hydro¿elowych mikrokapsu³ek opracowano wykorzystuj¹c model matematyczny zaproponowany przez Ritgera i Peppasa. Uzyskane dla wszystkich analizowanych kombinacji du¿e wartoœci wspó³czynnika korelacji R od 0,9254 do 0,9997 wskazuj¹ na bardzo dobre dopasowanie danych eksperymentalnych z zastosowanym modelem. Stwierdzono, i¿ iloœæ uwolnionego metazachloru z hydro¿elowych mikrokapsu³ do œrodowiska wodnego wzrasta wyk³adniczo w czasie i zale¿y od rodzaju œrodowiska wodnego i temperatury przechowywania. Otrzymane czasy T_{50} pozwalaj¹ na stwierdzenie, i¿ szybkoœæ uwalniania metazachloru zale¿y zarówno od temperatury procesu, jak i w³aœciwoœci fizykochemicznych wód. Najwiêksze wartoœci czasu T_{50} metazachloru, w przypadku obu temperatur, uzyskano dla wody destylowanej. Uzyskany dla wód naturalnych czas T_{50} herbicydu jest znacznie ni¿szy i uleg³ zmniejszeniu o 83–90 % w temp. 20 ^{o}C i o 69–96 % w temp. 4 ^{o}C.

S³owa kluczowe: uwalnianie metazachloru, hydro¿elowe mikrokapsu³ki, alginian sodu, œrodowisko wodne

Varia

Invitation for ECOpole '11 Conference

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 20th annual Central European Conference ECOpole '11, which will be held in 13–15 X 2011 (Thursday–Saturday) at the Conference Center "Rzemieślnik" in Zakopane, PL.

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

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

The Conference language is English.

The Conference Opening Lecture:
"Atmospheric Chemistry and Climate in the Anthropocene"
will be delivered by the Nobel Prize Winner
Professor Dr. Paul Jozef CRUTZEN.

Contributions to the Conference will be published as:

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

Additional information one could find on the Conference website:

ecopole.uni.opole.pl

The deadline for sending the Abstracts is 31.08.2011 and for the Extended Abstracts: 1.10.2011. The actualised list (and the Abstracts) of the Conference contributions

accepted for presentation by the Scientific Board, one can find (starting from 15.07.2011) on the Conference website.

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

At the Reception Desk each participant will obtain a CD-ROM with abstracts of the Conference contributions as well as Conference Programme (the Programme will be also published on this site).

Further information is available from:
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Zapraszamy
do udzia³u w Œrodkowoeuropejskiej Konferencji
ECOpole '11
w dniach 13–15 X 2011

SUBSTANCJE CHEMICZNE W ŒRODOWISKU PRZYRODNICZYM

Bêdzie to dwudziesta z rzêdu konferencja poœwiêcona badaniom podstawowym oraz dzia³aniom praktycznym dotycz¹ca ró¿nych aspektów ochrony œrodowiska przyrodniczego. Odbêdzie siê ona w Oœrodku Konferencyjno-Wypoczynkowym „Rzemieœlnik" w Zakopanem.

Doroczne konferencje ECOpole maj¹ charakter miêdzynarodowy i za takie s¹ uznane przez Ministerstwo Nauki i Szkolnictwa Wy¿szego.

Obrady konferencji ECOpole '11 bêd¹ zgrupowane w piêciu sekcjach:
- SI Chemiczne substancje w œrodowisku przyrodniczym oraz ich monitoring
- SII Odnawialne Ÿród³a energii i jej oszczêdne pozyskiwanie oraz u¿ytkowanie
- SIII Zarz¹dzanie œrodowiskiem w warunkach kryzysowych
- SIV Forum M³odych (FM) i Edukacja proœrodowiskowa
- SV Wp³yw zanieczyszczeñ œrodowiska oraz ¿ywnoœci na zdrowie ludzi.

Pan Profesor Dr Paul Jozef CRUTZEN – Laureat Nagrody Nobla
wyg³osi referat inauguracyjny:
„Atmospheric Chemistry and Climate in the Anthropocene"

Materia³y konferencyjne bêd¹ opublikowane w postaci:
- abstraktów (0,5 strony formatu A4) na CD-ROM-ie;
- rozszerzonych streszczeñ o objêtoœci 4-6 stron w pó³roczniku *Proceedings of ECOpole*;
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Termin nadsy³ania angielskiego i polskiego streszczenia o objêtoœci 0,5–1,0 strony (wersja cyfrowa + wydruk) planowanych wyst¹pieñ up³ywa w dniu 31 sierpnia 2011 r. Lista prac zakwalifikowanych przez Radê Naukow¹ Konferencji do prezentacji bêdzie sukcesywnie publikowana od 15 lipca 2011 r. na stronie webowej

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Przewodnicz¹ca Komitetu Organizacyjnego
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