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CONTENTS

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ECOLOGICAL CHEMISTRY AND ENGINEERING A

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Elżbieta BOLIGŁOWA[1]

IMPACT OF ABIOTIC FACTORS ON *FUSARIUM* MYCOTOXIN OCCURRENCE IN CEREAL GRAIN

WPŁYW CZYNNIKÓW ABIOTYCZNYCH NA WYSTĘPOWANIE MIKOTOKSYN FUZARYJNYCH W ZIARNIE ZBÓŻ

Abstract: The paper focuses on an analysis of the abiotic factors impact on *Fusarium* mycotoxin cumulation in cereal grain. The mycotoxins most frequently determined in cereal grain include deoxynivalenol (DON), zearaleon (ZEN) and fumonisins. There are numerous causes of the presence and cumulation of these toxic compounds. The main factors promoting *Fusarium* fungi parasitising and mycotoxin production comprise the weather conditions and region of cultivation, species and cultivar susceptibility, the wrong forecrop, shallow placement of harvest residue or leaving it on the soil surface, as well as abandoning plant protection during the vegetation period.

Keywords: mycotoxins, grain, cereals, abiotic factors

The presence of mycotoxins in cereal grain poses a hazard to humans and animals causing diseases and economic losses [1]. The problem of mycotoxin presence in food and animal feeds is still important. According to FAO, about 25 % of world food production is significantly contaminated with mycotoxins. The mycotoxins most frequently determined in the grain of Polish cereals are trichothecenes, zearaleon (ZEN) and fumonisins, which are counted to *Fusarium* mycotoxins [2–5]. These metabolites are produced by fungi of the genus *Fusarium* [2, 3, 6]. Trichothecenes comprise highly toxic compounds of A type (T-2, HT-2, diacetoxyscirpenol – DAS, neosolaniol – NEO) and less toxic B type (deoxynivalenol – DON and nivalenol – NIV [7]). The production of mycotoxins by fungi of the genus *Fusarium* depends on many environmental factors and on the resistance of plant species and cultivars [3, 8, 9]. *Fusarium* mycotoxins may be produced already during the plant vegetation period [3, 4, 8, 10, 11]. Apart from the weather conditions, the *Fusarium* fungi development is promoted by simplified

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crop rotation, harvest residue left on the soil surface or a delayed crop harvest date [8, 12, 13]. The problem becomes serious particularly in the areas where cereal share the cropping system is high and out of necessity the crops are cultivated one after another, which favours the development of among others *Fusarium*. Among cereals, wheat is the most susceptible to fungal infections [14] and triticale, rye, barley and oat place next.

Fusarium fungi which infect plants during the vegetation period cause pre- and after-emergence blight, stem base rot and *Fusarium* ear disease [13]. Production of mycotoxins is usually connected with plant susceptibility to *Fusarium* head blight. Numerous authors [6, 11, 15] consider this disease the most serious. In the opinion of Perkowski et al [2], Perkowski [3] and Arseniuk and Goral [9], in Poland *Fusarium* head blight is caused by a complex of various *Fusarium* species (*F. graminearum, F. culmorum, F.avenaceum, F. poae*). However, the research conducted so far [9–11] indicates that an individual *Fusarium* species starts to dominate on the infected ears depending on the temperature, which is often connected with the region where the plants are cultivated [16]. *F. culmorum* prevails in the fungi species structure in the areas with moderate climate, whereas *F. graminearum* attacks cereal ears more in warmer regions, with higher air temperature. The hitherto conducted investigations [2–4] reveal that *F. garminearum* and *F. culmorum* are mainly responsible for deoxynivalenol (DON) and zearaleon presence in cereal grain. On the other hand fumosins found in maize grain are produced by *F. moniliforme* and *F. proliferatum*.

Literature data indicate a considerable diversity in mycotoxin cumulation in cereal grain. Hooker et al [8] think that DON cumulation depends mainly on the weather conditions during the plant flowering period, whereas according to Weber [13] ears become infected by fungi of *Fusarium* genus particularly during the prolonged flowering period.

In many countries considerable amounts of cumulated DON are assessed in cereal grain, whereas in Canada the presence of this toxin in wheat has been monitored since 1980 [10]. In his research, Tekauz [10] detected a similar level of DON presence in grains of wheat, barley and oats when the plants were growing in the same conditions (western Canada). Langseth and Rundberget [11] think that the presence of this mycotoxin depends on the plant species. The research results were confirmed by reports of Perkowski [2, 3]. Under natural conditions the greatest quantities of toxins accumulated in oat grain, next in barley and wheat grain (Fig. 1). The amounts assessed in the territory of Poland are comparable with the data obtained for other countries with similar climatic conditions. More intensive occurrence of *Fusarium* head blight (5 % of infected ears per plantation) poses a hazard of *Fusarium* toxin accumulation (for DON > 1 mg/kg). According to Perkowski [3] and Arseniuk and Goral [9] the proportion of grain samples or cereal products contaminated with mycotoxins is high (Table 1). The latest research of Perkowski et al [17] revealed the dependence of trichothecene content in oat grain on the region of its cultivation (Fig. 2) and as a result on climatic conditions.

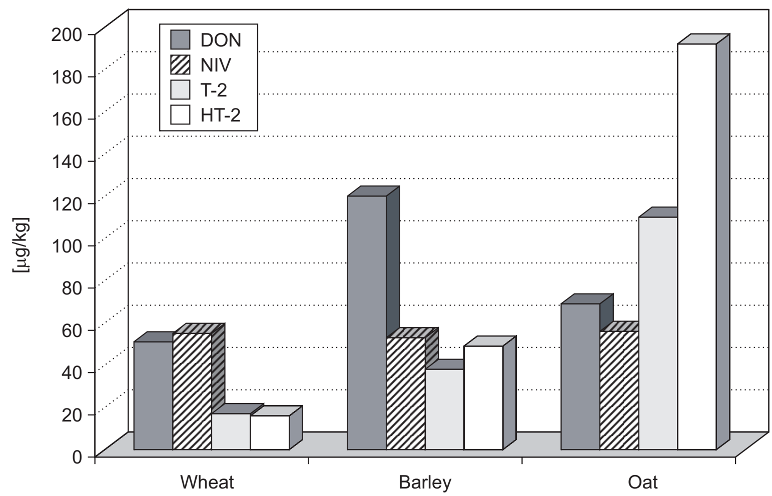

Fig. 1. Mean content of *Fusarium* toxins in Polish cereals [2]

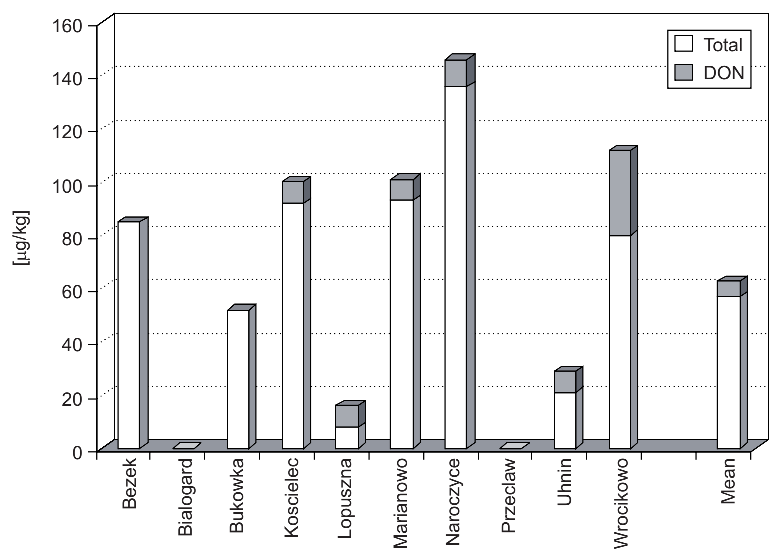

Fig. 2. Mean content of trichothecenes in grain of three oat varieties in the regions of Poland [17]

According to Solarska [5], the presence of *Fusarium* mycotoxins depends not only on the species but also on the cultivar. Winter maize Kobra c.v. revealed greater susceptibility to *Fusarium* head blight and higher cumulation of mycotoxins in grain. Busko et al [18] registered a higher DON accumulation in grain of 37 winter triticale breeding lines (Table 2). However, they assessed a significant correlation between ear infection by *F. culmorum* and DON content only in 2001.

Table 1

List of cereal and maize grains containing trichothecenes [9]

Toxin	Product or group of products with highest contamination (percent of samples with toxin presence)
Deoxynivalenol (DON)	Maize (89 %), wheat (61 %, including flour)
Nivalenol	Maize (35 %), oat (21 %), wheat (14 % including flour)
T-2	Maize (28 %), oat (21 %), wheat (21 %)
Zearalenon	Maize (79 %), milled maize (51 %), wheat (30 %), milled wheat (79 %)
Fumonisin B1	Maize (66 %), maize flour (79 %), wheat (79 %)
Fumonisin B2	Maize (51 %)

Table 2

Deoxynivalenol (DON) accumulation in triticale grain infected by *Fusarium culmorum* [18]

Year	Specification	Fusarium head blight [%]	DON [mg/kg]
2000	Average	21.53	41.86
	Maximum value	36.40	86.25
	Minimum value	10.30	6.25
2001	Average	24.76	75.82
	Maximum value	56.50	128.63
	Minimum value	11.50	21.39

In the opinion of Arseniuk and Goral [9] a majority of winter and spring wheat cultivars listed in the Polish cultivar register are prone to head blight and may accumulate mycotoxins in grain. Therefore, it is necessary to continue breeding experiments aimed to improve Polish cereal cultivars' resistance to head blight. *Fusarium* disease of maize cobs is counted to the most dangerous diseases, although it does not cause any major yield losses [19]. According to Tekiela [19, 20] even a small *Fusarium* infection of maize cobs leads to fumonisine cumulation in grain (Table 3).

Table 3

Percentage of infected maize cobs and mean content of fumonisines (B1, B2) in 2006 [19]

Cultivar	Wielkopolska		Podkarpacie	
	Cobs infected by *Fusarium* [%]	Content of fumonisines [ppb]	Cobs infected by *Fusarium* [%]	Content of fumonisines [ppb]
DKC 3420	74	4727	21	56
DKC3421YG	56	947	27	0
PR38F70	42	1330	51	288
PR39D81	24	304	50	847
PR39F58	80	1559	28	18

Conducted mycological analysis revealed mainly the presence of *Fusarium subglutions* [(Wollenw. et Reiking) Nelson et al], *F. graminearum* (Schwabe) and *F. culmorum* [(W. G. Smith.) Sacc.].

So far no monitoring of the mycotoxin content in maize grain has been conducted in Poland. On the basis of dispersed research it may be assumed that between several and 30 % of maize grain samples may contain the amounts of harmful substances exceeding permissible standards [21]. The content of mycotoxins in maize grain may be limited not only through proper protection of this crop, but also by cultivating genetically modified varieties because GMO grains did not contain or had only small amounts of fumonisines in comparison with maize initial forms [19, 20].

Among the abiotic factors, crop rotation has a considerable effect upon plant infection by *Fusarium* ssp. It is a common view that wheat cultivation after forecrops (cereals or maize), which are *Fusarium* pathogen hosts poses a greater risk of head blight occurrence and grain contamination with mycotoxins. However, Obst et al [22] registered lower DON values in wheat grain cultivated in monoculture than on the stand after potatoes or beetroots. A similar dependence was noticed by Fernandez et al [23], but Dill-Macky and Jones [24] obtained different results. Higher amounts of DON were assessed in wheat grain cultivated after maize, than on the stand after soybeans. The right crop rotation, ie the right selection of plant succession decreases the level of *Fusarium* inoculums and as a result the presence of mycotoxins in grain. The experiments conducted by Mazurkiewicz and Solarska [25] confirmed this thesis. Conventional and organic cultivation of barley after onion forecrop reduced grain contamination with *Fusarium* trichothecenes. However, grain originating from organic cultivation revealed a higher content of these mycotoxins. On the other hand, Vanova et al [26] did not assess any difference in DON cumulation in winter wheat grain cultivated in conventional and organic crop rotation.

The other factor which affects the increased plant infection by *Fusarium* is the tillage method. *Fusarium* fungi survive on harvest residue and are the source of infection for the subsequent crops. Their deep covering using the tillage system increases the soil biological activity and a growth of antagonist microorganisms occurs leading to lesser plant infection by diseases [27, 28]. On the other hand, leaving harvest residue on the soil surface or its shallow mixing, favours development of pathogens. Nitzsche et al [29] demonstrated that simplified tillage and direct sowing promote DON cumulation in wheat grain (Table 4).

Table 4

DON content [μ/kg] depending on tillage [29]

Cultivar	Soil cultivation method		
	Tillage	Simplified	Direct sowing
Petrus	210	220	960
Banit	940	1050	1600

Obst et al [22] found that simplified tillage caused a 10-fold increase in DON in wheat grain after maize forecrop. Dill-Macky and Jones [24] obtained a similar result, in comparison with conventional tillage, while cultivating wheat in monoculture or after maize in a no-tillage system. However, they revealed that the depth of harvest residue placing has no significant impact on DON cumulation in wheat grain grown on the stand after soybeans.

Research was also conducted on the nitrogen fertilization effect of cumulation of selected trichothecenes in cereal and maize grain. However, the results concerning the doses and forms of fertilization are not unanimous. Beta et al [30] and Bladino et al [31] registered ZEN increase in maize with increasing nitrogen dose. On the other hand, Yoshida et al [32] did not find any significant influence of fertilizer doses on wheat head blight and cumulation of mycotoxins. Further research is advisable in this field. Plants which are left unprotected against pathogenic fungi are most exposed to *Fusarium* mycotoxins presence in cereal and maize grain.

Conclusion

The presented material shows that beside the climatic conditions, cultivar and species plant resistance, also application of good agricultural practices – crop rotation, deep cover of harvest residue, proper plant protection against agrophages during the vegetation period) may limit the occurrence of *Fusarium* fungi and provide a basis to reduce cereal contamination with *Fusarium* toxins.

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WPŁYW CZYNNIKÓW ABIOTYCZNYCH NA WYSTĘPOWANIE MIKOTOKSYN FUZARYJNYCH W ZIARNIE ZBÓŻ

Katedra Ochrony Środowiska Rolniczego
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Praca dotyczy analizy wpływu czynników abiotycznych na kumulację mikotoksyn fuzaryjnych w ziarnie zbóż. Najczęściej w ziarnie zbóż wykrywa się deoksyniwalenol (DON), zearalenon (ZEN) oraz fumonizyny. Przyczyn obecności i kumulacji tych toksycznych związków jest wiele. Do podstawowych czynników, które sprzyjają pasożytowaniu grzybów *Fusarium* i produkowaniu mikotksyn, należy zaliczyć: warunki pogodowe i rejon uprawy, podatność gatunkową i odmianową roślin, nieodpowiedni przedplon, płytkie umieszczenie resztek pożniwnych w glebie lub pozostawienie ich na powierzchni oraz rezygnacja z ochrony roślin w okresie wegetacji.

Słowa kluczowe: mikotoksyny, ziarno, zboża, czynniki abiotyczne

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 5–6 2011

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and Grzegorz FORMICKI[1]

ACCUMULATION OF HEAVY METALS AT EARLY STAGES OF *Phrynohyas resinifictrix* (GOELDI) ONTOGENESIS

KUMULACJA METALI CIĘŻKICH WE WCZESNYCH STADIACH ONTOGENEZY *Phrynohyas resinifictrix* (GOELDI)

Abstract: The research focused on an assessment of interrelations between copper, zinc, magnesium and cadmium regarding the rate of their accumulation in *Phrynohyas resinifictrix* organisms. Group A consisted of organisms subjected to the effect of solution containing 2 mg/dm^3 Mg^{2+}, Zn^{2+} and Cd^{2+}, whereas group B to 4 mg/dm^3 Mg^{2+}, Zn^{2+} and 2 mg/dm^3 Cd^{2+}. Significant changes in copper and zinc concentrations were registered during the experiment. Copper content in group A was 5.97 μg/g d.m. after 2 hrs and 367.521 μg/g d.m. after a week ($p = 0.002$). In the group B copper concentration was 12.914 μg/g d.m. after 2 hrs and 234.372 μg/g d.m. ($p = 0.013$) after 2 weeks. Zinc level in group A was 282.779 μg/g d.m. after 2 hrs and 750.051 μg/g d.m. after a week ($p = 0.003$).

Keywords: mineral composition, Cd, Cu, Zn, Mg, amphibian, *Phrynohyas resinifictrix* larvae

Heavy metal concentrations in surface waters may differ greatly and their bioavailability is connected with water chemical composition [1]. Inorganic metal compounds show the strongest toxic properties, since they are readily soluble and strongly dissociating, therefore they easily penetrate through cell membranes. Interrelations between heavy metals, eg Zn-Cu-Cd play a special metabolic role. Mutual biochemical relations between these metals occur both at their natural and toxic contents. Cadmium present in the blood serum and corpuscules dislodges zinc, which is subsequently bound by metallothioneins. This protein serves to maintain zinc homeo-

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stasis, it is also a factor protecting organism against toxic effect of cadmium. Biochemical mechanisms of cadmium and copper antagonism involve, similar as in the relationship between cadmium and zinc, replacement of copper by cadmium in proteins. The metabolic disturbances connected with low level or lack of copper may be an indirect result of cadmium toxicity.

Material and methods

The experiment focused on the measurement of degree and rate of copper, zinc, magnesium and cadmium cumulation at early stages of *Phrynohyas resinifictrix* ontogenesis. The research was conducted on 105 larvae from the culture initiated in the zoological garden in Plock and continued at the Department of Vertebrate Zoology of the Pedagogical University in Krakow. Analyzed were 105 larvae divided into three groups. The control group consisted of larvae living in a spring water. In group A the tadpoles were placed in a solution containing 2 mg/dm^3 Mg, Zn and Cd and in group B in a solution with 4 mg/dm^3 Mg and Zn and 2 mg/dm^3 Cd. 5 tadpoles were captured from each group after 2 hrs, 4 hrs, 6 hrs, 8 hrs and 16 hrs, after a week and two weeks later. The organisms were killed and dried in a thermostat (at 105 ^{o}C) until their dry mass was established. Subsequently the material was incinerated in a muffle furnace (at 450 ^{o}C), the ash was dissolved in 2 cm^3 of nitric acid(V) in 65 % concentration. When the prepared mixture was poured into volumetric flasks, the samples were filled up with (spectrally pure) distilled water to the volume of 5 cm^3 and thoroughly mixed. The solutions prepared in this way served for an assessment of heavy metal concentrations. The content of cadmium and copper were determined using EA9C electrochemical analyzer, while magnesium and zinc concentrations using BUCK200A atomic absorption spectrophotometer. The data were given in milligrams per a gram of dry mass, and subjected to statistical analysis using Friedman ANOVA and Kruskal-Wallis ANOVA tests.

Results

A comparison of the control groups revealed statistically significant differences only in copper content ($p = 0.022$). For group A statistically significant changes in the contents of analysed metals were registered for copper ($p = 0.002$) and zinc ($p = 0.003$). For group B the only statistically significant differences were noted for copper ($p = 0.013$). Copper content in group A after 2 hrs was 5.97 μg/g d.m. and after 1 week 367.521 μg/g d.m., whereas in group B copper concentrations were 12.914 μg/g d.m. after 2 hrs and 234.372 μg/g d.m. after 2 weeks. Zinc contents in group A were 282.779 μg/g d.m. after 2 hrs and 750.051 μg/g d.m. after 1 week ($p = 0.003$).

Statistical analysis based on Kruskal-Wallis ANOVA test shows statistically significant differences between groups for copper after 4 hrs ($p = 0.031$), 16 hrs ($p = 0.039$), after 1 week ($p = 0.009$) and after 2 weeks ($p = 0.007$). After 4 hrs the highest copper concentrations were noted in group B and the lowest in the control group. On the other hand, after 16 hrs the highest copper content was registered in group A. The results obtained after 1 and 2 weeks point to the greatest differences in this element content between groups, where the highest increase was found in groups A and B. Changes in copper content were presented in Figs. 1–4.

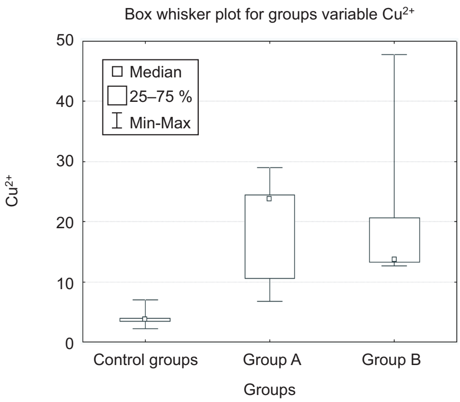

Fig. 1. Comparison of copper content in *Phrynohyas resinifictrix* larvae organisms between groups after hrs of experiment

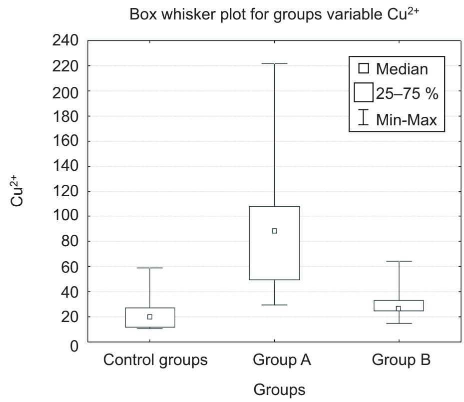

Fig. 2. Comparison of copper content in *Phrynohyas resinifictrix* larvae organisms between groups after 16hrs of experiment

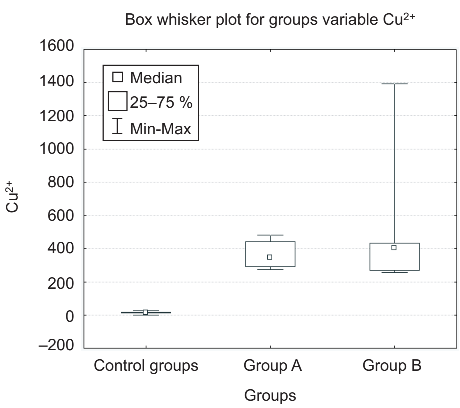

Fig. 3. Comparison of copper content in *Phrynohyas resinifictrix* larvae organisms between groups after 1 week of experiment

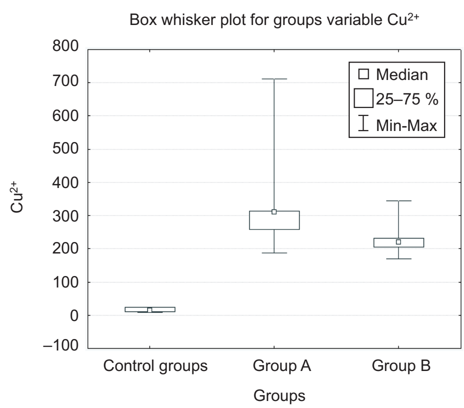

Fig. 4. Comparison of copper content in *Phrynohyas resinifictrix* larvae organisms between groups after 2 weeks of experiment

For zinc, statistically significant differences were registered after 1 week ($p = 0.003$) and after 2 weeks ($p = 0.026$). These measurements noted the highest concentrations in groups A and B. Changes in zinc contents were shown in Figs. 5 and 6.

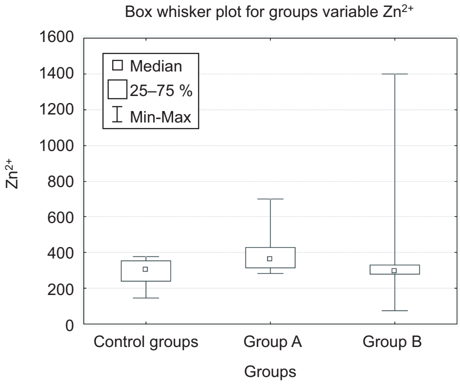

Fig. 5. Comparison of zinc content in *Phrynohyas resinifictrix* larvae organisms between groups after 16 hrs of experiment

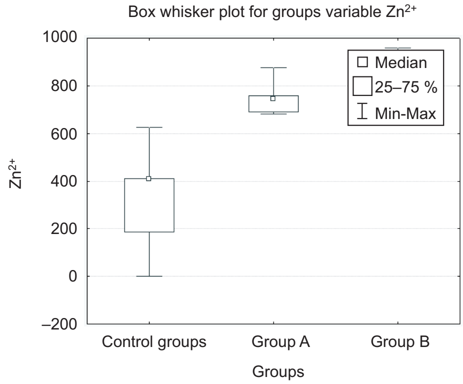

Fig. 6. Comparison of zinc content in *Phrynohyas resinifictrix* larvae organisms between groups after 1 week of experiment

Discussion

Our research revealed that copper concentrations increased during the experiment. Copper is an element crucial for organism functioning. However, copper excess is strongly toxic. The degree of this metal assimilation depends on its form in feed but also on the organism species, its development stage and also on the content of other elements in the diet, among others zinc and cadmium. Increasing copper concentrations may be connected with the organism protection against toxic effect of cadmium [2]. The most frequent is Cu-Zn antagonism, which explains many symptoms associated with copper deficiency. It involves the competition between both cations in the process of absorption from the alimentary system. Generally, a balance between these metals is maintained in the organism for the benefit of zinc, which is often associated with increased copper excretion from the organism, and also with increased zinc to copper ratio in the adipose tissue. Greater zinc content in food leads to a decrease in copper concentration in the liver, heart and blood serum. The result of copper and zinc linking is less toxic than their separate effects. This result may be ascribed to a replacement and competition of these elements for available bonds in protein synthesis, but also to the species properties [3]. On the basis of the above-mentioned information and the data obtained from the discussed experiment, it may be assumed that after 1 week and 2 weeks antagonistic effect of Zn and Cu becomes apparent in the organisms of tadpoles. Application of 4 mg/dm^3 Zn in group B points mainly to the effect of zinc on copper content, but interactions between copper and cadmium are also known.

Zinc is an element crucial for the proper development of larvae [4]. In animal organisms zinc cooperates in metabolic processes with other elements, mainly with copper, whose metabolism is disturbed by cadmium. Both zinc and cadmium reveal similar properties in forming metal-protein complexes and therefore their physiological effect is antagonistic. Zinc may have a limiting effect on cadmium cumulation [5]. The analysis of zinc ions influence on cadmium level in group A may suggest that after one and two weeks significant changes in zinc content in tadpole organisms led to a slight decrease in cadmium level after two weeks. However, it may be supposed that this process is not only the effect of zinc activity. Changes in zinc and cadmium concentrations in group B are not significant, but the duration of zinc effect on cadmium caused similar changes in its content as in group A.

The analysis of magnesium concentrations did not reveal any marked changes in the test groups. However, it is difficult to determine the direct influence of magnesium on the other elements [6]. Most probably solutions with 2 mg/dm^3 and 4 mg/dm^3 of magnesium applied for the experiment and considering high level of this element in the control did not have any marked effect on the level of other metals cumulation in the organisms.

The antagonism between cadmium and copper occurs especially in young organisms, for which even low concentrations of cadmium in diet affect copper content in the liver and kidneys. Elevated copper concentrations in organism lowers the rate of cadmium uptake and even contributes to its elimination [7]. In the presented experiment such interactions might have occurred in group A, where copper reveals significant changes of its contents measured after 4 hrs, 16 hrs, after 1 week and 2 weeks, whereas cadmium in larvae organisms does not reveal any differences in cumulation. It means that changes in cadmium concentrations in the analyzed test group are not significant despite almost constant cumulation rate of this metal in all registered measurements, whereas copper reveals high concentrations after 16 hrs and after one week. The analysis of group B concerning the antagonism between copper and cadmium shows changes similar to those which occur in group A.

Biochemical function of cadmium in animal organisms has not been fully explained so far. Selvi [8] and Flament [9] stated that increasing level of this metal accumulation in amphibian organisms affects the metamorphosis, the course of gonadogenesis and behavioural changes. In our experiment we have registered increased cadmium cumulation in larvae organisms already after 16 hrs and after 1 week, and high levels persisting after 2 weeks in groups A and B. On the other hand, we cannot state a direct effect of cadmium because of tadpoles exposure to cadmium in the presence of zinc which we applied in our experiment. We may only assume that if cadmium concentration were higher, such phenomena would probably occur.

The interactions found between the individual elements are more pronounced when early stages of *Phrynohyas resinifictrix* ontogenesis were treated with 1:1:1 (Mg : Zn : Cd) ratio than for 2:2:1 (Mg : Zn : Cd) ratio.

Conclusions

1. A significant increase in copper content after 4 hrs, 16 hrs, after 1 week and 2 weeks later, and elevated zinc content after 1 and 2 weeks were assessed in the organisms of *Phrynohyas resinifictrix* larvae subjected to the effect of 2 mg/dm^3 Mg^{2+}, Zn^{2+} and Cd^{2+} and 4 mg/dm^3 Mg^{2+}, Zn^{2+} and 2 mg/dm^3 Cd^{2+}.

2. No significant changes of cadmium or magnesium contents were assessed in *Phrynohyas resinifictrix* larvae organisms.

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KUMULACJA METALI CIĘŻKICH WE WCZESNYCH STADIACH ONTOGENEZY *Phrynohyas resinifictrix* (GOELDI)

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Abstrakt: Badania dotyczyły oceny współzależności między miedzią, cynkiem, magnezem i kadmem w odniesieniu do tempa ich kumulacji w organizmach *Phrynohyas resinifictrix*. Grupę A stanowiły organizmy poddane działaniu roztworu zawierającego 2 mg/dm^3 Mg^{2+}, Zn^{2+} i Cd^{2+}, a grupę B 4 mg/dm^3 Mg^{2+} i Zn^{2+}, 2 mg/dm^3 Cd^{2+}. W czasie trwania eksperymentu stwierdzono istotne zmiany zawartości miedzi i cynku. Zawartość miedzi w grupie A wynosiła 5,97 μg/g s.m. po 2 h, a po 1 tygodniu 367,521 μg/g s.m. (p = 0.002). W grupie B zawartość miedzi wynosiła 12,914 μg/g s.m. po 2 h, a po 2 tygodniach 234,372 μg/g s.m. (p = 0,013). Poziom cynku w grupie A wynosił 282,779 μg/g s.m. po 2 h i 750,051 μg/g s.m. po 1 tygodniu (p = 0.003).

Słowa kluczowe: mineralny skład, Cd, Cu, Zn, Mg, płaz, larwy *Phrynohyas resinifictrix*

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Vol. 18, No. 5–6 2011

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EFFECT OF EXTRACTS OF SOILS FROM VARIOUS DISTANCES FROM BLACK LOCUST (*Robinia pseudoaccacia*) SHELTERBELTS ON *Trichoderma* FUNGI

WPŁYW WYCIĄGÓW Z GLEB Z RÓŻNEJ ODLEGŁOŚCI OD ZADRZEWIEŃ ROBINII AKACJOWEJ (*Robinia pseudoacacia*) NA GRZYBY Z RODZAJU *Trichoderma*

Abstract: The aim of the paper was determining the effect of extracts of soils from various distances from black locust shelterbelts on growth, spore germination and antagonism of selected *Trichoderma* spp. isolates. The soil was characterized by the highest organic carbon content in the 0–2 m zone, whereas total nitrogen content was decreasing with the distance from the shelterbelts. The extract of soil from the zone situated 0–2 m from black locust significantly decreased growth rate and stimulated formation of sporogenous hypha. Extracts of soils originating from various distances from black locust shelterbelts caused changes of *Trichoderma* spp. activity towards *B. cinerea*.

Keywords: *Trichoderma* spp., black locust

Fungi of the *Trichoderma* genus play an important role in agricultural environment, because they commonly settle the soil and act antagonistically towards numerous pathogens causing plant diseases. Common occurrence of these fungi is connected with their easy utilization of carbon and nitrogen from various available sources. Demand for carbon and energy is covered by mono- and polysaccharides but also by purines, pyrimidines, fatty acids and even by methanol and methylamine. The sources of nitrogen are ammonia, amino acids, urea, nitrates(V) and nitrates(III). Differences in the species composition of *Trichoderma* spp. in soil and their antagonistic effect in the first place depend on the soil physico-chemical properties. The most important factors comprise: the temperature, humidity, nutrient content, soil pH and presence of other

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microorganisms. Like a majority of soil microorganisms, *Trichoderma* fungi are sensitive to changes of their living conditions and provide early evidence of even slight changes in soil long before the changes of chemical composition and soil physical properties actually take place [1–4].

One of plants frequently occurring in the shelterbelts is black locust. The plant affects an increase in nitrogen content in soil and its shelterbelts and plays a humus forming role. Moreover, the roots of black locust release substances with allelopathic effect. This plant's impact on the soil environment is visible primarily in the arable field zones and it decreases with increasing distance from them [5, 6].

The paper aimed to determine the effect of soil extracts from soils originating from various distances from black locust shelterbelts on the growth and germination of spores and on the antagonism of selected *Trichoderma* spp. isolates.

Material and methods

The following species of antagonistic fungi were selected for the research: *Trichoderma harzianum* Rifai, *Trichoderma pseudokoningii* Rifai, *Trichoderma viride* Pers. ex Gray and pathogenic: *Botrytis cinerea* Pers. and *Rhizoctonia solani* Kühn.

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

Table 1

Selected properties of soils sampled from particular zones

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

Soils extracts were prepared by pouring 90 cm^3 of sterile distilled water over 10 g of soil. The solution was shaken for 24 hours, then filtered through filter paper and passed through bacteriological filters. The extracts prepared in this way were added to PDA medium in the amount allowing to obtain 20-fold dilution. The effect of the extracts on *Trichoderma* fungi growth rate was determined by inoculation of selected *Trichoderma* ssp. inoculum on glucose-potato medium (PDA) with added soil extracts. Incubation was conducted until the moment when mycelium overgrew the whole Petri dish in the control combination (PDA medium without the soil extract supplement). The growth rate index was computed on the basis of results obtained from daily measurements of the colonies' diameters [7]. *Trichoderma* spore germination ability in the soil extract solutions was also assessed using the method presented in this paper. Spore collected from the two-week-old *Trichoderma* spp. cultures were placed in the soil extracts. After 48 hours of incubation germinating process was stopped by adding a drop of formalin. Subsequently the process of spore germination was assessed on the scale and the spore germination index was computed on the basis of the obtained results [7].

The experiment was conducted in 4 replications and the results were elaborated using ANOVA for two-factor experiments (factor A – soil extraction, factor B – *Trichoderma* fungus). The significance was verified by the Duncan test.

The effect of soil extracts on the interrelations between antagonistic fungi treated with the tested factors and *Botrytis cinerea* and *Rhizoctonia solani* was assessed with the biotic series method after Manka [8]. *Trichoderma* fungus inoculum was inoculated in the center of a Petri dish, on PDA medium with added soil extracts and 2 cm away from a pathogenic fungus (4 replications). In the control combinations the medium did not contain soil extracts. After 10 days of incubation each combination was assessed on a scale considering three parameters: the degree to which one fungus colony was surrounded by the other, the inhibition zone and the diminishing of a colony. The highest assessment on the 8 point scale denoted a total lack of growth of one fungus. If the *Trichoderma* spp. fungus was dominating, it was assigned "+" sign (positive effect), when the pathogenic fungus was dominating, "–" (negative result) sign was used. If no prevalence of any colony was visible, the "0" mark was assigned. The obtained marks jointly gave the individual biotic effect (IBE), illustrating the effect of *Trichoderma* spp. on the pathogen growth.

Results and discussion

The obtained results point to existing differences in the tested *Trichoderma* spp. isolates' influence on organic matter content in soil. The most sensitive isolate was *T. harzianum.*

The tested soil extracts did not affect the increase in *T. pseudokoningii* or *T. viride* fungi colony (Fig. 1). On the other hand it was noticed that the extract of soil from the zone situated 0–2 m from black locust markedly decreased the growth rate of *T. harzianum*.

It was also noted that the studied extracts caused a decline in the spore germination index of *T. pseudokoningii* and *T. viride* isolates (Fig. 2). On the other hand the soil extract from the 0–2 m zone from the shelterbelt stimulated the process of sporogenous hypha formation in *T. harzianum* isolate.

Extracts from soils situated at various distances from black locust did not diminish *Trichoderma* spp. antagonism towards *R. solani* (Table 2). On the other hand, these extracts caused changes in *Trichoderma* spp. activity towards *B. cinerea*. It was noticed

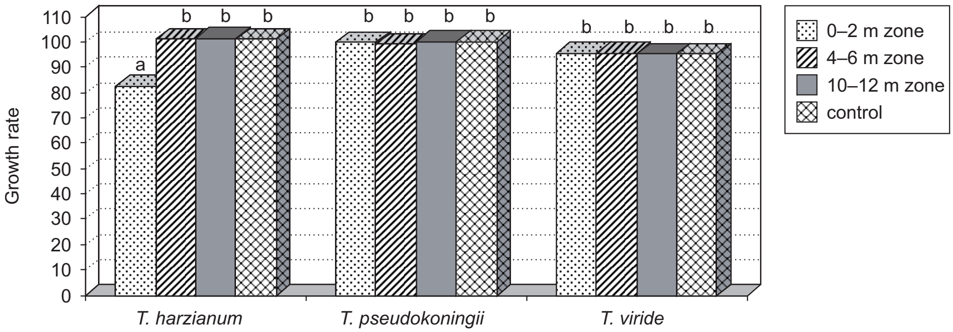

Fig. 1. Growth rate of *Trichoderma* fungi on medium with addend soil extracts
* Columns marked with the same letters do not differ significantly acc. to Duncan test (p = 0.05)

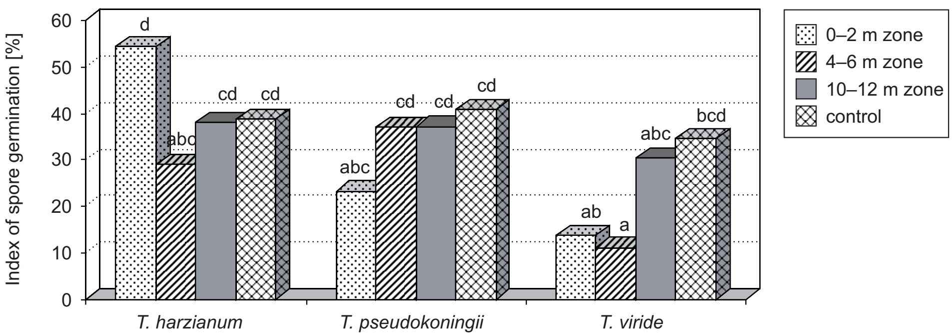

Fig. 2. *Trichoderma* fungi spore germination in soil extracts
* Columns marked with the same letters do not differ significantly acc. to Duncan test (p = 0.05)

that the extract from the soil situated at the distance of 10–12 m from black locust caused a decrease in IBE to +3. However, the activity of *T. pseudokoningii* and *T. viride* isolates was increasing under the influence of soil extracts from the 0–2 m zone from black locust.

Table 2

The impact of soil extracts on individual biotic effect of *Trichoderma* fungi on pathogens

Extract from soils distanced from black locust shelterbelts	*T. harzianum*		*T. pseudokoningii*		*T. viride*	
	Botrytis cinerea	*Rhizoctonia solani*	*Botrytis cinerea*	*Rhizoctonia solani*	*Botrytis cinerea*	*Rhizoctonia solani*
0–2 m zone	+6	+7	+7	+8	+7	+6
4–6 m zone	+5	+8	+5	+8	+2	+8
10–12 m zone	+3	+8	+6	+8	+2	+8
Control without extract	+6	+8	+5	+8	+1	+6

The results obtained in this work confirm numerous reports that microorganism activity depends on many physical and chemical properties of soil, including also mechanical composition, pH, contents of nitrogen and organic matter. Reaction close to acid creates better conditions for *Trichoderma* fungi development [1–4, 9, 10]. Life and activities of soil microflora are strictly connected with plant life. Microorganisms are especially influenced by the rhizosphere, where root secretions of individual plant species modify soil microorganism communities and pathogen antagonist relationship. Black locust roots secrete allelopathic substances, such as robinetin, myricetin and quercitin [6].

Black locust shelterbelts affect also nutrient content in soil. In the zone most distanced from the trees the amount of organic carbon and total nitrogen content were decreasing. The greatest amount of leaves, branches and pods find their way to the area 10 m distant from the shelterbelts. Fallen black locust leaves revealing a low C:N ratio are fast decomposed providing an additional source of nitrogen in soil [5]. It should be added that black locust lives in symbiosis with *Bacilus radiola* nodule bacteria and *Rhizobium* bacteria, therefore it markedly affects an increase in nitrogen concentrations in the substratum. Nitrogen is transformed into humus substances with the highest degree of humification [5, 11]. At growing nitrogen concentrations in substratum a reaction of forming conidia and chlamydospores was observed [9].

Higher content of plant residue in the soil surface creates better conditions for *Trichoderma* development. These fungi are characterized by a great ability of activating and absorbing nutrients from soil in comparison with other microorganisms. The efficient utilization of available nutrients is the basic ability of *Trichoderma* spp. for ATP acquisition from the metabolism of various carbohydrates originating from polymers widely dispersed in the soil environment, such as cellulose, glucan and chitine [10]. Increased soil abundance in carbon causes that the C:N ratio is more advantageous for the development of fungi revealing antagonism towards pathogens including *Trichoderma* fungi. A low C:N ratio causes the disappearance of the competitive effect between fungi responsible for many diseases and organisms revealing considerable fungistatic activity at a high C:N ratio [12, 13].

Plant cover and soil environment are factors which may modify development and antagonistic activity of *Trichoderma* spp. Therefore, the application of these fungi in agricultural practice for plant protection against pathogens meets obstacles.

Conclusions

Black locust (*Robinia pseudoacacia*) caused changes in carbon and nitrogen content in soil and affected mycelium growth, spore germination and antagonism of *Trichoderma harzianum*, *T. pseudokoningii* and *T. viride*.

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WPŁYW WYCIĄGÓW Z GLEB Z RÓŻNEJ ODLEGŁOŚCI OD ZADRZEWIEŃ ROBINII AKACJOWEJ (*Robinia pseudoacacia*) NA GRZYBY Z RODZAJU *Trichoderma*

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Abstrakt: Celem pracy było określenie wpływu wyciągów z gleb pochodzących z różnych odległości od zadrzewień robinii akacjowej na wzrost, kiełkowanie zarodników i antagonizm wybranych izolatów *Trichoderma* spp. Gleba charakteryzowała się największą zawartością węgla organicznego w strefie 0–2 m, a wraz z odległością od zadrzewień malała zawartość azotu ogólnego w glebie. U izolatu *T. harzianum* wyciąg z gleby ze strefy położonej 0–2 m od robinii znacznie obniżał tempo wzrostu i stymulował proces tworzenia strzępek kiełkowych. Wyciągi z gleb pochodzących z różnych odległości od zadrzewień robinii akacjowej powodowały zmiany aktywności *Trichoderma* spp. w stosunku do *B. cinerea.*

Słowa kluczowe: *Trichoderma* spp., robinia akacjowa

Vol. 18, No. 5–6 2011

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IMPACT OF THE MUNICIPAL LANDFILL SITE ON BACTERIA PARTICIPATING IN TRANSFORMATION OF SOIL NITROGEN

WPŁYW SKŁADOWISKA KOMUNALNEGO NA BAKTERIE BIORĄCE UDZIAŁ W PRZEMIANACH AZOTU GLEBOWEGO

Abstract: Field research on the subject presented in the paper were conducted from March 2006 until September 2007. For the experimental reasons 8 research plots for soil sampling were established on each side of the municipal waste landfill site in Tarnow in two zones: 50–200 and 250–500 m from its boundaries. Spring wheat, Zura c.v. was cultivated on the plots. An additional experimental plot was set up in the reclaimed part of the landfill site. Analysis of the obtained results points to differences in the quantitative composition of microflora participating in nitrogen metabolism in the analyzed soils. It was found that on the experimental plots under wheat the number of proteolytic bacteria in 1 g of the soil dry mass ranged from 2 300 to 96 700 cfu, ammonifying bacteria from 122 000 to 4 860 000 and bacteria from Azotobacter genus from 0 to 330 cfu. Over the whole period of investigations also *Clostridium pasteurianum* bacteria counts were determined within the range of 0.01 to 0.00001, the values of nitrification process index from 0.01 to 0.000001 and denitrification index from 0.001 to 0.00001 were assessed in the whole analyzed soil environment.

Keywords: municipal landfill sites, soil, bacteria

Soil microflora is the component of its biocenosis, which most rapidly grows and responds to changes of environmental parameters. Microorganisms change their biomass, metabolic activity and microbiocenotic composition in response to numerous stressors and stimulants which may occur in the soil environment [1, 2]. Even non-farmed soil is not a neutral environment without any interactions, either. It is the area where the conditions, biotic and abiotic factors often determine change in the number of the organisms living in it [3]. It should be remembered that soils belong to

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non-renewable or hardly renewable resources [4]. Therefore, a thorough identification of biocenotic systems formed in the soil environment and allowing for observation of the scale of changes in soil microorganism biodiversity gains a crucial importance [5]. Microorganisms are an important element, necessary for the proper functioning of biological systems, because any damage to microorganisms in soils, whatever its cause, would always lead to recession of individual either eco- or agrosystem [6]. Microorganisms which mobilize unavailable biogen forms enable the growth and development of higher plants conditioning the functioning of whole terrestrial ecosystems [7].

Therefore, the present work aimed at an assessment of the impact of municipal waste landfill site on the occurrence of bacteria participating in nitrogen transformation process.

Material and methods

The research presented in the paper was conducted in the area and in the neighbourhood of municipal waste landfill site in Tarnow during the period from March 2006 until September 2007. The research was conducted on a field experiment model. Therefore, two zones were marked out: I – 250 m and II – between 250 and 500 m from its boundaries. A total of 8 experimental plots, on which spring wheat, Zura c.v. was cultivated, were set up in the established zones. An additional ninth plot was situated in the landfill site area, in the reclaimed sector. The marking of sites was presented in Table 1.

Table 1

Plots situated in the vicinity of municipal waste landfill site in Tarnow

Plot	Plot localization zone [m]	Soil pH	Range [%]
WI	50–200	5.1	6.7–19.4
WII	250–500	5.1	6.1–17.7
NI	50–200	5.6	9.2–23.7
NII	250–500	4.8	7.5–25.8
EI	50–200	4.8	8.9–27.4
EII	250–500	4.9	7.3–24.3
SI	50–200	7.5	9.8–31.3
SII	250–500	4.7	9.7–29.1
Z	Reclaimed sector area	4.7	12.4–39.6

The soil was sampled for analyses from the arable layer (0–20 cm) from the experimental plots under spring wheat at different stages of its growth, from March 2006 until September 2007. Collected soil samples were brought to microbiological laboratory at the Microbiology Department, University of Agriculture in Krakow, where moisture and pH were measured and microbiological analyses were performed. These comprised determination of proteolytic microorganism count (medium acc. to Pochon), ammonifying bacteria count (medium acc. to Rougieux) and aerobic assimilators of atmospheric nitrogen of *Azotobacter* genus (Ashby's medium) and well as bacteria assimilating atmospheric nitrogen in anaerobic conditions – *Clostridium pasteurianum* (medium acc. to Rougieux). Moreover, the course of nitrification processes (medium acc. to Winogradsky) and denitrification process was determined (medium acc. to Giltay). The number of cfu (*colony forming units*) of microorganisms was determined using dilution seeding method and converting the result into a gram of the soil dry matter, or determining the count in diluted soil starting from 10^{-1}, ie in 0.1 g.

Results and discussion

A current opinion states that "no healthy society is possible without a healthy soil". Healthy soil is connected with healthy food and to a great extent also with healthy water, air, microclimate and green areas surrounding our housing estates and workplaces [7]. While seeking solutions to these problems, microbiological analyses were conducted on the soils under spring wheat cultivated on the experimental plots situated in the area and around the municipal waste landfill site in Tarnow. They revealed a diversified occurrence of bacteria participating in nitrogen metabolism. Mineralization of nitrogen containing organic compounds, occurring in the environment, carried on by proteolytic, ammonifying, nitrifying and denitrifying bacteria is the basic microbiological process providing nitrogen in a mineral form easily available to plants [8–10].

Analytical data on the number of investigated soil microorganisms show apparent differences dependant on the plot location in relation to the landfill site. Considering the assessed number of proteolytic bacteria present in the experimental plots it may be seen that their number ranged from 2 300 top 96 700 cfu (Table 3). During the experiment their highest number was assessed in July 2007 in the soil from the experimental plot situated in the zone immediately adjoining the landfill site – SI plot, where also the highest average number was registered throughout the whole period of the experiment (Table 2). On the other hand, the lowest number of proteolytic bacteria was noted in September 2006 on the experimental plot situated in the zone located 250 m west of the landfill site – WI plot.

Considering the research results on the number of ammonifying bacteria in the soil of the established plots under spring wheat, which were given in Table 3, it was found that their number ranged from 122 000 to 4 860 000 cfu per 1 g of the soil dry mass. The highest number of ammonifying bacteria was noted in July 2006 in the soil of the experimental plot situated on the northern side of the landfill – NI plot, whereas the lowest number was revealed in March 2007 on the experimental plot located in the reclaimed sector, and slightly higher was registered on the WI plot located in the zone below 250 m in front of the entrance to the researched object. Much higher number of the analyzed bacteria was found in the investigated soil in the summer months (summer of 2006 and 2007) in comparison with the other periods. The average values show that the highest number of ammonifying bacteria occurred in the soil of the plots situated on the northern side of the landfill (plots NI and NII). The lowest average number (366 400 cfu) was registered in the soil of experimental plot on the western part of the landfill –

Table 2

Average number of bacteria participating in nitrogen metabolism in the soil under spring wheat in the vicinity of municipal waste landfill site in Tarnow

Analyzed microorganisms	Average number of cfu per 1 g of soil d.m.								
	Plots								
	W I	W II	N I	N II	E I	E II	S I	S II	Z
Proteolityc bacteria	9 566	18 902	15 790	32 391	28 980	40 209	40 960	20 176	34 949
Ammonifying bacteria	366 400	1 129 345	2 041 500	1 897 985	737 313	704 625	1 132 204	923 300	881 550
Bacteria of *Azotobacter* genus	14	123	72	64	0	16	124	28	0

Table 3

Number of bacteria participating in processes of nitrogen transformation in soils under Zura spring wheat in the vicinity of municipal waste landfill site in Tarnow (cfu per 1 g soil d.m/count)

Date of analysis	2006				2007			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Experimental plot – WI								
Proteolytic bacteria	7 300	9 400	5 389	2 300	10 980	15 400	19 560	6 200
Ammonifying bacteria	420 300	478 300	678 000	415 500	127 800	155 000	372 100	284 200
Bacteria of *Azotobacter genus*	0	0	36	30	0	0	45	0
Nitrification	0.0001	0.001	0.00001	0.00001	0.001	0.0001	0.001	0.01
Denitrification	0.00001	0.00001	0.00001	0.0001	0.0001	0.000001	0.00001	0.0001
Clostridium pasteurianum	0.01	0.001	0.00001	0.001	0.001	0.001	0.001	0.001

Table 3 contd.

Date of analysis	2006				2007			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Experimental plot – WII								
Proteolytic bacteria	14 247	26 300	15 300	11 470	19 400	16 000	35 300	13 200
Ammonifying bacteria	923 000	1 126 000	1 510 000	1 684 660	805 780	405 000	1 865 320	715 000
Bacteria of *Azotobacter genus*	104	264	330	0	0	110	178	0
Nitrification	0.00001	0.00001	0.0001	0.000001	0.000001	0.00001	0.0001	0.0001
Denitrification	0.0001	0.0001	0.0001	0.001	0.0001	0.000001	0.00001	0.0001
Clostridium pasteurianum	0.001	0.0001	0.0001	0.001	0.001	0.0001	0.01	0.001
Experimental plot – NI								
Proteolytic bacteria	21 500	26 400	16 680	19 000	11 000	7 000	16 700	8 040
Ammonifying bacteria	1 241 000	2 312 000	4 860 000	1 734 000	1 004 000	1 200 000	2 837 000	1 144 000
Bacteria of *Azotobacter genus*	0	167	200	205	0	0	0	0
Nitrification	0.00001	0.001	0.001	0.00001	0.001	0.001	0.001	0.001
Denitrification	0.001	0.00001	0.000001	0.000001	0.00001	0.001	0.00001	0.0001
Clostridium pasteurianum	0.001	0.01	0.001	0.0001	0.001	0.001	0.001	0.001
Experimental plot – NII								
Proteolytic bacteria	12 200	46 390	13 670	24 100	36 800	53 000	46 400	26 570
Ammonifying bacteria	829 280	1 994 000	3 935 000	3 290 000	766 200	1 790 000	1 490 200	1 089 200
Bacteria of *Azotobacter genus*	0	40	0	0	48	140	224	58
Nitrification	0.0001	0.00001	0.0001	0.000001	0.000001	0.00001	0.000001	0.00001
Denitrification	0.00001	0.00001	0.000001	0.0001	0.0001	0.000001	0.000001	0.00001
Clostridium pasteurianum	0.001	0.001	0.001	0.001	0.01	0.0001	0.001	0.0001

Table 3 contd.

Date of analysis	2006				2007			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Experimental plot – EI								
Proteolytic bacteria	24 800	68 300	73 000	15 600	8 790	12 602	18 070	10 680
Ammonifying bacteria	739 000	1 000 400	830 000	1 380 400	282 500	345 200	842 100	478 900
Bacteria of *Azotobacter genus*	0	0	0	0	0	0	0	0
Nitrification	0.00001	0.00001	0.000001	0.000001	0.0001	0.000001	0.000001	0.01
Denitrification	0.001	0.001	0.0001	0.0001	0.001	0.001	0.00001	0.0001
Clostridium pasteurianum	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Experimental plot – EII								
Proteolytic bacteria	33 800	69 000	91 000	11 500	15 090	25 980	55 400	19 900
Ammonifying bacteria	402 000	685 200	1 985 000	399 500	192 800	295 000	1 495 000	182 500
Bacteria of *Azotobacter genus*	12	22	0	0	20	75	0	0
Nitrification	0.00001	0.0001	0.000001	0.00001	0.001	0.00001	0.000001	0.00001
Denitrification	0.0001	0.00001	0.0001	0.0001	0.0001	0.000001	0.00001	0.0001
Clostridium pasteurianum	0.0001	0.001	0.001	0.001	0.0001	0.0001	0.0001	0.001
Experimental plot – SI								
Proteolytic bacteria	25 800	53 400	46 100	26 000	16 630	35 500	96 700	27 550
Ammonifying bacteria	937 800	1 192 300	1 805 600	1 627 000	592 000	194 333	1 488 600	1 220 000
Bacteria of *Azotobacter genus*	0	0	226	270	0	185	263	48
Nitrification	0.00001	0.0001	0.001	0.000001	0.001	0.00001	0.00001	0.001
Denitrification	0.00001	0.00001	0.00001	0.000001	0.00001	0.000001	0.00001	0.00001
Clostridium pasteurianum	0.001	0.01	0.0001	0.0001	0.001	0.001	0.001	0.001

Table 3 contd.

Date of analysis	2006				2007			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Experimental plot – SII								
Proteolytic bacteria	20 400	25 300	32 000	22 510	11 800	9 000	18 700	21 700
Ammonifying bacteria	1 102 300	1 664 800	1 466 900	1 167 000	358 000	395 000	765 100	467 300
Bacteria of *Azotobacter genus*	0	0	148	0	0	40	34	0
Nitrification	0.00001	0.0001	0.001	0.01	0.0001	0.000001	0.0001	0.0001
Denitrification	0.00001	0.00001	0.000001	0.000001	0.0001	0.000001	0.000001	0.0001
Clostridium pasteurianum	0.001	0.001	0.0001	0.0001	0.001	0.001	0.0001	0.0001
Experimental plot – Z								
Proteolytic bacteria	19 060	74 700	60 000	11 600	16 330	29 000	49 400	19 500
Ammonifying bacteria	830 000	1 230 000	2 630 000	1 232 000	122 000	200 800	423 800	383 800
Bacteria of *Azotobacter genus*	0	0	0	0	0	0	0	0
Nitrification	0.001	0.0001	0.00001	0.001	0.01	0.0001	0.0001	0.0001
Denitrification	0.0001	0.00001	0.00001	0.000001	0.00001	0.00001	0.00001	0.0001
Clostridium pasteurianum	0.01	0.001	0.01	0.01	0.001	0.01	0.001	0.01

WI plot (Table 2). The obtained results confirm that the course of ammonification process depends on various environmental factors, among others soil type, total contents of carbon and organic nitrogen, mineral and organic fertilization [9].

Bacteria active in atmospheric nitrogen assimilation comprise among others those from *Azotobacter* genus. When their number in the soil of the experimental plots under spring wheat changed, the changes were considerable, fluctuating from zero presence in the soils of all plots to 330 cfu per 1 g d.m. of soil (summer 2006) on WII plot situated in front of the entrance gate (west side) in the zone between 250–500 from the landfill boundaries (Table 3). Considering their number in the soil of the individual experimental plots, no presence was found in the analyzed soil environment on the plots situated in the reclaimed sector and in the area immediately adjoining the landfill from the east (plot EI). It may be due to the fact that the soil microflora is the fastest growing and responding to the changes of environmental parameters element of biocenosis. It is conditioned by typical for microorganisms diversity of biochemical functions and exceptionally high physiological activity [2, 7]. The analysis of data based on the obtained average values for these bacteria revealed, that *Azotobacter* bacteria were the most numerous on the experimental plots considerably distanced from the active part of the landfill site, most frequently to the west and south – plots WII and SII (Table 2). A comparison of these bacteria number in the experimental model reveals an apparent increase in their number in summer months of 2006–2007. It confirms that number of *Azotobacter* bacteria depended on the soil pollution, which had a toxic impact on it by significantly decreasing or leading to a total lack of these microorganisms in the soil [11].

Beside atmospheric nitrogen fixing aerobic microorganisms the research comprised also atmospheric nitrogen assimilators living in anaerobic conditions. As results from the data presented in Table 3, during the entire period of the experiment, the value of *Clostridium pasteurianum* in the soil of the experimental plots under spring wheat ranged from 0.01 (all experimental plots except plots EI, EII and SII) to 0.00001 on the experimental plot localized in the area west of the landfill, in the zone 250 m from its boundaries – WI plot. An apparent prevalence of the analyzed bacteria was visible in the soil of the plots situated on the southern part of the landfill – plots SI and SII in comparison with the other plots localized on different sides of the landfill. The lowest bacteria count was the most frequently noted in the soil of the plot localized in the reclaimed sector.

Attention should be also paid to the course of nitrification and denitrification processes in the analyzed soil. As seen in Table 3, the nitrification index determined in the soil of experimental plots localized in the neighbourhood of the municipal waste landfill site in Tarnow was on the level between 0.01 (all cultivated plots except WI) and 0.000001 (all plots except SI and SII). A markedly lower occurrence of nitrification process is observable in the soil of the experimental plots situated on the southern side of the landfill – plots SI and SII and in the reclaimed sector – plot Z. A tendency of increasing nitrification index was noticed during the summer – July of 2006 and 2007 but its decline in the final period of the investigations in autumn 2007. The obtained research results demonstrate that nitrification process may be disturbed by chemical compounds present in soil. The course of this process may be misshapen also when there are other conditions in soil, unfavourable for nitrificators. Nitrification process is determined by pH, organic matter content and heavy metal concentrations in soil [12].

Concerning the value of denitrification index, it was found that it ranged from 0.001 (plots NI and EI) to 0.00001 on all experimental plots except the one situated on the eastern side of the landfill – plot E I (Table 3). General seasonal changes of the nitrification process in soil under spring wheat were little regular. The analyzed soil of the experimental model shows that the lowest soil denitrifying activity occurred on the experimental plot localized in the area immediately adjoining the landfill on its eastern side, plot EI. The analyses proved that nitrifying and denitrifying soil activity depended both on the localization of the experimental plot and the period of plant growth. According to the literature data, the age and development of plants affect the character of secretions, which in consequence has a considerable effect on microorganism populations. Soil is not only a substratum, the place of plant planting or a reservoir of biogenic elements, but also the habitat of numerous coexisting and mutually protective organisms [13, 14].

In the analyzed soil environments pH was assessed from 4.7 on SII and Z plots to 7.5 on the experimental plot SI. Considerable moisture fluctuations were registered from 6.1 % to 39.6 %, particularly in the reclaimed landfill sector, where between 12.4 % and 39.6 % were noted (Table 1). While comparing the number of bacteria participating in nitrogen metabolism on all plots, one may observe an apparent increase in their number during the plant growth period in May, July 2006–2007, which might have been caused by an intensive mineralization of soil organic matter in result of thermal and moisture conditions in this period usually favouring the microflora development [15]. The presented experiments corroborate also reports of other researchers who revealed the presence of microorganisms in soil in the area and in the neighbourhood of public utility facilities and different level of environmental contamination [1, 13, 16, 17].

Conclusions

1. The factor diversifying the presence of bacteria participating in nitrogen metabolism in the soil was the localization of the experimental plot.
2. Comparing the number of the analyzed bacteria, their successively increasing number was observed during the plant growth period, ie in the summer months, which might have been the result of favourable thermal and moisture conditions for microflora development in this period.
3. On the basis of conducted experiments it may be stated that the areas immediately adjoining the municipal waste landfills site (in the zone below 250 m) should be excluded from agricultural use.
4. The obtained results demonstrate that further extensive research should be continued in the vicinity of municipal waste landfill sites to select the plant positively affecting the beneficial soil microorganism groups which may contribute to the improvement of biological activity of the soils surrounding the operating municipal facilities.

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WPŁYW SKŁADOWISKA KOMUNALNEGO NA BAKTERIE BIORĄCE UDZIAŁ W PRZEMIANACH AZOTU GLEBOWEGO

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Abstrakt: Badania terenowe związane z tematem pracy prowadzono w okresie od marca 2006 do września 2007 r. W tym celu z każdej strony składowiska odpadów komunalnych w Tarnowie w dwóch strefach

50–200 i 250–500 metrów od jego granic wyznaczono 8 stanowisk badawczych (poletek) do pobrania próbek gleby, na których uprawiano pszenicę jarą odmiany Żura. Dodatkowe poletko doświadczalne założono na terenie zrekultywowanej części składowiska. Analiza otrzymanych wyników wskazuje na występowanie w badanych glebach różnic w ilościowym składzie mikroflory biorącej udział w metabolizmie azotowym. Na poletkach doświadczalnych pod uprawą pszenicy stwierdzono, że w 1 g suchej masy gleby liczba bakterii proteolitycznych kształtowała się w granicach od 2 300 do 96 700 jtk, bakterii amonifikacyjnych od 122 000 do 4 860 000 jtk, oraz bakterii z rodzaju *Azotobacter* od 0 do 330 jtk. Określano również w ciągu całego okresu badawczego w badanym środowisku glebowym wartości miana bakterii *Clostridium pasteurianum* w przedziale od 0,01 do 0,00001, wartości miana procesu nitryfikacji od 0,01 do 0,000001 oraz denitryfikacji od 0,001 do 0,00001.

Słowa kluczowe: składowiska odpadów komunalnych, gleba, bakterie

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HEAVY METALS IN SOILS OF HENRYK JORDAN PARK IN KRAKOW

METALE CIĘŻKIE W GLEBACH PARKU IM. HENRYKA JORDANA W KRAKOWIE

Abstract: Dr. Henryk Jordan Park in Krakow, considering its location in the city centre, is exposed to a strong anthropogenic influence. The conducted research aimed to assess the degree of pollution of soils from the park with Cd, Ni, Cr, Cu, Pb and Zn. A majority of the studied soils were characterized by elevated zinc and cadmium levels but there occurred soils slightly polluted with these metals too. Anthropogenic influence exerted its impact on the content of lead, copper and especially nickel in a lesser degree in studied soils, and in a major part of soils there were natural contents of them.

Keywords: Krakow, urban soils, heavy metals, pollution

Progressing industrialization and urbanization affect negatively the urban environment. The spatial development of towns takes place at the sacrifice of green areas. The non-built-up areas, and urban parks which belong to them, in turn are exposed to a range of transformations including chemical ones. Therefore, in urban parks studies concerning pollution of their soils with heavy metals have been carried out [1–4].

Dr. Henryk Jordan Park, having the surface of more than 20 ha, is one of the biggest and the most willingly visited green areas in the centre of Krakow. However, due to its location it is exposed to the impact of transport, municipal and industrial pollutions.

The aim of the undertaken research was to assess the degree of pollution with Cd, Ni, Cr, Cu, Pb and Zn of Henryk Jordan Park soils in Krakow. The presented studies are the continuation of issues regarding the pollution of urban parks in Krakow [4] and they may be used for the evaluation of the urban green lands quality.

Material and methods

In the area of dr. Henryk Jordan Park 13 research points were established – taking into account their regular distribution (Fig. 1), of which from the surface layer (0–20 cm)

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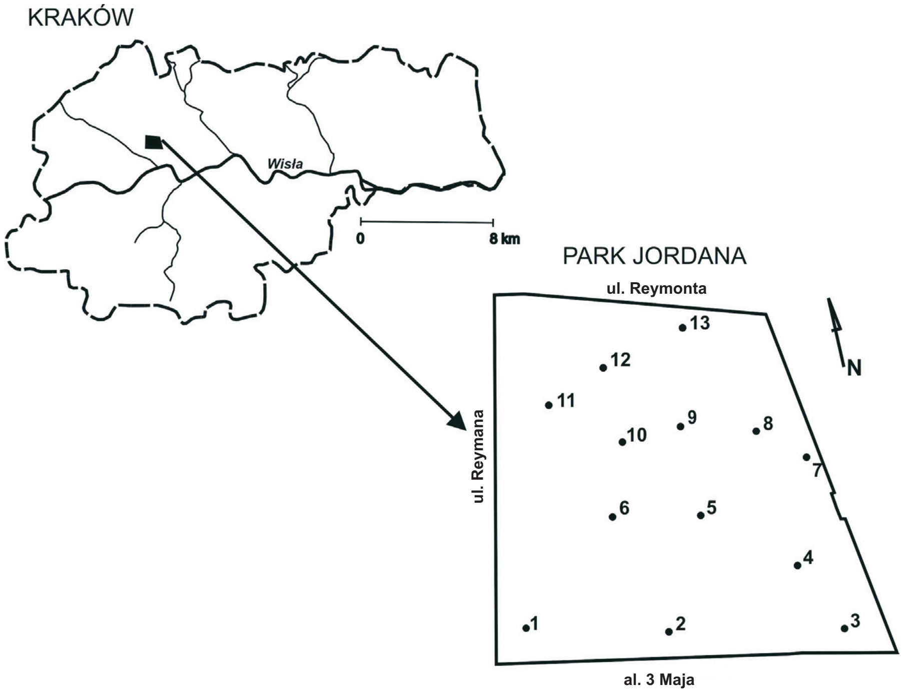

Fig. 1. Location of Jordan Park in Krakow and sites of taking soil samples

soil samples were taken. In dried and sifted through a plastic sieve with 2 mm mesh soil samples pH in 1 mol · dm^{-3} KCl solution [5], soil texture by the densimetric-sieve method [6], organic carbon content with the modified Tiurin method, hydrolytic acidity after the Kappen method [7] and the sum of exchangeable bases in 0.5 mol · dm^{-3} NH_4Cl [8] were determined. On the basis of the two last values a degree of *base saturation* (BS) was calculated. Total studied heavy metal contents after previous mineralization of soil samples in concentrated nitric(V) and perchloric(VII) acids were determined by AAS method [9] (excluding nickel, in case of which the ICP-AES technology was applied).

The interdependence between the contents of the analyzed heavy metals and selected soil properties was determined by computing the correlation coefficient (r) according to Spearman's rank with the use of *STATISTICA* program. Graphical presentation of the spatial distribution of selected heavy metals in soils of the park was performed using Surfer 8 program.

Results and discussion

Results of basic soil properties assessments which affect heavy metal mobility, their fitoavailability and contents were presented in Table 1. Studied soils differed in texture.

In the southern part of the park (from 3 Maja Av.) there occurred common silts (points 1–3 and 5) and in the remaining part soils had strong loamy sand or light loam textures but with a big share of silt fraction. Only the soil in point 13 had the texture of silty light loamy sand. The presence or absence of calcium carbonate influenced pH values of studied soils. It ranged from 5.3 to 7.0. Non-carbonated, acid soils occurred in the central part of the park (points 6 and 8–11). The high pH of remaining soils, besides natural causes, could be affected by the introduction of the anthropogenic substances of alkaline character considering the vicinity of buildings and roads. The alkalization of soil environment is one of the characteristic transformations of soils in urban areas [4, 10]. A degree of sorption complex saturation with basic cations was high and on average exceeded 90 %. The humus content in the analyzed soils was also high, which evidences organic C content amounting on average to 25.6 $g \cdot kg^{-1}$.

Table 1

Soil reaction, degree of sorption complex saturation with bases, content of fraction ∅ < 0.002 mm, organic carbon and heavy metals

Point No.	pH_{KCl}	BS [%]	Fraction < 0.002 mm [%]	org. C [$g \cdot kg^{-1}$]	Cd	Ni	Cr	Cu	Pb	Zn
					[$mg \cdot kg^{-1}$]					
1	6.3	91.6	5	19.51	0.80	11.4	28.2	9.9	28.6	93.6
2	7.0	96.5	6	24.94	1.20	14.9	39.3	29.2	77.3	320.4
3	6.9	95.5	6	22.65	0.90	13.0	37.2	19.8	52.2	138.6
4	6.8	95.8	7	24.59	1.06	15.8	41.2	142.6	62.8	214.0
5	6.8	95.7	10	30.13	0.89	17.8	49.6	36.9	61.7	232.2
6	5.3	80.8	5	22.39	1.15	11.0	33.4	11.1	43.3	97.7
7	7.0	97.5	5	22.76	0.61	11.3	33.2	23.2	70.9	161.5
8	5.7	89.3	12	25.24	1.30	17.4	51.1	14.3	57.5	132.9
9	5.5	83.2	8	34.35	1.27	12.4	42.9	15.3	55.1	130.6
10	5.6	85.5	6	28.09	0.87	12.8	28.7	11.0	41.2	91.5
11	5.4	75.6	8	22.35	0.64	11.8	31.7	8.7	29.7	69.1
12	6.5	94.3	7	35.50	0.75	13.7	41.7	15.7	53.7	129.3
13	6.8	94.6	3	20.70	0.55	10.5	24.5	20.0	76.1	112.8

Soil environment of Henryk Jordan Park was exposed to anthropogenic influence. There occurs at least a few soils with contents of each of determined heavy metals, higher than recognized as natural. However, it must be clearly emphasized that these amounts are not worrying. Taking into consideration criteria proposed by IUNG [11] most of studied soils are characterized by natural contents of nickel, copper and lead. In the case of nickel and lead, a few, particularly lighter soils, revealed their elevated contents. A strong pollution with copper occurred only in point 4 (IV degree of pollution, the highest in these studies), which is surely the effect of a point introduction of this element. Investigated soils were characterized, compared to soils from Krakowski Park [4] by relatively high amounts of chromium and therefore they should be recognized as elevated [12].

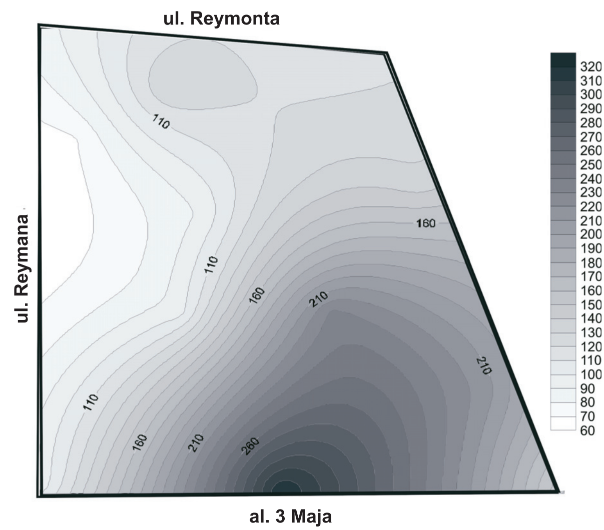

Fig. 2. Spatial distribution of zinc content [mg · kg^{-1}] in Jordan Park soils

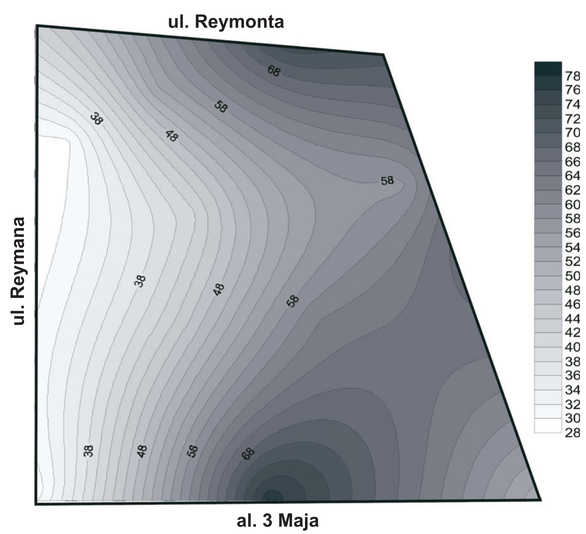

Fig. 3. Spatial distribution of lead content [mg · kg^{-1}] in Jordan Park soils

In more than a half of studied soils an elevated content of cadmium occurred, soils from points 1, 3, 5 and 12 had its natural contents while soils from points 6 and 9 should be recognized as slightly polluted with Cd [11]. A considerable majority of soils in Jordan Park revealed an elevated content of zinc, three soils (points 2, 4 and 9) were polluted in a slight degree and only soils from points 2 and 11, taking into account IUNG classification [11], can be counted among soils with natural contents of this element. According to the Regulation of Minister of the Natural Environment on the soil quality standards and earth quality standards [13] studied soils should be recognized as unpolluted.

Lead and especially zinc were these heavy metals which contents oscillated within a relatively large range, with smaller amounts of them occurring in the western parts of the park (Fig. 2 and 3). It results surely from the fact that Reyman street is characterized by incomparable smaller traffic capacity than 3 Maja Avenue and Reymont street. Considering the high abundance of the park soils in humus, it is supposed to have a great importance in immobilization of heavy metals.

In studied soils significant relations between nickel and chromium, and clay and humus contents occurred (Table 2), which in the case of Ni – clay relation is confirmed in the literature [12]. In studied soils Cu, Pb and Zn contents were positively correlated with pH and amount of bases.

Table 2

Spearman's rank correlation coefficients (r) determining the relationship between heavy metal contents and selected properties of the analyzed soils (n = 13)

	Cd	Ni	Cr	Cu	Pb	Zn
∅ < 0.002 mm	0.479	0.772**	0.797**	0.025	–0.047	0.220
pH_{KCl}	–0.170	0.275	0.071	0.797**	0.676*	0.775**
BS	–0.132	0.302	0.121	0.863***	0.736**	0.819***
C_{org}	0.396	0.643*	0.747**	0.280	0.209	0.368

* $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$.

Conclusions

1. Soils of dr. Henryk Jordan Park, in dependence of which part of it they were taken, differed in texture and reaction, and they revealed also a high degree of sorption complex saturation with bases and a high content of humus.
2. A majority of park soils were characterized by elevated zinc and cadmium contents, but there occurred also soils slightly polluted with them.
3. In studied soils anthropogenic influence affected in a lesser degree lead, copper and especially nickel contents, there occur only a few soils enriched in these elements.
4. Basing on the Regulation of Minister of the Natural Environment on the soil quality standards and earth quality standards dated 2002 soils from the park should be considered as unpolluted with analyzed heavy metals.

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METALE CIĘŻKIE W GLEBACH PARKU IM. HENRYKA JORDANA W KRAKOWIE

Katedra Gleboznawstwa i Ochrony Gleb
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Park im. dr. Henryka Jordana w Krakowie, ze względu na swą lokalizację w centrum miasta, jest narażony na silne antropogenne oddziaływanie. Przeprowadzone badania miały na celu określenie stopnia zanieczyszczenia gleb parku Cd, Ni, Cr, Cu, Pb i Zn. Większość gleb parku charakteryzowała się podwyższoną zawartością cynku i kadmu, występowały również gleby słabo nimi zanieczyszczone. Antropogenne oddziaływanie wpłynęło w mniejszym stopniu na zawartość w badanych glebach ołowiu, miedzi a zwłaszcza niklu, w większości gleb była naturalna ich zawartość.

Słowa kluczowe: Kraków, gleby miejskie, metale ciężkie, zanieczyszczenie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 5–6 2011

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EFFECT OF ACTIVE COMPOUNDS FROM PESTICIDES APPLIED TO SOIL ON THE CALIFORNIA EARTHWORM *Eisenia fetida*

WPŁYW AKTYWNYCH ZWIĄZKÓW ŚRODKÓW DOGLEBOWYCH NA DŻDŻOWNICĘ KALIFORNIJSKĄ *Eisenia fetida*

Abstract: This study was aimed at estimating the effect of selected pesticides on the California earthworm *E. fetida* its survival and reproduction. Parameters that were taken into account included:

1) changes in the density, mortality, biomass and hampered life activity after application of pesticides as compared with the control,

2) the number of laid cocoons and hatching.

Selected pesticides contained the following active chemical compounds: dichlobenyl (herbicide), diazinon (insecticide) and carbendazin and tiuram (fungicide). Earthworms responded to soil pesticides in different ways. Diazinon caused remarkable loss of body weight. Most toxic was fungicide which resulted in 100 % mortality of *E. fetida* after 48 h contact.

Keywords: *Eisenia fetida*, pesticides, soil

Soil oligochaetes are important for proper functioning of the soil fauna. They play a role in the production and transport of humus and in the improvement of water and air relations in the soil [1, 2]. Natural occurrence of oligochaetes is affected by the activity of man and his desire to obtain maximum income from farming. Chemical treatments result in changes of the proportion of particular trophic groups of animals due to application of new plant protection chemicals. Side effects of pesticide treatments are not always obvious.

Earthworms became one of the basic ecotoxicological study objects to evaluate environmental changes [3] focussed particularly on various herbicides which are now inseparable part of agricultural production [4–7].

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Material and methods

Plant protection chemicals applied to the soil in concentrations recommended by the producer to control weeds, insects and for seed protection were used in the study.

a) Herbicide – dichlobenyl (Casoron 6,75 GR) (a compound from the benzonitriles group) at a concentration of 6–7 g/m^2;

b) Insecticide – diazinon (Basudin 600EW) (a compound from the phosphoorganic group) at a concentration of 4 $cm^3/10\ dm^3$ water;

c) Fungicide – carbendazin (a compound from benzamidazoles) and tiuram (a compound from the dithiocarbamate group) (Funaben T preparation) at a concentration of 3 $g/1\ dm^3$ water.

Thirty individuals of the earthworm *Eisenia fetida* with well developed clitellum from own culture of the Department of Zoology, Warsaw Agricultural University were placed in sterile soil in laboratory conditions. Animals were left in a box for 24 hours at room temperature to let them disperse freely in the soil and to release them from stress caused by capturing and transferring to the laboratory conditions. After 24 hours the soil was sprinkled with pesticides at a concentration and dose used in practise.

Animal behaviour was observed. Appearance of the body wall and mortality after 1, 24 and 48 hours was adopted as criteria of animal response to herbicides.

The next stage of the study was checking the ability of reproduction and hatching after ca 3 weeks since the beginning of the experiment. Each experimental variant was triplicated. Animals placed in the same conditions but in soil sprinkled with distilled water instead of chemicals served as control. Obtained results were tested with one-way ANOVA and LSD tests.

Results

Plant protection chemicals affected primarily earthworms' body walls from small changes in their appearance to heavy burns as compared with control animals. Most effective was the fungicide containing carbendazin and tiuram which caused the death of earthworms within 72 hours. Earthworms were observed to move out to the soil surface and release celomic fluid directly after fungicide application.

Diazinon appeared to act less drastically though it also contributed to partial paralysis of *E. fetida* and to damage of its body wall. This insecticide limited also the reproductive potential of earthworms. The number of laid cocoons and hatched young individuals in relation to the control was low (Table 1).

Table 1

The effect of active substances from plant protection products on the number of laid cocoons and hatching in the California earthworm *E. fetida*

	Control	Dichlobenyl	Diazinon	Carbendazin + tiuram
Number of cocoons/ind.	97.7	115.7	18.0	0.0
Number of hatched young/cocoon	112.3	123.3	30.0	0.0

The least toxic was dichlobenyl, though it also resulted in earthworm mortality, decreased mobility and metabolic rates (loss of weight compared with the initial at the same feeding system as in the control) (Fig. 1). However, the preparation had a positive effect on reproduction (mean 2.8 cocoons/ind.) and hatching (mean 0.7 cocoons/ind.) as compared with diazinon (Table 1).

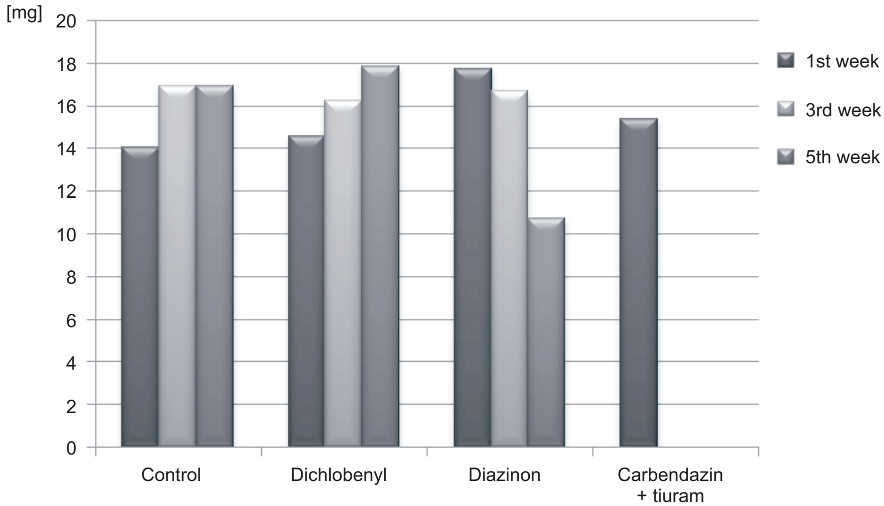

Fig. 1. The effect of active substances from plant protection products on the biomass of California earthworm [mg]

Statistical analysis showed significant differences in the number of laid cocoons and hatched young earthworms between treatments with various active substances from plant protection chemicals ($p < 0.05$).

Discussion

Reproductive abilities and weight loss of earthworm's body is largely affected by the class of toxicity of a given pesticide and the time of its persistence in the soil. *Lumbricus terrestris* treated with diazinon (from Basudin 10G) by Kostecka and Wojcikiewicz [8] first decreased their body weight but later an increase in animal weight was observed. In our experiment, however, systematic loss of the body weight was observed regardless of the treatment (Table 1). This was caused by permanent contact of the California earthworm *E. fetida* with active substances and by application of the latter in dissolved forms. Reproductive potential of animals is used as a response indicator. Pesticide treatment first of all decreased population density since applied preparations made earthworms produce less cocoons (Fig. 1, Table 1). The time of production of one cocoon under such circumstances is longer [9]. Many studies on the effect of various insecticides, fungicides or herbicides demonstrated remarkable influence of these chemicals on various species of soil oligochaetes [10–13]. Zablocki et al [14] showed that preparations persisting long in the soil at high concentrations exerted stronger effects. Earthworms being soil animals of limited ability to migrate are sensitive to various chemicals [1, 3].

An important aspect of the studies on mesofauna is to estimate how the chemicals introduced to the environment during agrotechnical works affect mortality and, first of all, an ability to reproduce and migrate [7, 10, 11].

Summary

Plant protection chemicals (dichlorobenyl, diazonon and carbendazin with tiuram) applied to soil showed significant impact on mortality, biomass and reproduction of the California earthworm *E. fetida*. These substances acted both externally and internally causing burn of the body walls and partial paralysis of earthworms. Body weight loss and the flow of coelomic fluid of the body segments were also observed.

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WPŁYW AKTYWNYCH ZWIĄZKÓW ŚRODKÓW DOGLEBOWYCH NA DŻDŻOWNICĘ KALIFORNIJSKĄ *Eisenia fetida*

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Abstrakt: Celem przeprowadzonych badań było określenie wpływu wybranych środków ochrony roślin doglebowych na dżdżownicę kalifornijską *E. fetida* na jej zdolność do przeżywania w środowisku oraz reprodukcję. Pod uwagę wzięto takie parametry jak:

1) zmiany w liczebności po zastosowaniu środka chemicznego w stosunku do kontroli (śmiertelność, wpływ na biomasę, ograniczenie aktywności życiowej),

2) liczbę składanych kokonów i wylęg.

Jako materiał chemiczny wybrano związki ochrony roślin zawierające następujące substancje czynne: dichlobenyl (herbicyd), diazon (insektycyd) oraz karbendazyn i tiuram (fungicyd).

Reakcja dżdżownic na zastosowane pestycydy doglebowe była różna. Zaobserwowano znaczne zmniejszenie masy ciała po wpływem diazynonu. Najbardziej toksyczny był fungicyd, który spowodował 100 % śmiertelność *E. fetida* po 48 godz. kontakcie.

Słowa kluczowe: *E. fetida*, gleba, pestycydy

ECOLOGICAL CHEMISTRY AND ENGINEERING A

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BIOMONITORING OF HEAVY METALS IN THE BIESZCZADY NATIONAL PARK USING SOIL AND *Fagus sylvatica* L. LEAVES

BIOMONITORING METALI CIĘŻKICH W BIESZCZADZKIM PARKU NARODOWYM PRZY UŻYCIU GLEBY I LIŚCI *Fagus sylvatica* L.

Abstract: The investigation of zinc, cadmium, lead and copper content was carried out in the soil (from the level 0–10 cm) and in the leaves of European beech (*Fagus sylvatica* L.) in the Bieszczady National Park.

Samples of soil and plants material were collected in 2007 from 63 selected sites. The level of Zn, Cd and Cu in the plants leaves and soil was below the values considered as toxic levels for the protected area. The lowest and the average (4,4 μg/g d.m.) concentrations of copper in the leaves of *Fagus sylvatica* L. were definitely lower than the normal level, which indicated a deficiency of that element. In the analyzed soil samples it was noted that the concentration of Pb (mean 31 μg/g d.m.) which as higher than the normal level for the protected area.

Keywords: heavy metals, *Fagus sylvatica* L., Bieszczady National Park

National parks usually include well-preserved and unspoilt parts of nature, shaped by the specificity of particular environment and adapted to it in the long process of evolution [1].

Forest ecosystems, including protected reserves, are subject to different dangers whose scale increases all the time [2]. Nowadays some disadvantageous changes can be observed in the protected parts [3]. They are caused by various factors. The most important is the industrial pollution, above all heavy metals and sulfur, which affect the ecosystems over a long period of time.

75 % of Polish parks are situated in the threatened areas, ie where the environment contamination by the toxic metals and gases repeatedly exceeds the standards for the protected areas [4]. One of them is the Bieszczady National Park, which protects the nature of the Eastern Carpathians.

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European beech (*Fagus sylvatica* L.) in the Bieszczady National Park is the most frequent and common species. Next to oaks, it is the most important deciduous species which contributes to the forest expansion. The chemical analysis of the assimilation apparatus and the bark of European beech (*Fagus sylvatica* L.) is often used in the biomonitoring of the environment [1, 5, 6].

The objective of this elaboration was to determine a load factor of the chosen heavy metal (Zn, Cu, Pb, Cd) in the Bieszczady National Park on the basis of a chemical analysis of the upper layer of the soil as well as the assimilation apparatus of European beech (*Fagus sylvatica* L.)

Material and method

The investigation was carried out in the soil and leaves of European beech (*Fagus sylvatica* L.) in the Bieszczady National Park. Samples of soil (from the level 0–10 cm) and leaves of European beech (*Fagus sylvatica* L.) were collected from 63 previously set stands in the whole area of the park in the vegetation season of 2007. On the map of the Bieszczady National Park (Fig. 1) marked location research points in the Bieszczady National Park. In order to determine the heavy metals concentration, plant material was dried in 105 ^oC to a constant weight, ground to powder, then mineralized and dissolved in 10 % HNO_3. After filtration Cd, Pb, Zn, Cu and Fe content was determined using flame *Atomic Absorption Spectrometry* (AAS) [7]. Soil was air dried and extracted with 10 % HNO_3 then measured using the conventional Atomic Absorption Spectrometry (AAS) [7].

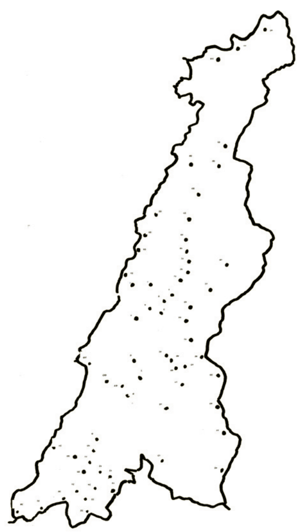

Fig. 1. Location of research points in the Bieszczady National Park

The quality of the analytical procedures was controlled using the reference material (Certified Reference Material CTA – OTL – 1 Oriental Tabacco Leavs). Figures 2–7 were drawn with the use of Surfer 8 program.

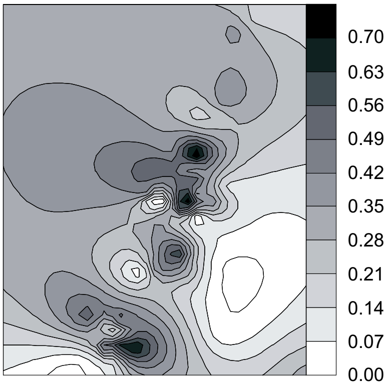

Fig. 2. Distribution of Pb concentrations in beech leaves from BdNP [µg/g]

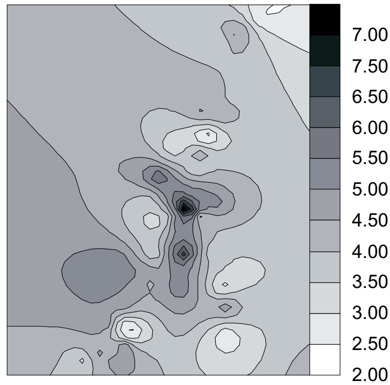

Fig. 3. Distribution of Cu concentrations in beech leaves from BdNP [µg/g]

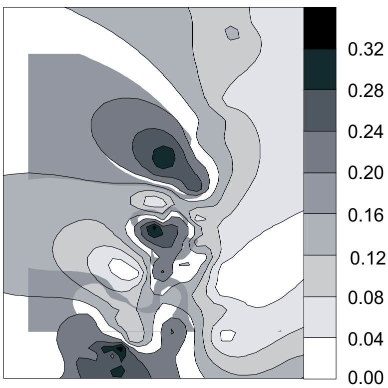

Fig. 4. Distribution of Cd concentrations in beech leaves from BdNP [µg/g]

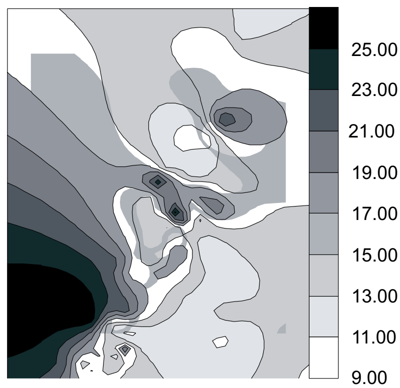

Fig. 5. Distribution of Zn concentrations in beech leaves from BdNP [µg/g]

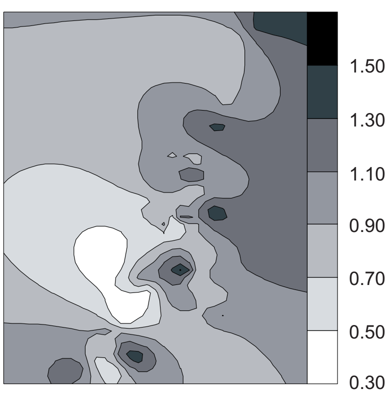

Fig. 6. Distribution of Cd concentrations in soil from BdNP [µg/g]

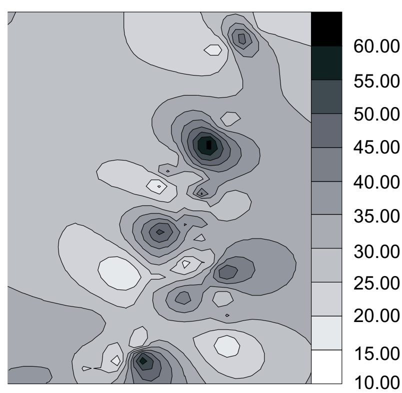

Fig. 7. Distribution of Pb concentrations in soil from BdNP [µg/g]

Results and discussion

The content of heavy metals in the leaves of European beech (*Fagus sylvatica* L.) and in the top layer of soil was presented by means of isolines at the Figs. 2–7. The lowest values of concentration were marked with the bright color and the highest with the dark one.

The average zinc concentration in the soils from different countries is within the limits of 30–120 μg/g. The average zinc content for the non-polluted soils in Poland is 40 μg/g [8]. The permitted content of zinc in the soils of protected areas amounts to 100 mg/kg.

In the investigated soil material the content of zinc was within the range of 7.54–26.7 μg/g. The average concentration of zinc in the Bieszczady National Park was 13.7 μg/g [9].

In the top layer of soil in the Roztocze National Park Rycman noted 3.10–6.85 μg Zn/g [10]. Kuc found the content of zinc in the Tatra National Park within the range 15.90–150.47 μg/g. In the Slotwina reserve in the Podkarpacie Region the average content of zinc was 3 μg/g. Ciepal and Lipka [11] found 350 μg Zn/g in the surface layer of soil in the Gora Chelm Reserve and in the Smolen Reserve 200 μg Zn/g. Lukasik [5] it the upper level of soil in the Parkowe Reserve found that an average concentration of zinc equals 85 μg/g. On Babia Gora the content of zinc was from 105.0 to 215 μg/g and on Mt Pilsko 185.0–325.0 μg/g [12].

The obtained results concerning zinc contents in the soil of the Bieszczady National Park are within the average content of zinc for the non-polluted areas and the permitted zinc content was not exceeded.

In case of plants, zinc is necessary for proper growth. To cover physiological requirements of plants, its concentration in the leaves at the level of 15–30 μg/g d.m. is sufficient and in the aboveground parts of a plant, which are not affected by pollution, it should be around 10–70 μg/g [8].

Zinc concentrations in the examined leaves are within the range of 8.51–32.1 μg/g [9].

In the area of the Babia Gora National Park Sawicka-Kapusta noted content level of zinc 15–30 μg/g [13]. Ciepal observed the content of zinc in the reserve of the Beskid Slaski within the range of 95.5–170.4 [1], and Lukasik determined the zinc concentrations in the area of Wyzyna Slasko-Krakowska at 33–99 μg/g [5].

The content of zinc in European beech (*Fagus sylvatica* L.) leaves from the Bieszczady National Park area does not exceed the norms and even shows zinc deficiency.

The average content of cadmium in the soils of Poland amounts to 0.2 μg/g [8].

In the top layer of soil in the Bieszczady National Park the content of cadmium varied from 0.13–1.75 μg/g and slightly exceeded permitted concentration of this element in the soil in the protected areas.

The results of the soil analysis carried out in this research concerning the concentration of cadmium are comparable with the results obtained by Rycman in the Roztocze National Park (0.9 μg/g) [10] and Kuc in the Tatra National Park (1.12–2.83 μg/g) [14].

Kimsa and others [15] determined in the surface layer of soil in the the Swietokrzyski National Park a cadmium concentration amounting to 2.7–3.4 μg/g. Ciepal and others [16] noted 2.0 μg/g of Cd in the top layer of soil in the Bukowica Reserve, whereas in the Lipowiec Reserve it was 4.0 μg/g of Cd. Ciepal and Lipka [11] present the cadmium content in the top layers of soil in the Smolen Reserve at the level of 17.0 μg/g and for Gora Chelm 16 μg/g. Lukasik [5] noticed the concentration of Cd in the soil of the Parkowe Reserve at the level of 1.5 μg/g.

In the case of plants, cadmium is considered highly toxic. Sawicka-Kapusta [13] presents a range of cadmium occurrences in plants from not contaminated areas: 0.12–0.5 μg/g d.m., whereas Kabata-Pendias and Pendias [8] state that in such areas content of cadmium does not exceed 1 μg/g d.m. Content of cadmium at the level of 5–10 μg/g d.m. for susceptible plants and 10–30 μg/g d.m. for resistant plants is considered phytotoxic [17].

The average content of cadmium for the investigated area was 0.2 μg/g d.m. [9].

Szarek and others [18] discovered the average amount of cadmium 2.6 μg/g d.m in plants of ground cover in the beech forest in the area of the Ojcow National Park. Ciepal and Rycman in an analogous material from the Roztocze National Park noted that the content of cadmium was within the range from 0.6 to 1.05 μg/g d.m. [19]. The obtained results indicate that content of cadmium in European beech (*Fagus sylvatica* L.) leaves does not exceed the level characteristic for plants from non-polluted areas.

Copper belongs to the biogenic group of elements used by plants in many metabolic traces [8]. In the upper layer of Polish soils copper total is within the range 1–60 μg/g and usually it amounts to 10–30 μg/g [20].

Content of Cu in investigated soil was 1.99–11.43 μg/g.

The average, physiological content of copper in the leaves of different species is at the level of 5–30 μg/g d.m.

The concentrations of copper in the analysed samples are within the normal ranges from 1.97–8.9 μg/g [9].

Witkowski determined in the Bieszczady National Park the averange content of copper at the level 3–4 μg/g [6]. In another protected area of Poland Rycman described the range of copper occurrences in plants from the Roztocze National Park at 7.25–11.62 μg/g [10]. In the examined plant material, concentrations of copper do not exceed the permitted values and in some part of the Bieszczady National Park present even a deficiency of copper in the assimilation apparatus of European beech (*Fagus sylvatica* L.).

Similarly to the case cadmium, lead is also considered a very toxic element. Kabata-Pendias and Pendias [8] describe an average content of lead for soils of Poland not exceeding 20 μg/g. Permitted concentration of Pb in soils for the protected area on the basis of law regulations regarding protection of nature amounts to 50 μg/g.

Kimsa and others [14] determined in the surface layer of soil in the Swietokrzyski National Park lead concentration amounting to 18.0–19.0 μg/g. Ciepal informs that this value for the protected areas in Silesia and Malopolska is 60–350 μg/g [1].

The obtained results concerning the lead contents in the upper layers of soil in the Bieszczady National Park are between 11.8–66.7 μg/g, which indicates that the permitted content of lead for the protected areas was exceeded.

The regular content of lead in plants varies from 5 to 14 μg/g d.m., and the amount of 30 μg Pb/g d.m. is considered toxic [8].

The concentration of lead in the analyzed material falls between 0.01–0.9 μg/g [9].

Witkowski in the Bieszczady National Park found content of lead amounting to 6–7 μg/g [6]. Rycman for the Roztocze National Park noted it as ranging from 0.25 to 1.25 μg Pb/g [10].

The results concerning lead content in European beech (*Fagus sylvatica* L.) leaves from the Bieszczady National Park indicate an insignificant threat of this metal.

Conclusion

The results obtained in the research show that there is no excessive heavy metals load in the Bieszczady National Park. The values of the investigated heavy metals concentration are different depending on the region of the Park. However, their concentrations in the upper layer of soil and in the leaves of European beech (*Fagus sylvatica* L.) in most cases were lower than the average values noted in the sources and the values permitted for the protected areas. Only lead displays excessive concentration in the soil, which indicates that the soil is polluted with this metal.

On the basis of this research investigating the content of heavy metals in the soil and in the leaves it can be stated that the Bieszczady National Park is a good control stand for such studies.

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BIOMONITORING METALI CIĘŻKICH W BIESZCZADZKIM PARKU NARODOWYM PRZY UŻYCIU GLEBY I LIŚCI *Fagus sylvatica* L.

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Abstrakt: Przeprowadzono badania dotyczące zawartości metali ciężkich (Zn, Cd, Cu i Pb) w liściach *Fagus sylvatica* L. i w wierzchniej (0–10 cm) warstwie gleby na terenie Bieszczadzkiego Parku Narodowego.

Próbki gleby oraz materiał roślinny został zebrany w sezonie wegetacyjnym 2007 z 63 wyznaczonych wcześniej stanowisk. Zawartości Zn, Cd i Cu w liściach i glebie nie przekraczały dopuszczalnych norm dla terenów niezanieczyszczonych. Najniższe i średnie (4,4 μg/g s.m.) stężenie miedzi w liściach *Fagus sylvatica* L. jest zdecydowanie niższe niż stężenie określane jako „normalne", co świadczy o niedoborze tego pierwiastka. Stwierdzono przekroczenie (śr. 31 μg/g s.m.) dopuszczalnego poziomu Pb dla terenów chronionych analizowanych próbkach gleby.

Słowa kluczowe: metale ciężkie, *Fagus sylvatica* L., Bieszczadzki Park Narodowy

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Małgorzata KŁYŚ[1]

EMIGRATION ACITIVITY OF RICE WEEVIL *Sitophilus oryzae* L. (*Coleoptera, Curculionidae*) IN CONDITIONS OF REDUCED TEMPERATURE

AKTYWNOŚĆ EMIGRACYJNA WOŁKA RYŻOWEGO *Sitophilus oryzae* L. (*Coleoptera, Curculionidae*) W WARUNKACH OBNIŻONEJ TEMPERATURY

Abstract: The subject of the research was rice weevil *Sitophilus oryzae* L. – dangerous pest of grain crops storage. Research was held in a laboratory at the temperature 31 °C, optimal for this insect species, and reduced to 22 °C, and 70 ± 5 % relative humidity (R.H.). The main research problem concerned the emigration activity of *S. oryzae* in conditions of temperature reduction. Population dynamics and sex structure of population were also analyzed. It was affirmed that rice weevil shows a very high emigration activity in temperatures both 31 °C and 22 °C. Whereas the temperature reduction to 22 °C causes drop of emigration activity to 40 % only in two time ranges after 60 and 120 days of raise ing. A higher female emigration was also observed.

Keywords: *Sitophilus oryzae* L., emigration activity, population dynamics, sex ratio

Each population during its lifetime is a subject of fluctuations in number and rate and intensity of changes depend on biological characteristic of species, environmental factors and also development stage of the population. Environmental factors which influence a the population numbers are mainly the temperature and humidity. They determine the rate and process occurring in the population and also influence the possibility of its occurrence, reproduction and survival.

Research included rice weevil *Sitophilus oryzae* L. – an insect which in the mild climate lives mainly in granary and storehouse groceries. In countries of the subtropical and tropical climate it is a dangerous pest of grain crops. It is brought to Poland mainly with rice and corn grains and is passively spread with the infected products [1]. The main target of presented research was learning about the emigration activity of population of *S. oryzae* in conditions of the reduced temperature in relation to optimal

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for developing of this insect species. Furthermore dynamism of number and sex structure of the population were analyzed.

Materials and methods

Research was carried out in laboratory conditions at the temperature 31 °C (known as optimal for this species) later reduced to 22 °C and at relative humidity about 70 %. A set of experimental raising vessels was used, allowing emigration of *S. oryzae* adult individuals. The set consisted to two plastic vessels. 40g of wheat was placed in a bottom of the smaller vessel, with a diameter of 28 cm^2 in the presence of 40 adult weevils of the same age, obtained from the basic cultures. The vessel was placed in to a bigger one with 50 cm^2 bottom surface. The bottom of the big vessel was covered with glycerine layer, which was a trap for insects emigrating from the small vessel. The inner vessel was closed with perforated lid, which allowed the air to come in [2]. The experiment lasted seven months and was repeated six times. The influence of the reduced temperature on migration activity of *S. oryzae* population was estimated according to analysis of the population number, migration rate and sex ratio. Analysis of statistic indexes was conducted using Chi-square test from Statistica 8.0 program.

Results and discussion

In applied environmental conditions allowing one way movement of *S. oryzae* individuals shows a very high emigration activity both at the temperature 31 °C and at 22 °C. A very high emigration takes place in optimal for this insect thermal-humidity space conditions ie at the temperature of 31 °C and *relative humidity* (RH) 70 % it is 97 % as early as after 30 days of the test. This leads to disappearance of the population in a small vessel as early as after 60 days of the culturing. By that time every insect has emigrated from the initial population. However, at the temperature of 22 °C emigration of initial population does not lead to total leaving the mother vessel. In spite of 100 % emigration after 30 days the experiment duration, insect eggs are laid in wheat seeds in small vessel and the population spreads although its number is low. However the number of emigration groups is high, mainly in time range from 150 to 210 days of raising. That time emigration index is about 90 %. The temperature reduction to 22 °C causes lower emigration activity to 40 % within two time ranges after 60 and 120 days of raising. However, in the other time ranges emigration keeps on a high level (Fig. 1, Table 1). Chi-square test analysis shows that the difference between emigrant number at the temperature of 31 °C comparing with emigrant number at the temperature of 22 °C are statistically significant. Only after 60 days the results were statistically insignificant.

Beckett et al [3] studied *R. dominica* and *S. oryzae* mortality at moderate temperature. The data obtained indicate that all life cycle stages survive longer at a given temperature as grain moisture increases and the effect of moisture on survival increases as temperature decreases.

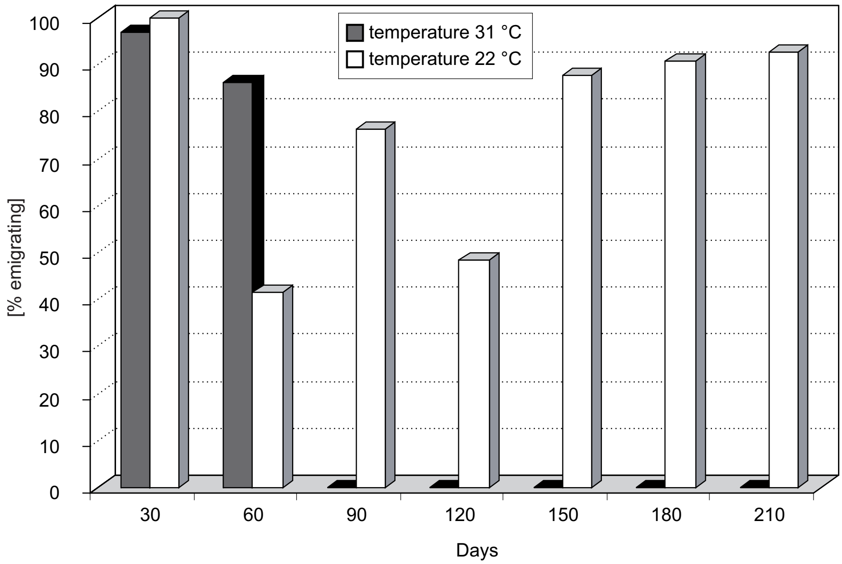

Fig. 1. Emigrational activity of the population *S. oryzae*

Table 1

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

Temperature 31 °C					Temperature 22 °C			
Days	Initial population	SD	Emigration groups	SD	Initial population	SD	Emigration groups	SD
30	0	0	38.7	1.9	0	0	49	1.9
60	0	0	25.3	1.5	32	4.7	22.7	2.8
90	0	0	3.3	1.7	19	6.4	61	5.6
120	0	0	0	0	33.3	0.9	32.3	4.7
150	0	0	0	0	44.7	4.8	350.3	34.7
180	0	0	0	0	34.7	4.0	370	27.4
210	0	0	0	0	44	6.1	568	43.6

SD – standard deviation.

In turn Ciesielska [4] testing emigration process of *Sitophilus oryzae* L., *Sitophilus granarius* L. and *Oryzaephilus surinamensis* L. at the temperature 27 °C affirmed a big migration activity of this species but only at the first period of the colonisation of grain.

Also Kłyś [5] testing influence of the temperature reduced to 22 °C on Rhizopertha dominica F. migration activity shows increase in emigration of this pest during the first four months of the research. That time insects intensively move outside the area of the initial population in spite of the food excess.

Table 2

Sex ratios in emigratin condition of *S. oryzae*

Days	Temperature 31 °C		Temperature 22 °C	
	Initial population	Emigration groups	Initial population	Emigration groups
30	0	0.5	0	0.9
60	0	1.1	1	0.6
90	0	2	1.1	0.5
120	0	0	1.3	0.9
150	0	0	1.5	0.7
180	0	0	1.1	0.9
210	0	0	1	0.8

One of the mechanisms controlling population number is its sex structure. It has an influence on a rate of reproduction and on a population number [6]. Results which were gained in research on population of *S. oryzae* sex structure show a very high emigration activity of the females confirmed by the sex ratio < 1 (Table 2). Ciesielska [7] affirmed that *S. oryzae* females are twice as active as males and more likely that female will infect stored grain.

Conclusions

1. *S. oryzae* shows a very high emigration activity both at the temperature of 31 °C and at 22 °C.

2. The temperature reduction to 22 °C causes reduction of the emigration activity to 40 % only in two time ranges after 60 and 120 days of the conduct culture.

3. At the temperature of 22 °C females show a very high emigration activity.

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AKTYWNOŚĆ EMIGRACYJNA WOŁKA RYŻOWEGO
***Sitophilus oryzae* L. (*Coleoptera, Curculionidae*)**
W WARUNKACH OBNIŻONEJ TEMPERATURY

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Abstrakt: Obiektem badań był wołek ryżowy *Sitophilus oryzae* L. – groźny szkodnik magazynowanego ziarna zbóż. Badania prowadzono w laboratorium w temperaturze 31 °C optymalnej dla tego gatunku owada i w obniżonej do 22 °C, oraz w wilgotności względnej powietrza 70 ± 5 % r.h. Główny problem badawczy dotyczył aktywności emigracyjnej wołka ryżowego w warunkach obniżonej temperatury. Analizowano także dynamikę liczebności i strukturę płciową populacji *S. oryzae*. Stwierdzono, że wołek ryżowy wykazuje bardzo dużą aktywność emigracyjną zarówno w temperaturze 31 °C, jak i 22 °C. Obniżenie temperatury do 22 °C powoduje spadek aktywności emigracyjnej do 40 % tylko w dwóch przedziałach czasowych po 60 i 120 dniach prowadzenia hodowli. Zaobserwowano również większą emigrację samic.

Słowa kluczowe: wołek ryżowy, aktywność emigracyjna, liczebność populacji, wskaźnik płci

Vol. 18, No. 5–6 2011

Józef KOC[1] and Marcin DUDA[1]

ROLE OF THE RETENTION RESERVOIR IN SZABRUK FOR THE CHLORINE ION MIGRATION FROM ITS AGRICULTURAL CATCHMENT

ZNACZENIE ZBIORNIKA RETENCYJNEGO SZĄBRUK W MIGRACJI JONÓW CHLORU ZE ZLEWNI ROLNICZEJ

Abstract: The role of a retention reservoir in chlorine migration from an agricultural catchment area was analyzed during the hydrological years 2005/2007. The investigated retention reservoir is situated in a valley, in the lower course of the Szabruk stream flowing into Lake Wulpinskie located in north-eastern Poland, in the Olsztyn Lakeland mesoregion. The chlorine content of water evacuated from the catchment was determined in the range of 3.0 mg Cl · dm^{-3} to 43.0 mg Cl · dm^{-3}, and it was determined by the type and intensity of catchment use. The highest chlorine levels were noted in agricultural catchments connected to a drainage network (20.6 mg Cl · dm^{-3} on average), lower concentrations were found in farming areas drained via ditches (11.4 mg Cl · dm^{-3}), while the lowest Cl content of water was determined in outflows from afforested catchments (5.3 mg Cl · dm^{-3} on average). Chlorine concentrations were lower in the growing season in all studied catchment types. The chlorine load evacuated from the catchment was determined by the type of catchment use. The greatest chlorine loss per hectare of the catchment area was noted in the agricultural catchment connected to a drainage network (13.8 kg Cl · ha^{-1} · $year^{-1}$), a smaller Cl load was evacuated from the catchment drained via ditches (6.2 kg Cl · ha^{-1} · $year^{-1}$), while the smallest loss was observed in the afforested catchment (4.1 kg Cl · ha^{-1} · $year^{-1}$). Chlorine concentrations increased by 10 %, from 8.2 mg Cl · dm^{-3} to 9.0 mg Cl · dm^{-3} following the passage of the stream's waters through the retention reservoir. The above resulted from the inflow of drainage water with a high chlorine content as well as higher Cl concentrations due to evapotranspiration. The chlorine content of water remained unchanged after the Szabruk stream passed through the retention reservoir and the band ditch, indicating that chlorine is a good tracer of water movement through drainage facilities.

Keywords: retention reservoir, agricultural catchment area, chlorine

In nature, chlorine is found primarily in the reduced form of the chloride anion (Cl^-), and a naturally occurring form of potassium perchlorate ($KClO_4$) has been determined only in the Chile saltpetre. Chloride is abundant in living organisms and it is the main mineral anion in plants [1]. There are very few publications addressing the effect of

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chloride on water quality. Most studies investigating water pollution with mineral components in rural areas focus on nitrogen compounds and phosphates. The absence of reports discussing the role of chloride hinders the determination of the origin of polluting substances, it prevents making pollution forecasts and taking adequate recovery measures. The chloride content of water is an important indicator of water quality. Chloride ions differ significantly from nitrates, ammonium and phosphates. Chlorides do not undergo change in soil and water, they are not absorbed by soil material or bottom deposits, therefore they remain fully soluble in water and soil solutions. Chlorides are absorbed by plants and they are easily leached from the soil, including surface runoffs, which makes them a natural tracer of water and water-borne substances [2].

In rural areas, chlorides reach water bodies from various sources, mainly human settlement, farming production and precipitation. Introduced in the form of kitchen salt, chloride is an indispensable component of food products. The average daily consumption of chloride reaches 6–12 g, of which only up to 1 % is ingested with water [3]. Chlorides found in detergents are evacuated nearly in their entirety with household sewage water, while chlorides in unconsumed food and other types of household waste are deposited in dumps. Sodium chloride and calcium chloride are used in road thawing. Road-side snow contains up to 4000 mg Cl $\cdot$ m^{-3} on average [3]. Farming production is a major source of chloride that reaches water resources. Potassium fertilizers are administered mostly in the form of potassium chloride. Kitchen salt is added to animal feed with other mineral supplements, it is also available in the form of salt licks in pastures. Every year, an estimated 180 000 Mg Cl are evacuated to soil in Poland. Organic fertilizers are also an abundant source of chloride. The average chloride content of slurry is 1040 mg Cl $\cdot$ dm^{-3} [4, 5]. Unused components of mineral and organic fertilizers are leached out from the soil, transported to the water-bearing horizon or directly to surface water. For this reason, farming production is an important determinant of the quality of surface and groundwater in rural areas [6, 7]. Chloride levels throughout the entire country are also dependent on atmospheric precipitation. In Poland, wet precipitation supplies approximately 12 kg Cl $\cdot$ ha^{-1} with average concentration of 3.12 mg Cl $\cdot$ dm^{-3} [8–10].

This study analyses the effect of catchment use on the chlorine content of water outflows and the role of a retention reservoir in chlorine migration to surface waters.

Examination methodology

The role of a retention reservoir in chlorine migration from an agricultural catchment area was analyzed during the hydrological years 2005/2006 and 2006/2007. The investigated retention reservoir is situated in a valley, in the lower course of the Szabruk stream in north-eastern Poland, in the Masurian Lakeland macroregion and the Olsztyn Lakeland mesoregion. The catchment area of the Szabruk stream consists of an agricultural and an afforested part. The afforested part with an area of 4.4 km^2 occupies 33 % of the total catchment area of 13.2 km^2 (Fig. 1). Household sewage from two wastewater treatment plants located in the nearby residential areas was discharged to the stream until June 2005. Wastewater was not evacuated to the stream during the experimental period. A retention reservoir with a total area of 24.80 ha and the maximum depth of 1.51 m is situated in the lower part of the Szabruk stream valley. The reservoir was built 25 years ago, it is enclosed by a dike and is equipped with an outlet box. In the western part, the reservoir is enclosed by a band ditch which controls water flow by evacuating excess water from the Szabruk stream. Outflows from the reservoir pass through the terminal section of the Szabruk stream to Lake Wulpinskie. The study covered the Szabruk stream, the inflows and outflows of the retention reservoir (Fig. 1) and two drained catchment areas.

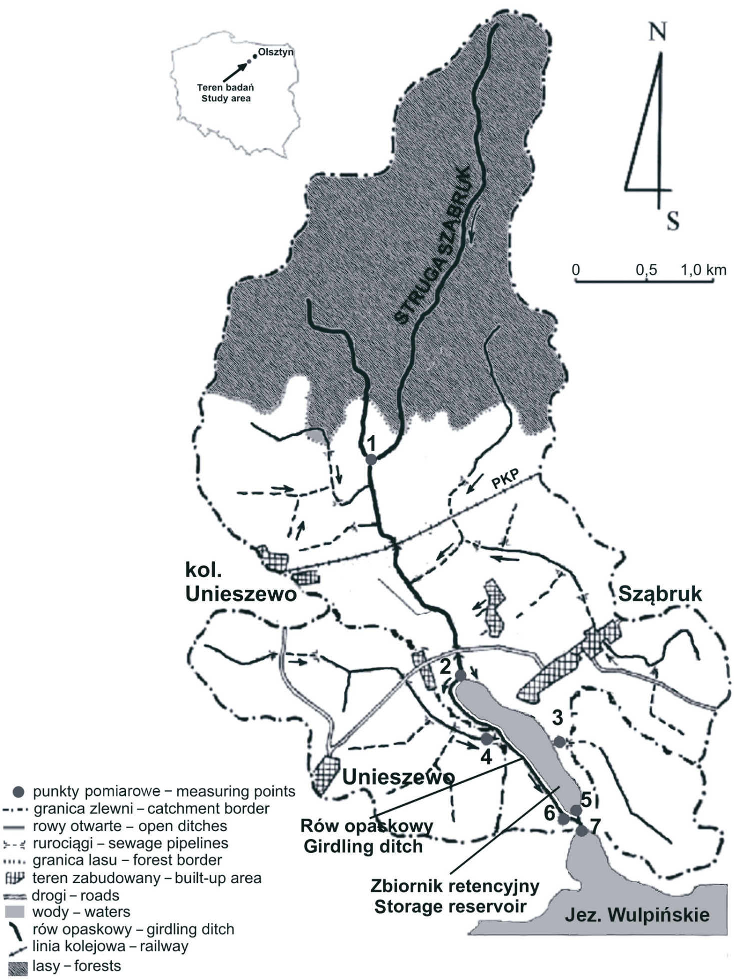

Fig. 1. Map showing the Szabruk creek catchment; 1 – Szabruk creek downstream of forest catchment, 2 – Szabruk creek upstream of storage reservoir, 3 – inflow from drained catchment to storage reservoir, 4 – inflow from drained catchment to girdling ditch, 5 – outflow from storage reservoir, 6 – outflow from girdling ditch, 7 – inflow to Wulpinskie Lake

The experiment involved hydrological measurements and laboratory analyses. The flows of the Szabruk stream were measured below the afforested catchment, at the reservoir inflow (outflow from the agricultural catchment, inflow to the retention reservoir and to the band ditch), at the drainage outflow to the band ditch, at the drainage outflow to the retention reservoir, at the outflow from the reservoir, at the outflow from the band ditch and at the inflow to Lake Wulpinskie. Flow measurements were performed weekly with the use of a VALEPORT electromagnetic flow-meter. The volumetric method was applied at low flow levels (below 2 $dm^3 \cdot s^{-1}$). Water samples for physical and chemical analyses were collected every two months at flow measurement points, and Cl^- levels were determined by the argentometric method in line with the generally observed standards [11].

The chlorine load evacuated from the catchment, supplied to and discharged from the reservoir was calculated by summing up the product of monthly flows and the corresponding chlorine concentrations. The growing season was the period from April to October.

Results and discussion

The results of the study, carried out from 2005 to 2007, showed significant variations in chlorine concentrations in different sections of the Szabruk stream, the inflows and outflows from the retention reservoir, the band ditch and the inflows to Lake Wulpinskie, subject to the type of catchment area (Table 1). High fluctuations in chlorine levels were determined in various periods of the study in the range of 3.0 mg $Cl \cdot dm^{-3}$ to 43.0 mg $Cl \cdot dm^{-3}$. Chlorine concentrations were always lower in the growing season than in the winter. The maximum Cl concentrations determined *per annum* were twice higher than the lowest chlorine levels noted in each catchment. The lowest chlorine content was reported in the Szabruk stream, in the outflow from the afforested catchment, in the range of 3–7 mg $Cl \cdot dm^{-3}$, with an average of 5.3 mg $Cl \cdot dm^{-3}$. Chlorine concentrations were lower in the summer (3–5 mg $Cl \cdot dm^{-3}$) and higher in the winter (5–7 mg $Cl \cdot dm^{-3}$). The above findings are similar to the average chlorine concentration determined in European rivers in 1959 at 6.9 mg $Cl \cdot dm^{-3}$ [12] which was adapted as the background concentration for fresh water resources [2]. Higher chlorine levels were observed in runoffs from farming areas at 11.4 mg $Cl \cdot dm^{-3}$ on average, leading to an increase in average Cl concentrations to 8.2 mg $Cl \cdot dm^{-3}$ in the stream's outflow from the combined afforested and agricultural catchment.

Significant seasonal variations in the chlorine content of water were found. Average concentrations in the summer were 20 % lower than in the winter and reached 7.2 mg $Cl \cdot dm^{-3}$. Higher Cl levels in the runoffs from agricultural catchments could be due to the use of mineral and organic fertilizers. Chlorides not absorbed by plants move deeper into the soil profile and reach surface waters. High Cl concentrations in the range of 12 to 43 mg $Cl \cdot dm^{-3}$, with an average of 20.6 mg $Cl \cdot dm^{-3}$, were reported in drained catchments which directly supply the retention reservoir and the band ditch. The highest chlorine levels were determined in the drainage ditch feeding into the band ditch around farm buildings where Cl concentrations were five times higher than in the outflows from the afforested catchment and twice higher than in the drained farming area. The above indicates that fertilizer storage, human settlement and animal production significantly contribute to chlorine pollution of water. An insignificant increase in Cl levels was noted after the passage of water through the retention reservoir, from 8.2 mg Cl · dm^{-3} at the inflow to 9.0 mg Cl · dm^{-3} at the outflow. The above increase resulted from the influx of drainage water with higher Cl concentrations as well as deposition due to atmospheric precipitation. Evapotranspiration could also be a potential cause of raised chlorine levels [2].

Table 1

Means of concentrations of chlorine in chosen points of catchment

Measuring points	Cl [mg · dm^{-3}]					
	Hydrological year 2005/2006 and 2006/2007		Outside vegetation period		Vegetation period	
	Mean	Ranges	Mean	Ranges	Mean	Ranges
Szabruk creek downstream of forest catchment	5.3	3.0–7.0	5.8	5.0–7.0	4.6	3.0–5.0
Szabruk creek upstream of storage reservoir	8.2	5.0–11.0	9.0	7.0–11.0	7.2	5.0–8.0
Inflow from drained catchment to storage reservoir	16.7	12.0–26.0	17.7	14.0–26.0	15.3	12.0–19.0
Inflow from drained catchment to girdling ditch	24.5	13.0–43.0	25.8	17.0–43.0	22.3	13.0–38.0
Outflow from storage reservoir	9.0	7.0–11.0	9.2	7.0–11.0	8.8	7.0–11.0
Outflow from girdling ditch	13.7	9.0–20.0	13.5	11.0–20.0	11.6	9.0–15.0
Inflow to Wulpinskie Lake	11.0	8.0–16.0	11.8	10.0–16.0	10.0	8.0–11.0

A comparison of water samples collected from the Szabruk stream after passage through the retention reservoir and through the band ditch indicates that chlorine concentrations were lower in the water evacuated from the reservoir than in the water flowing through the band ditch. The above was due to a high share of drainage water marked by the highest Cl concentrations in the catchment (24.6 mg Cl · dm^{-3}). Following the mixing of the outflows from the retention reservoir and the band ditch, the average chlorine content of water at the inflow to Lake Wulpinskie was 11.0 mg Cl · dm^{-3}.

Average chlorine concentrations and flow data gathered at various measuring sites were used to develop an annual chlorine balance in different parts of the Szabruk stream's catchment area (Table 2). The results of the study indicate that along a section stretching from the stream's source to a point situated above the retention reservoir, Szabruk's waters carried 5018 kg Cl · $year^{-1}$. The outflows from the afforested catchment carried 1823 kg Cl · $year^{-1}$, of which 56 % was evacuated in the growing season. The above findings supported the conclusion that the Szabruk stream collected 3195 kg Cl · $year^{-1}$ during its passage through the agricultural catchment. Annual chlorine outflows per hectare of the catchment area increased after the stream's waters had passed from the afforested catchment to farmed areas. Chlorine outflows from afforested catchments reached 4.1 kg Cl · year^{-1} and from agricultural catchments – 6.2 kg Cl · year^{-1}. The mixing of waters from farming and afforested areas resulted in average chlorine runoffs of 5.2 kg Cl · year^{-1}, including 52 % in the growing season. Outflows from drained catchments reached 13.8 kg Cl · year^{-1}, of which 62 % was evacuated during the summer drainage outflow. The total chlorine loss from the Szabruk stream catchment to Lake Wulpinskie was 6612 kg Cl · year^{-1}, with an average of 5.0 kg Cl · year^{-1}. A greater chlorine load (53 % of total load) was evacuated from the catchment in the growing season due to more intense outflows from the drainage network.

Table 2

Load of chlorine forms in chosen points of catchment

Measuring points	Total load [Cl kg · year^{-1}]			Unit load [Cl kg · ha^{-1} · year^{-1}]
	Hydrological year 2005/2006 and 2006/2007	Outside vegetation period	Vegetation period	Hydrological year 2005/2006 and 2006/2007
Szabruk creek downstream of forest catchment	1823	789	1024	4.1
Szabruk creek upstream of storage reservoir	5018	2402	2616	5.2
Inflow from Szabruk creek to storage reservoir	3381	1599	1782	—
Inflow from Szabruk creek to girdling ditch	1637	803	834	—
Inflow from drained catchment to storage reservoir (agricultural catchment)	404	170	234	11.2
Inflow from drained catchment to girdling ditch (agricultural catchment)	1173	451	722	16.4
Outflow from storage reservoir	3601	1606	1995	—
Outflow from girdling Ditch	3011	1539	1472	—
Inflow to Wulpinskie Lake	6612	3145	3527	5.0

The Szabruk stream was divided above the retention reservoir, as the result of which a part of its waters was fed directly to the reservoir, while the remaining flow was evacuated via the band ditch. According to the annual balance, the retention reservoir discharged 184 kg Cl · year^{-1} less chlorine than the inflow. Cl outflows in the band ditch were 201 kg Cl · year^{-1} higher than total inflows. The above results support the conclusion that approximately 5 % of water with dissolved chlorine percolated from the retention reservoir to the band ditch.

The calculated chlorine balance showed that its amount did not change after the passage with the flowing water along the retention reservoir. It results from little plant demand for the component as well 0.2–0.5 % dry mass [13] as the fact that it is not cumulated by vegetation. The vegetation did not accumulate chlorine in spite of a significant share of macrophytes and helophytes in the water area as well as a relatively high biomass production. Chlorine, in general, does not form undissolved salts. Thus, it is hardly deposited in bottom sediments. In the sediments of the studied reservoir, where 2500 Mg of sediment is accumulated, the chlorine deposition is 70 kg [14] estimated as 1 % of exported that from its catchment by 25 years.

Conclusions

1. The chlorine content of water evacuated from the catchment was determined in the range of 3.0 mg Cl · dm^{-3} to 43.0 mg Cl · dm^{-3}, and it was determined by the type and intensity of catchment use. The highest chlorine levels were noted in agricultural catchments connected to a drainage network (20.6 mg Cl · dm^{-3} on average), lower concentrations were found in farming areas drained via ditches (11.4 mg Cl · dm^{-3}), while the lowest Cl content of water was determined in outflows from afforested catchments (5.3 mg Cl · dm^{-3} on average). Chlorine concentrations were lower in the growing season in all studied catchment types.

2. The chlorine load evacuated from the catchment was determined by the type of catchment use. The greatest chlorine loss per hectare of the catchment area was noted in the agricultural catchment connected to a drainage network (13.8 kg Cl · ha^{-1} · $year^{-1}$), a smaller Cl load was evacuated from the catchment drained via ditches (6.2 kg Cl · ha^{-1} · $year^{-1}$), while the smallest loss was observed in the afforested catchment (4.1 kg Cl · ha^{-1} · $year^{-1}$).

3. Chlorine concentrations increased by 10 %, from 8.2 mg Cl · dm^{-3} to 9.0 mg Cl · dm^{-3} following the passage of the stream's waters through the retention reservoir. The above resulted from the inflow of drainage water with a high chlorine content as well as higher Cl concentrations due to evapotranspiration.

4. The amount of chlorine contained in the water of the Szabruk stream did not change after passing the reservoir and sourrounding ditch what is the evidence of the balance between the amounts of the ion uptaken by plants and deposited in bottom sediments and the amounts released from decayed material and sediments.

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ZNACZENIE ZBIORNIKA RETENCYJNEGO SZĄBRUK W MIGRACJI JONÓW CHLORU ZE ZLEWNI ROLNICZEJ

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Abstrakt: Badania nad znaczeniem zbiornika retencyjnego w określeniu migracji chloru ze zlewni rolniczej prowadzono w latach hydrologicznych 2005/2007. Do badań szczegółowych wytypowano zbiornik retencyjny położony w dolinie końcowego biegu strugi Sząbruk wpadającej do Jeziora Wulpińskiego, położonej w północno-wschodniej Polsce w mezoregionie Pojezierza Olsztyńskiego. W wyniku przeprowadzonych badań stwierdzono, stężenie chloru w wodzie odpływającej ze zlewni mieściło się w granicach od 3,0 mg Cl · dm^{-3} do 43,0 mg Cl · dm^{-3} i zależało od sposobu i intensywności jej użytkowania. Najwyższe stężenie chloru stwierdzono w wodzie zlewni rolniczych odwadnianych siecią drenarską (średnio 20,6 mg Cl · dm^{-3}), niższe z użytków rolnych odwadnianych rowami (11,4 mg Cl · dm^{-3}), a najniższe natomiast w przypadku odpływu ze zlewni leśnej (średnio 5,3 mg Cl · dm^{-3}). We wszystkich zlewniach cząstkowych mniejsze stężenia chloru stwierdzono w okresie wegetacyjnym niż poza nim. Ładunek chloru odprowadzany z obszaru zlewni był uzależniony od sposobu jej zagospodarowania. Największy odpływ chloru z jednostki powierzchni stwierdzono w zlewni rolniczej zdrenowanej (13,8 kg Cl · ha^{-1} · rok^{-1}), mniejszy ze zlewni odwadnianej rowami (6,2 kg Cl · ha^{-1} · rok^{-1}) i najmniejszy ze zlewni leśnej (4,1 kg Cl · ha^{-1} · rok^{-1}). W wyniku przepływu wody przez zbiornik retencyjny następowało podwyższenie w niej stężenia chloru o 10 %, z 8,2 mg Cl · dm^{-3} do 9,0 mg Cl · dm^{-3}, co było efektem zasilania wodami drenarskimi o wyższych stężeniach chloru i zatężenia roztworu w wyniku ewapotranspiracji.

Słowa kluczowe: zbiornik retencyjny, zlewnia rolnicza, chlor

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PHOSPHORUS FARMING OF MORPHOLOGICALLY DIFFERENT PEA (*Pisum sativum*) VARIETIES IN POTASSIUM DEFICIENCY SOIL CONDITIONS

GOSPODARKA FOSFOROWA ZRÓŻNICOWANYCH MORFOLOGICZNIE ODMIAN GROCHU (*Pisum sativum*) W WARUNKACH NIEDOBORU POTASU W PODŁOŻU

Abstract: In a two-year pot experiment the effect of potassium deficiency on the dynamics of the concentration and accumulation of phosphorus in two morphologically different pea varieties – Agra and Bursztyn – was investigated. The experiment was conducted in Mitscherlich pots, in an experimental greenhouse in IUNG-Pulawy. Potassium was applied in the following doses per pot: 0, 500 and 1500 mg K (as K_2SO_4). Phosphorus was applied in equal quantities per pot: 1000 mg P (as $NaH_2PO_4 \cdot 2H_2O$). Other components of mineral nutrients were applied in equal amounts to ensure proper growth and development of plants. Plants were harvested at 5 development stages, and divided into component parts. The percentage content of phosphorus in the dry weight of component parts was then determined. The potassium fertilization applied in the experiment resulted in differences in the concentration and accumulation of phosphorus in particular component organs and in whole plants at analyzed stages of tested pea varieties. Potassium deficiency limited plant growth, distribution and accumulation of phosphorus in comparison with plants which were optimally fertilized with K (the effect was stronger for Agra variety). Regardless of the dose of potassium, plants of Bursztyn variety accumulated more phosphorus and gave of larger biomass yield compared with plants of Agra variety.

Keywords: pea varieties, phosphorus farming, potassium deficit

Pea, like most legumes, has high nutritional requirements, among other things, in relation to phosphorus due to high N_2 symbiotic fixation processes [1–3]. The contents of this element in the various species of leguminous plants, plant parts and varieties is highly variable [4, 5]. There are no data from literature, or are very fragmented, in respect of phosphorus farming of morphologically different new pea varieties. There is also no data on the collection and management of this component in the above-mentioned pea varieties in potassium deficiency soil conditions. As it is known, most

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of the soils of our country have a relative shortage of K [6], so it seems an interesting issue. Some results of studies concerning the collection and management of potassium and yield of plants have already been published by the author [7]. The present work focuses on the phosphorus farming of two pea varieties.

The aim of this work is to investigate the state of phosphorus supply in morphologically different pea varieties and the phosphorus farming during growth, development and maturation of plants in potassium deficiency soil conditions.

Material and methods

The object of research were two morphologically different pea varieties: Bursztyn – a traditional form and Agra – semi-leafless with leaves partially changed at tendrils, growing in different conditions of potassium fertilization. The experiment was conducted in an experimental greenhouse in IUNG – Pulawy, during two years (1998 and 1999), in Mitscherlich pots filled with 6.5 kg of light soil, low in potassium and phosphorus. Seeds of peas were inoculated with *Rhizobium leguminosarum* biotype *viceae* and were sown in early April. Five plants were grown in each pot. Humidity was maintained at the ground level of 60 % field water capacity.

Potassium in the experiment was varied and was used in the following 3 doses per pot: 0, 500 and the optimum dose of K – 1500 mg K (K as K_2SO_4). Plants received equal amounts of phosphorus in all facilities, at a dose of 1000 mg P in pot (as $NaH_2PO_4 \cdot 2H_2O$). Half of the dose was given before sowing to the soil, and half at the 5–6 knots phase. Other components of the mineral nutrient were administered in equal amounts to ensure proper growth and development of plants. Soil reaction in pots was close to pH_1n_{KCl} 6.5.

Plants were collected at five dates in the following phases of development, as defined by BBA scale [8]: I – phase 9 knots (29), II – creation of flower buds (59), III – flowering (65), the development of pods (79), full maturity (95). Collected plant material was divided into organs, dried and weighed to determine dry matter and then analyzed including the content of P. Phosphorus was determined by flow spectrophotometry. Phosphorus determination was made in two parallel repetitions, the relative method error was within 5–8 %. Research results in this paper are averages of two years of research. Yields of dry mass of plants were subjected to statistical analysis using variance analysis Tukey test at the level $\alpha = 0.05$ and have been already published [7].

Results and discussion

The differential in the experiment of potassium fertilization led to differences in concentration and accumulation of phosphorus in various organs and whole plants, as analyzed in the test phases of growth of the pea varieties (Table 1 and 2). Phosphorus in young plants, in the early stages of growth and development of plants in both pea varieties regardless of feeding potassium, was collected in larger quantities and concentrations in roots than in the overground parts of plants. It was only in the generative phase, the phase of full maturity of seed that phosphorus accumulated more in seeds in relation to the roots, leaves and other organs. Location of phosphorus in bodies of peas was strictly dependent on the development phase and the physiological role of this component in the plant [9]. In the young plants to a large extent phosphorus is responsible for the nodule bacteria survival and N_2 symbiotic fixation, hence the high content of this component in the roots [2]. In mature plants the accumulated phosphorus was mainly concentrated in the seeds, at the expense of vegetative organs of which it was phased out (Table 1).

Table 1

Phosphorus content in particular organs of plants, in successive harvests of two varieties of pea in relation to potassium fertilization

Harvest*	Plant organ	Bursztyn variety			Agra variety		
		K dose [mg K · pot^{-1}]					
		0	500	1500	0	500	1500
		P content [% d.m.]					
I (29)	Leaves + stems	0.48	0.44	0.46	0.43	0.48	0.50
	Roots	0.88	0.89	0.86	0.61	0.67	0.65
II (59)	Leaves + stems	0.46	0.41	0.39	0.38	0.37	0.39
	Roots	1.12	1.05	0.98	0.83	0.90	0.91
III (65)	Leaves +stems	0.49	0.43	0.43	0.39	0.40	0.42
	Roots	1.10	1.10	0.91	0.94	0.99	0.96
IV (79)	Leaves + stems	0.30	0.28	0.30	0.32	0.34	0.38
	Flowers + pods	0.49	0.45	0.44	0.55	0.48	0.53
	Roots	0.83	0.75	0.74	0.97	0.87	0.79
V (95)	Leaves + stems	0.38	0.09	0.08	0.31	0.19	0.16
	Seeds	0.60	0.44	0.47	0.56	0.49	0.47
	Hulls	0.43	0.14	0.09	0.41	0.15	0.13
	Roots	0.54	0.27	0.33	0.35	0.30	0.38

* Harvest – development stage (the code according to BBA): (29) – 9 nod stage, (59) – bud formation, (65) – flowering, (79) – pod development, (95) – complete maturity.

Potassium deficiency in soil limited absorption of phosphorus from the soil only in older plants and, despite the high concentration of this component in different organs of plants, the total uptake of P in plants from these objects was lower in both pea varieties in relation to the plants optimally fertilized with that component. In mature plants, there were substantial differences in the concentration and accumulation of phosphorus between K deficiency objects and objects fertilized sufficiently with this component. Both pea varieties unfertilized with potassium (K = 0), despite the generally higher concentration of P in all organs, have accumulated much less of this component, especially in the seeds (Table 2) as compared with the plants optimally fertilized with potassium (K = 1500). Phosphorus in plants from these first objects (K = 0) remained in leaves, hulls and other organs. The shortage of potassium in the soil led to a change in the formulation of distribution and accumulation of phosphorus in plants, compared with plants fertilized optimally with K (objects fed 1500 mg K per pot). Seeds of Agra variety from the latter objects accumulated about 75 % and seeds of Bursztyn variety as much as 85 % of the total pool of phosphorus accumulated in the plant. In contrast plants from deficiency objects, especially where no potassium was given, accumulated significantly less of this element in the seeds, about 52 % (Agra) and only 32 % (Bursztyn) of the total pool of phosphorus accumulated in plants. According to Marshner [5] and Starck [9] in plants sufficiently fed with potassium processes of distribution and redistribution of P are more efficient and the main pool of phosphorus is accumulated in seeds at the expense of the removal of this element from leaves and other organs. P uptaken by plants growing in the potassium deficiency ground could not be sufficiently used as a shortage of K limits growth and yielding of plants [7, 10, 11].

Table 2

Accumulation of phosphorus in particular organs of plants, in successive harvests of two varieties of pea in relation to potassium fertilization

Harvest*	Plant organ	Bursztyn variety			Agra variety		
		K dose [mg K · pot^{-1}]					
		0	500	1500	0	500	1500
		P uptake [mg P · pot^{-1}]					
I (29)	Leaves + stems	41	39	35	18	24	23
	Roots	40	44	33	14	16	14
	Whole plant	81	83	68	32	40	37
II (59)	Leaves + stems	55	52	46	33	37	37
	Roots	57	59	51	32	36	34
	Whole plant	112	111	97	65	73	71
III (65)	Leaves + stems	84	96	84	46	54	57
	Roots	75	81	65	35	39	39
	Whole plant	159	177	149	81	93	96
IV (79)	Leaves + stems	103	109	151	64	93	89
	Flowers + pods	78	93	103	36	53	40
	Roots	59	65	85	47	50	44
	Whole plant	240	267	338	147	197	173
V (95)	Leaves + stems	131	32	30	55	38	37
	Seeds	106	276	368	95	172	181
	Hulls	48	16	13	19	8	8
	Roots	43	11	21	11	8	14
	Whole plant	328	334	432	180	226	240

* explanations under table 1.

The obtained values of P concentration in particular organs at the successive phases of plants development (from all objects) were in range of sufficient value for the pea [4, 12]. Similar results and values, in respect of faba bean, were acquired in their studies by Dietrych-Szostak et al [13] and Labuda [14].

Tested pea varieties showed significant differences in the size of the uptaken and accumulated phosphorus (Table 2). Mature plants of both pea varieties from K deficiency objects accumulated much less P as compared with plants fertilized optimally with potassium. Bursztyn variety, regardless of potassium fertilization, in all development phases accumulated much more P compared with Agra variety. This was due to, first of all, a significant difference in yield of dry weight of plants: traditional Bursztyn variety produced a much greater biomass and seed yield compared with the variety of "tendril-leaves" Agra [7].

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GOSPODARKA FOSFOROWA ZRÓŻNICOWANYCH MORFOLOGICZNIE ODMIAN GROCHU ZWYCZAJNEGO (*Pisum sativum*) W WARUNKACH NIEDOBORU POTASU W PODŁOŻU

Zakład Żywienia Roślin i Nawożenia
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Abstrakt: W dwuletnim doświadczeniu wazonowym badano wpływ niedoboru potasu na dynamikę pobierania i akumulacji fosforu roślin dwóch zróżnicowanych morfologicznie odmian grochu zwyczajnego: Agra oraz Bursztyn. Doświadczenie prowadzono w wazonach Mitscherlicha, w hali wegetacyjnej IUNG-PIB w Puławach. Potas zróżnicowano i zastosowano, w przeliczeniu na wazon, w 3 dawkach: 0, 500 i 1500 mg K (w K_2SO_4). Natomiast fosfor rośliny otrzymały w jednakowych ilościach we wszystkich obiektach, w dawce 1000 mg P/wazon (w postaci $NaH_2PO_4 \cdot 2H_2O$). Pozostałe składniki pożywki mineralnej podawano w jednakowych ilościach zapewniających prawidłowy wzrost i rozwój roślin. Rośliny zbierano w 5 fazach rozwojowych, dzielono na organy i w ich suchej masie oznaczano procentową zawartość fosforu. Zastosowane w doświadczeniu nawożenie potasem prowadziło do różnic w koncentracji i akumulacji fosforu w poszczególnych organach i w całych roślinach, w analizowanych w fazach wzrostu badanych odmian grochu. Niedobór potasu ograniczał wzrost roślin, dystrybucję i akumulację fosforu, w porównaniu z roślinami optymalnie nawożonymi K, bardziej u odmiany Agra. Niezależnie od dawki potasu, rośliny odmiany Bursztyn nagromadziły większą ilość fosforu i wydały większy plon biomasy w porównaniu do roślin odmiany Agra.

Słowa kluczowe: odmiany grochu, gospodarka fosforowa, niedobór potasu

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INFLUENCE OF MINE WATER ON WATER QUALITY OF PELCZNICA RIVER

WPŁYW WÓD KOPALNIANYCH NA JAKOŚĆ WÓD RZEKI PEŁCZNICY

Abstract: Pełcznica River is the right-bank tributary of the Strzegomka River. There is an inactive mine nearby which releases the outflow of mine waters. They connect with the Pelcznica River influencing its water quality. In 2007 five research localities were investigated: 1 – the source of the River on the eastern slope of Borowa massif approximately 625 m above sea level, 2 – 50 m above the point of mine waters discharge, 3 – the point of the mine waters drop, 4 – 50 m below the point of mine water drop and 5 – 200 m below the point of mine water drop and 50 m above the sewage treatment plant. The analysis of the results showed that mine waters have a considerable influence only on a few water parameters of the Pelcznica River especially related to electrolytic conductivity, nickel, chlorides, sulfates, calcium concentration and alkalinity or hardness.

Keywords: Pelcznica River, mine, water quality

Civilization development results in a considerable human influence on the natural environment [1]. An example of anthropopression may be found in the effect mine industry on the condition of surface waters. Mine industry results in topography, soil quality and fauna alternation along with the change of the amount of water and its quality [2]. Walbrzych region belongs to an area under great anthropopressure, where the contamination of local rivers and streams by industry is on the rise. Out-of-date technologies and the lack of human responsibility resulted in total degradation of the rivers in the region of Walbrzych. A few places at the bottom of the Pelcznica River were covered with thick sediment of coal dust which was a remnant of coal flotation. The Pelcznica River is reported to be the most polluted tributary of the Strzegomka River due to its long-term negligence.

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The collapse of Walbrzych mines posed another threat linked with possibilities of local undercuts. Creation of complex drainage system which would provide the drainage of inactive mines became necessary. The Friedrich-Wilhelm adit fulfilling a similar role till the middle of the XIX-th century was used. The adit was linked by the system of drifts with all the mines located in Walbrzych region allowing their drainage [3]. Mine waters flew into the Pelcznica River gravitationally without previous purification. An average flow of the Pelcznica River in the region of the mine waters drop was measured at 0.86 $m^3 \cdot sec^{-1}$ while an average flow of mine waters was 0.32 $m^3 \cdot sec^{-1}$ which comprised 59 % of an average flow of Pelcznica River [4]. Such a high contribution of mine waters in the River composition may have resulted in considerable change of physical and chemical properties.

Water Framework Directive 2000/60/WE obliges Poland to protection and improvement of water quality in Poland [5]. It would be advisable to conduct research on the types of contamination which flow into the water to determine possibilities of pollution prevention. The aim of the research was investigation of the mine waters influence on the condition of the Pelcznica River.

Materials and methods

The samples were assembled 3-fold in 2007 at five research sites: 1– the source of the River on the eastern slope of Borowa massif, 2– 50 m above the point of mine waters discharge, 3– the point of the mine waters drop into the Pelcznica River, 4–50 m below the point of mine water drop into the River and 5–50 m above the sewage treatment plant in the Piotrowski street. At each site 3 samples of water were taken. The water was examined in terms of the following physical and chemical parameters such as water reaction (pH-metr 204), electrolytic conductivity PN-EN 27888:1999, hardness (wersenian method PN-ISO 6059), alkalinity PN-90/ C-04540.03, calcium (versenate method PN-ISO 6058), magnesium PN-ISO 6059, copper, zinc, nickel, lead (using atomic absorption spectrometry with atomization in the flame PN-ISO 8288:2002), sulfates (titration method [6]), chlorides (argenometric Mohr method PN-ISO 9297, nitrates(III) using atomic absorption spectrometry method PN-EN 26777, nitrates(V) using atomic absorption spectrometry method PN-82/C-04576.08, ammonia (Nessler's solution method PN-C-04576-4), phosphates PN-EN 1189.

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

Results and discussion

Chemical substances comprised in mine waters resulted in the alternation of Pelcznica River reaction into more alkaline which confirmed the Pelcznica's tendency towards alkalinity [4, 7–9]. However, such tendency was not always common as Indian and American mines showed acid reaction [10]. An elevation of electrolytic conductivity was observed.

Although it was difficult to compare two different mines due to different location and variety of extracted fossils an elevation of electrolytic conductivity in the Pelcznica River was observed while in both the Indian and the American mines electrolytic conductivity was measured at a higher level [10]. A similar influence was observed in water hardness and alkalinity. It may suggest high concentration of dissolved salts [11] in mine water, especially calcium and magnesium, which was confirmed during the research (Table 1).

An elevation in nickel and zinc concentration was observed after the introduction of mine waters. Before the drop of mine water nickel content in the river was low and similar to the values occurring in the Scinawka River [12]. The mine water drop to the Pelcznica River resulted in a sharp increase in nickel concentration up to 0.105 mg $Ni \cdot dm^{-3}$ which was the highest reported value occurring in mine waters flowing into the Pelcznica in the last years [13]. Such a considerable growth in nickel content may have been caused by a ceramic plant where it is used. Despite the fact that zinc concentration rose 2-fold it was still measured at lower level in comparison with the zinc content in the Klodnica River which plays the same role –0.9 mg $Zn \cdot dm^{-3}$ [12] in a different region.

Copper concentration in the Pelcznica River was higher above the point of the mine water discharge than at the point of discharge. The Pelcznica River contained equalized concentration of cadmium and lead. It suggested a lack of fossil influence on these metals' concentration in the River.

Elevated sulfate content was characteristic for the mine water discharge, however, it was still considerably lower than in Klodnica or Bierawka Rivers where mine sewage was dropped too. It was measured for the Klodnica and the Bierawka at a maximum value of 766.6 mg $SO_4 \cdot dm^{-3}$ and 1009 mg $SO_4 \cdot dm^{-3}$, respectively, with the maximum concentration in the mine water discharge at 154 mg $SO_4 \cdot dm^{-3}$ [12] or even 5000 mg $SO_4 \cdot dm^{-3}$ [7]. In comparison to the previous years when sulfates content was measured at 927.3 $SO_4 \cdot dm^{-3}$ [13] it decreased (in mine waters). Sulphates could dissolve sulphides and created sediments, which are below the point of discharge. The research showed the discharge of mine waters was low in chloride content. In the Klodnica River it ranged from 685 to 2760 mg $Cl^{-} \cdot dm^{-3}$ while in the Bierawka River – 1265 to 6150 $Cl^{-} \cdot dm^{-3}$. Fisher [4] and Kowalski [13] reported a considerable growth in sulfates in mine waters. According to Kowalski [13] mine contamination may occur in the environment for ages. It provokes a duty of mine waters purification.

Both waters of the Pelcznica River and the discharge of mine waters was not reported rich in nitrate(III), however, the highest concentration of nitrate (12.0 mg $NO_3^{-} \cdot dm^{-3}$) was measured in mine water discharge. Although mine waters may be the reason for elevation of nitrate concentration other discharges show greater content of nitrates. The growth of nitrates concentration below the point of mine waters discharge may have been caused by a decrease in the ammonia content which transforms to nitrates during the nitrification process occuring in that point.

Before the introduction of mine waters into the Pelcznica River, phosphates concentration was quite high and mine waters discharge resulted in phosphate content decrease.

Table 1

Physical and chemical properties of the Pelcznica River at 5 surveyed sites

Water parameters	Uunit	Site no.									
		1		2		3		4		5	
		$\bar{x} \pm s$	range	$\bar{x} \pm s$	range	$\bar{x} \pm s$	range	$\bar{x} \pm s$	range	$\bar{x} \pm s$	range
Electrolytic conductivity	$\mu S \cdot cm^{-1}$	220 ± 69	154–292	1142 ± 250	854–1300	3597 ± 455	3290–4120	3287 ± 326	2950–3600	3140 ± 352	220 ± 69
Reaction	pH	7.2 ± 0.6	6.6–7.9	7.5 ± 0.4	7.1–7.9	7.4 ± 0.6	6.9–8.0	7.4 ± 0.5	6.9–8.0	7.4 ± 0.5	7.2 ± 0.6
Hardness	$mg\ CaCO_3 \cdot dm^{-3}$	85 ± 72	25–165	386 ± 275	127–675	1028 ± 109	904–1109	969 ± 56	931–1033	892 ± 56	85 ± 72
Alkalinity	$mg\ CaCO_3 \cdot dm^{-3}$	28 ± 0.0	28–28	112 ± 28	84–140	289 ± 56	280–392	289 ± 16	280–308	298 ± 16	28 ± 0.0
Calcium	$mg\ Ca \cdot dm^{-3}$	17 ± 10	6–24	78 ± 32	41–100	180 ± 55	144–243	91 ± 47	51–143	95 ± 59	17 ± 10
Magnesium	$mg\ Mg \cdot dm^{-3}$	10.3 ± 13.3	2.5–25.7	47.2 ± 54.9	6.1–109.5	140 ± 59.7	72.1–182.0	179.9 ± 32.7	142.4–202.3	159.1 ± 48.9	10.3 ± 13.3
Ammonnia	$mg\ NH_4^- \cdot dm^{-3}$	0.3 ± 0.2	0.2–0.6	7.1 ± 0.8	6.6–8.8	2.9 ± 0.3	2.8–3.6	2.7 ± 0.2	2.5–3.3	3.2 ± 0.3	0.3 ± 0.2
Nitrates	$mg\ NO_3^- \cdot dm^{-3}$	6.2 ± 0.6	5.5–6.7	8.0 ± 0.5	7.6–8.5	11.5 ± 0.5	11.0–12.0	9.5 ± 0.6	8.9–10.0	9.3 ± 0.7	6.2 ± 0.6
Nitrites	$mg\ NO_2^- \cdot dm^{-3} \cdot 10^{-3}$	3.0 ± 0.1	3.0–3.1	4.9 ± 0.3	4.6–5.2	6.1 ± 0.2	5.9–6.3	16.8 ± 1.9	14.6–18.1	31.7 ± 20.2	3.0 ± 0.1
Copper	$mg\ Cu \cdot dm^{-3} \cdot 10^{-3}$	22 ± 31	0–43.5	44 ± 36.5	18–70	5 ± 0.5	4.5–5.5	10 ± 2	9–11.5	11 ± 2.5	22 ± 31
Lead	$mg\ Pb^- \cdot dm^{-3} \cdot 10^{-3}$	3.9 ± 5.4	0.05–7.8	6.1 ± 2	4.2–7.9	6.1 ± 0.1	6.1–6.2	6.93 ± 0.01	6.8–6.9	4.0 ± 1.8	3.9 ± 5.4
Nickel	$mg\ Ni^- \cdot dm^{-3} \cdot 10^{-3}$	3.9 ± 5.4	0.05–7.8	0.75 ± 0.9	0.23–1.25	71.8 ± 40.5	38.2–105.8	78.2 ± 20.4	63.6–91.0	73.6 ± 22.7	3.9 ± 5.4
Cadmium	$mg\ Cd^- \cdot dm^{-3} \cdot 10^{-3}$	2.5 ± 3.5	0.0–4.9	3.6 ± 0.1	3.7–3.8	3.6 ± 0.1	3.9–3.6	4.1 ± 0.2	3.9–4.2	3.5 ± 1.2	2.5 ± 3.5
Zinc	$mg\ Zn^- \cdot dm^{-3} \cdot 10^{-3}$	29.3 ± 40.4	0.7–57.9	28.2 ± 37.6	1.6–54.8	50.8 ± 47.6	17.1–84.5	47.3 ± 41.4	18.0–76.5	50.5 ± 49.3	29.3 ± 40.4
Chlorides	$mg\ Cl^- \cdot dm^{-3}$	19.6 ± 0.4	16.0–23.0	33.7 ± 0.3	32.0–37.0	45.0 ± 0.3	42.0–47.0	43.7 ± 0.2	41.0–45.0	42.7 ± 0.2	19.6 ± 0.4
Phosphates	$mg\ PO_4^{-3} \cdot dm^{-3} \cdot 10^{-3}$	26.7 ± 20.8	10.0–50.0	693.4 ± 475	150–1030	60.0 ± 20	40–80	143.3 ± 160.9	0–300	450 ± 160.9	26.7 ± 20.8
Sulfates	$mg\ SO_4^{-3} \cdot dm^{-3}$	72.6 ± 21.4	54–96	74.6 ± 26.6	52–104	482 ± 167.6	308–642	422 ± 134.9	290–560	387 ± 87.2	72.6 ± 21.4

Conclusions

Mine waters chemical compound depend on types of mine minerals. That was the cause of huge differences among chemical compounds of mine waters [8–10, 14, 15]. The analysis of the obtained results revealed that mine waters have considerable influence only on some physical and chemical parameters especially connected with salinity of the Pelcznica waters such as elevation of the electrolytic conductivity, alkalinity, hardness, nickel, chlorides, sulfates and calcium content. The examinated mine waters do not cause higher cadmium, copper, lead, ammonia or phosphate contamination as the research showed a decrease in their content below the point of the mine water discharge. Constant monitoring of such waters should be advised.

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WPŁYW WÓD KOPALNIANYCH NA JAKOŚĆ WÓD RZEKI PEŁCZNICY

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Abstrakt: Rzeka Pełcznica stanowi prawobrzeżny dopływ Strzegomki. Z nieczynnej kopalni położonej nieopodal tej rzeki wypływają wody kopalniane. W roku 2007 zdecydowano się na przebadanie wody pobranej na pięciu stanowiskach badawczych: 1 – źródło rzeki na zachodnim stoku masywu Borowej ok. 625 m n.p.m, 2 – 50 m przed zrzutem wód kopalnianych, 3 – miejsce zrzutu wód kopalnianych, 4 – 50 m poniżej zrzutu wód kopalnianych oraz 5 – 200 m poniżej zrzutu wód kopalnianych, 50 m przed oczyszczalnią ścieków. Analiza uzyskanych wyników wykazała, iż wody kopalniane mają istotny wpływ tylko na niektóre parametry wód rzeki Pełcznica, związane z zasoleniem wody: konduktywność (przewodnictwo elektrolityczne), chlorki, azotany, siarczany, zasadowość, twardość, wapń, a także nikiel.

Słowa kluczowe: rzeka Pełcznica, kopalnia, jakość wody

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Vol. 18, No. 5–6 2011

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BIOACCUMULATION OF SELENIUM IN CHOSEN WATER PLANT FROM THE DRAWA RIVER

BIOAKUMULACJA SELENU W WYBRANYCH ROŚLINACH WODNYCH RZEKI DRAWY

Abstract: The Drawsko Military Training Ground is located in the area of the Drawienski National Park and the Drawsko Landscape Park. The Ground may be a serious source of natural environmental contamination with heavy metals. Researchers collected and examined water plant samples – Floating pondweed (*Potamogeton natans*) and Common reed (*Phragmites australis*). The samples were taken from places situated along the Drawa River (before and below the Military Training Ground). Selenium (Se) content in these plants was determined. The received results did not show essential difference of the metal content between plants from various places. The Training Ground does not cause contamination of the Drawa River with the investigated metal.

Keywords: military training ground, water plants, selenium, contamination

The Drawienski National Park (DNP) was founded in 1990 in the south-western part of Poland in Pomerania Lake District and it is one of 23 national parks in this country. Situated in the catchment of the Drawa River with its side stream – the Plociczna, the DNP is one of the forms of nature protection areas richest in rare species [1, 2]. Within this zone the *Drawsko Military Training Ground* (DMTG) is located – a place where ground and air forces have their tranings (Fig. 1). Such location of a military zone is rather rare. It has an area 36111 ha and it is one of the biggest military traning grounds in Europe [1, 3].

Deployment of the military action at this controversial site has an impact on the environment. Despite the implementation of the plan for compensation of damage and reduction of the harmful impact on the environment by the army, the influence of

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Fig. 1. Location of places where the tests plants were taken from

military training ground on the environment is significant [3]. The authors' last investigation suggests DMTG is a source of environmental contamination by Cu, Zn, Ni, Pb and Cd [4]. In addition, IOS studies in 2005 and 2006 showed the raised content of other toxic elements including selenium (Se) in the waters of the Drawa river flowing through the military area [5, 6]. Polish army refused giving any detailed information about this situation [3].

The aim of the work was to verify the hypothesis that the DMTG has a big influence on the Drawa River contamination by Se. The military zone is entirely closed for civilians and unavailable for environmental research, that is why authors used water plants, which have the ability to accumulate this metal.

Materials and methods

To achieve the purpose of the research 2 water plant species were used – Floating pondweed (*Potamogeton natans*) and Common reed (*Phragmites australis*). The plants were taken from 19 different places, which were situated along the Drawa River (12 places located before DMTG and 6 below it).

500 mg of material was put to special teflon containers. All samples were flooded by 5 cm^3 of concentrated nitrogenous acid 1:1 (SIGMA) and mineralized in microwave stove MARS-5. Addition HCl (SIGMA) caused Se reduction to the 4^{th} oxidation state.

Selenium concentrations were determined using hydride generation atomic absorption spectrophotometry (HG AAS) by means of a VARIAN Spectra 220 FS [7].

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

Selenium concentrations in water plants are included in Table 1 and 2.

Pontweed (submerged plant) accumulated significantly more ($p \le 0.01$) Se than reed (emers with a green mass drawn over the water). The Se average concentration in reed was (107.43 ± 35.42) $\mu g \cdot kg^{-1}$, in pondweed's tissues – (182.04 ± 42.21) $\mu g \cdot kg^{-1}$. For positions located above DMTG pontweed accumulated about 60.6 % more Se than the reed. Below military sources of pollution – difference in the Se content reached to 87.3 %. This results from the multiple absorption surfaces of weed in relation to the cane. First of them gathers all the components by the whole body surface, the second one – with just submerged root system [8–10].

There was no statistically significant difference ($p \le 0.01$) in the Se content in the samples collected above and below DMTG. The lowest concentration of the element was at position number 3 (inflow to the Krosino Lake) and 5 (Zerdno Lake). Drawa in this section has a wide shoreline covered with abundant water plants. There is also large quantity of submerged plants from both lakes. Due to the presence of such organisms the water reservoirs play the role of effective natural places where fitoremediation takes place [11, 12].

Table 1

The Se concentrations in water plants collected from places located above the military area [$\mu g \cdot kg^{-1}$]

Location number	Location name	River mileage	Common reed (*Phragmites australis*)	Floating pondweed (*Potamogeton natans*)
1	Drawsko Lake	167+700	83.208	206.473
2	below Drawsko	163+100	117.128	l.d.
3	above Krosino Lake	157+600	123.781	257.044
4	below Krosino Lake	154+600	108.873	l.d.
5	Zerdno Lake	152+900	175.240	176.547
6	Wilczkowo Lake	151+100	118.130	128.131
7	Złocieniec	149+800	134.149	137.827
8	below of inflow to Kokna	144+800	75.325	146.363
9	below Drawsko	128+200	93.350	162.318
10	Lubiec Lake	119+600	145.547	156.835
11	below Lubiec Lake	111+200	20.359^{A}	235.199
12	beginning of DMTG area	99+800	97.657	124.150
Average			*107.729*^{AB}	*173.089*^{B}
SD			*39.004*	*45.688*

l.d. – lack of data, not collected plants from the post; A, B, C – statistically significant differences ($p \le 0.01$).

Table 2

The Se concentrations in water plants collected from places located below the military area [$\mu g \cdot kg^{-1}$]

Location number	Location name	River mileage	Common reed (*Phragmites australis*)	Floating pondweed (*Potamogeton natans*)
13	Grazyna Lake	78+100	157.353	246.720
14	Adamowo Lake	75+300	l.d.	234.120
15	Baraninie	72+600	l.d.	249.040
16	inflow to Pstrag	39+300	95.396	179.317
17	above Kamienna	34+900	97.083	205.167
18	below Kamienna	30+400	87.845	166.677
19	above Przedborowo	15+700	95.918	201.800
Average			*106.719*^{C}	*199.936*^{C}
SD			*43.441*	*31.763*

l.d. – lack of data, not collected plants from the post; A, B, C – statistically significant differences ($p \le 0.01$).

The largest amount of Se was showed in the plants collected from the position number 11 and 12 (respectively for reed and pondweed). Both of them are under the direct influence of the military zone. The coastline of this section of is deeply anthropologenic transformed. A large supply of toxic elements limits development of water vegetation. Low Se capture from water of the Drawa River is the likely cause of the observed increase in the concentration of this element in the test plants [13–15].

Conclusions

An estimated amount of Se in the tissues of reed and pondweed correlated with the amounts of this element in aquatic plants presented by other authors [16–23]. Values considered to be reference in Poland [24, 25] do not show exceeding of the limit value for Se in investigated plants.

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BIOAKUMULACJA SELENU W WYBRANYCH ROŚLINACH WODNYCH RZEKI DRAWY

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Abstrakt: W obrębie Drawskiego Parku Krajobrazowego oraz Drawieńskiego Parku Narodowego zlokalizowany jest "Poligon drawski" – Centrum Szkoleniowe Wojsk Lądowych. Może on stanowić poważne źródło skażenia środowiska naturalnego parków metalami ciężkimi. Zebrano i przebadano próbki wybranych roślin wodnych, tj. rdestnicy pływającej (*Potamogeton natans*) i trzciny pospolitej (*Phragmites australis*). Materiał badawczy pobrano ze stanowisk leżących powyżej i poniżej poligonu, poprzez który przepływa rzeka Drawa. Oznaczono w nich zawartość selenu. Otrzymane wyniki nie różnią się istotnie statystycznie między badanymi lokacjami. Ilość pierwiastka nie przekracza dopuszczalnej normy zawartości Se w roślinach wodnych. Poligon wojskowy nie powoduje zanieczyszczenia rzeki Drawy selenem.

Słowa kluczowe: poligon wojskowy, rośliny wodne, selen, zanieczyszczenie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 5–6 2011

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MORTALITY AND PATHOGENIC PROPERTIES OF *Heterorhabditis bacteriophora* (POINAR 1976) FROM NEMATOP BIOPREPARATION AFTER CONTACT WITH AN INSECTICIDE

ŚMIERTELNOŚĆ I WŁAŚCIWOŚCI PATOGENNE *Heterorhabditis bacteriophora* (POINAR 1976) POCHODZĄCYCH Z BIOPREPARATU NEMATOP PO KONTAKCIE Z INSEKTYCYDEM

Abstract: The effect of Baycidal WP 25 insecticide on the mortality of *entomopathogenic nematodes* (EPN) *Heterorhabditis bacteriophora* from Nematop biopreparation was studied under laboratory conditions. The invasive larvae were kept in aquatic solutions of different concentrations of Baycidal WP 25. The concentration of 0.0013 g/cm^3 of insecticide (recommended by the manufacturer) unfavourably affected the survival of IJs. Moreover, it was found that their mortality was the lowest in the highest concentrations of Baycidal WP 25, except for the control group. In this study the effect of different concentrations of Baycidal WP 25 on pathogenic properties of entomopathogenic nematodes was also analysed.

Keywords: entomopathogenic nematodes, EPN, *Heterorhabditis bacteriophora*, Nematop, insecticide, Baycidal WP 25, *Alphitobius diaperinus*

Entomopathogenic nematodes are used in the production of biological preparations for controlling various pests [1]. Nematode species show different tolerance to insecticides used in agriculture [2]. Studies confirmed the possibility of parallel application of biological and chemical means, which increases the mortality of pest populations. The effectiveness of integrated methods of pest control often exceeds the effectiveness of one of these methods used alone [3]. Application of biological insecticides based on nematodes together with chemical means may be used to control a beetle of the family Tenebrionidae – *Alphitobius diaperinus* (Panzer 1797). It is a dangerous pest and a vector of many diseases. It propagates with poultry fodder and

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the conditions in henhouses favour its development. The most threatened group of animals are bred birds which have contact with insects brought to farm buildings [4].

Material and methods

The effect of the Baycidal WP 25 insecticide on mortality and pathogenic properties of entomopathogenic nematodes *Heterorhabditis bacteriophora* was studied in experimental conditions. Baycidal WP 25 is an insecticide from the group of insects' growth and development regulators. It is produced by BAYER CropScience AG in Germany. The preparation is mainly used for the control of flies and the lesser mealworm in farm houses. Three concentrations of Baycidal WP 25 were used in experiments: the dose recommended by the producer (0.0013 g/cm^3), ten times lower (0.00013 g/cm^3) and ten times higher (0.013 g/cm^3) than the recommended. *H. bacteriophora* originated from the biopreparation Nematop made by the German firm E-nema.

The experiment was carried out during 7 consecutive days under laboratory conditions at 25 ^{o}C. Larvae of the third invasive stadium (IJs) were placed in water solutions of the appropriate concentration of Baycidal WP 25. The control group consisted of the larvae kept in distilled water. Every day samples of the solution were taken and nematodes mortality was studied. Tests were made in 5 repetitions.

After 7 days the nematodes that survived the contact with the insecticide were separated by sedimentation. Live nematodes were used to infect various growth stages of *A. diaperinus* (four week larvae, pupae and adults). Experiments were performed in Petri dishes of the diameter of 9 cm lined with filter paper in which 10 insects from particular growth stages were placed. Each dish received 500 invasive larvae (IJs). Tests were made in 3 repetitions. Mortality was checked for 7 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 reason of their death. Experiment was carried out at 25 ^{o}C and 85–90 % relative moisture. The control consisted of insects in a respective growth stage infected with nematodes which did not contact Baycidal WP 25. The mortality, extensiveness and intensity of infection of insects by *H. bacteriophora* were analyzed.

The obtained results were statistically processed (ANOVA, chi square and Tukey test) with the SPSS 15 software. Statistical significance was tested at $p < 0.05$.

Results and discussion

Nematodes mortality in solutions of Baycidal WP 25 (0.00013; 0.0013 and 0.013 g/cm^3) was analysed every day during the 7 days of the experiment (Fig. 1). The highest concentration of 0.013 g/cm^3 caused the lowest (17 %) mortality in *H. bacteriophora*, the lowest (0.00013 g/cm^3) – 19 % mortality. A similar tendency for high heavy metal doses was observed for different groups of nematodes [5, 6]. It can be caused by excess of the threshold value of a toxic factor, in which defense mechanisms are activated. At the concentration recommended by the producer (0.0013 g/cm^3) nematodes mortality was the highest and amounted 28 %. Differences between

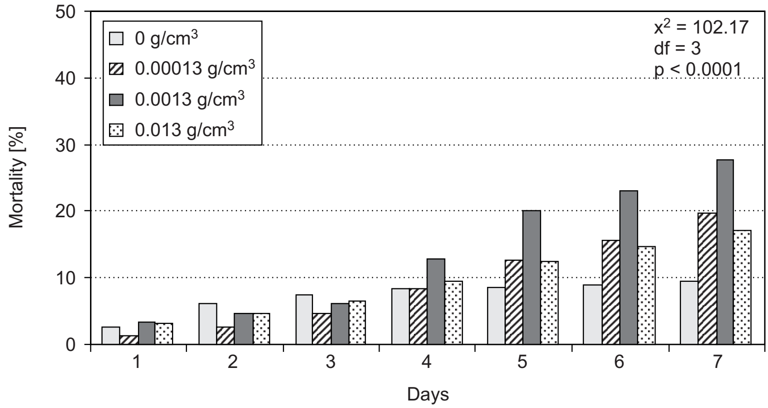

Fig. 1. The effect of various concentrations of Baycidal WP 25 on the mortality of *Heterorhabditis bacteriophora* larvae (test Chi^2 performed for the last day of experiment)

particular concentrations of Baycidal WP 25 measured on 7th day of the experiment were statistically significant in all cases.

The highest mortality and extensiveness of infection on the last day of the experiment were noted for the larvae of *A. diaperinus* (Fig. 2). A hundred per cent of dead insects were noted for nematodes exposed to 0.00013 g/cm^3 solution of Baycidal WP 25, the extensiveness of infection was 93 % in that case. The lowest percentage of mortality and extensiveness (83 and 33 %, respectively) was found at the highest concentration of insecticide.

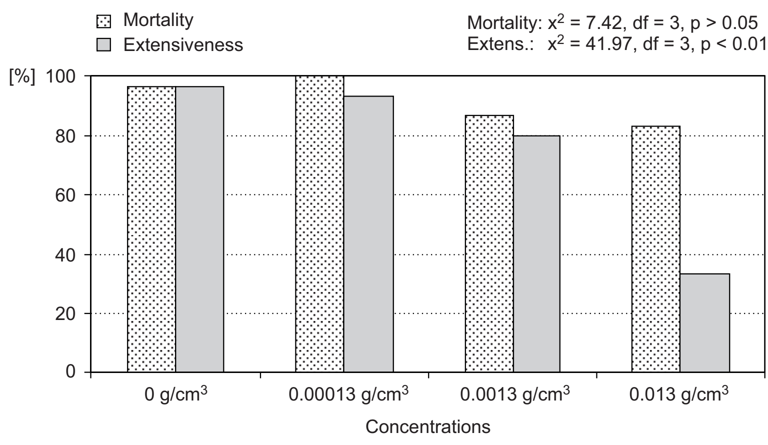

Fig. 2. The effect of Baycidal WP 25 on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 7 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* larvae) (test Chi^2)

High mortality (83 %) and extensiveness of infection (73 %) were noted in pupae after the contact of nematodes with the solution of a concentration of 0.0013 g/cm^3

(Fig. 3). The lowest values (40 and 27 %, respectively) were observed in the control group. For the highest concentrations, mortality and extensiveness of infection were respectively 63 and 33 %, which could be caused by lowered ability of EPN to penetrate hosts' tissues (fe damaging cuticule).

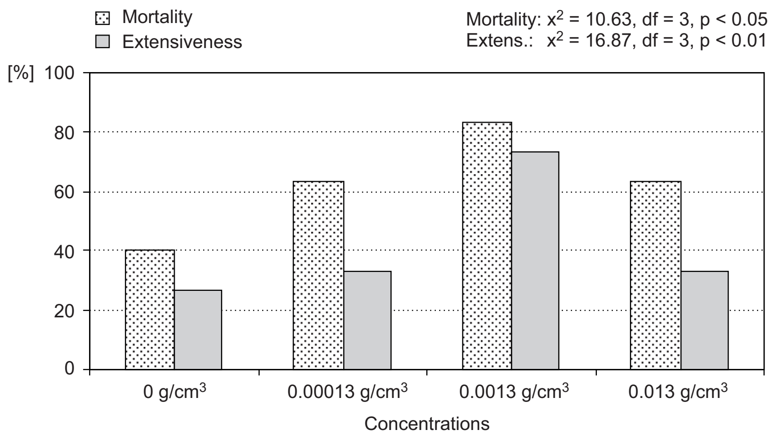

Fig. 3. The effect of Baycidal WP 25 on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 7 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* pupae) (test Chi^2)

The highest mortality of adult *A. diaperinus* (Fig. 4) was caused by nematodes exposed to concentrations of 0.013 and 0.0013 g/cm^3 (47 and 43 %, respectively). The extensiveness of infection at the concentration recommended by the producer was similar to mortality (43 %). The lowest mortality and extensiveness of infection (both equal to 13 %) was noted in the control.

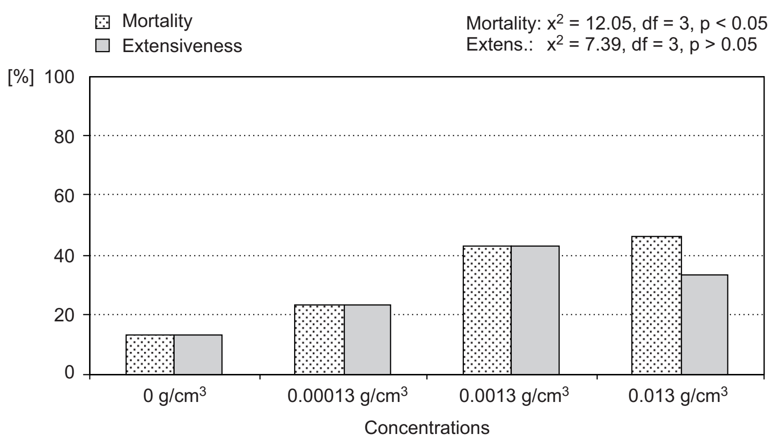

Fig. 4. The effect of Baycidal WP 25 on pathogenic properties of the nematode *Heterorhabditis bacteriophora* exposed for 7 days to solutions of various concentrations (the test of mortality percentage and extensiveness of infection of *Alphitobius diaperinus* imagines) (test Chi^2)

The presence of nematodes found after dissection of an insect's body evidenced that bacteria were the cause of its death. Microorganisms are the food base for entomopathogenic nematodes. Due to the lack of bacteria they do not reproduce [7]. Usually mortality is higher than the extensiveness of infection. This is because nematodes sometimes release bacteria from the alimentary tract and do not reproduce further. Therefore, their presence could not be found during dissection.

Intensity of infection is the mean number of invasive larvae of the nematode that entered the insect and developed to the L4 form plus hermaphroditic individuals. The intensity of infection for insect larvae was 6.87 at a concentration of 0.0013 g/cm^3 and 10.23 in the control (Table 1). Contribution of particular growth stages of nematodes to the population structure of the parasitic generation is shown in Table 2. Hermaphrodites dominated in the studied populations.

Table 1

The effect of Baycidal WP 25 on the intensity of infection of *Alphitobius diaperinus* by *Heterorhabditis bacteriophora* (Tukey test and ANOVA)

Insect developmental stage	Concentrations of Baycidal WP 25	Intensity of infection (Means)	ANOVA
Larvae	0 g/cm^3	10.23 B	$F3.116 = 9.53$, $p < 0.01$
	0.00013 g/cm^3	6.8 B	
	0.0013 g/cm^3	6.87 B	
	0.013 g/cm^3	0.97 A	
Pupae	0 g/cm^3	2.2 A	$F3.116 = 3.69$, $p < 0.05$
	0.00013 g/cm^3	4.87 AB	
	0.0013 g/cm^3	8.57 B	
	0.013 g/cm^3	1.87 A	
Adult insects	0 g/cm^3	0.97 A	$F3.116 = 2.42$, $p > 0.05$
	0.00013 g/cm^3	2.17 A	
	0.0013 g/cm^3	4.33 A	
	0.013 g/cm^3	1.7 A	

The highest intensity of infection (8.57) was noted for pupae (Table 1) at a concentration of 0.0013 g/cm^3, the lowest (1.87) – for the concentration 10 times lower than the recommended one. Contribution of particular growth stages of nematodes to the population structure of the parasitic generation is presented in Table 2. Hermaphrodites were the main component of the studied populations except for L4 larvae which attained a slight majority (4.43) at a concentration of 0.0013 g/cm^3.

The highest intensity of infection (4.33) in adult insects was noted at a concentration of 0.0013 g/cm^3 and the lowest – in the control (0.97) (Table 1). Contribution of particular growth stages of nematodes to the population structure of the parasitic generation is shown in Table 2. L4 growth stage was the main component of the studied populations except for hermaphrodites in the control group.

Table 2

The effect of Baycidal WP 25 on the population structure of the parasitic generation of *Heterorhabditis bacteriophora* in *Alphitobius diaperinus*

Insect developmental stage	Concentrations of Baycidal WP 25	Population structure of the parasitic generation (Means)	
		Hermaphrodite	L4
Larvae	0 g/cm^3	10.23	0.00
	0.00013 g/cm^3	6.40	0.40
	0.0013 g/cm^3	5.80	1.07
	0.013 g/cm^3	0.23	0.73
Pupae	0 g/cm^3	2.17	0.03
	0.00013 g/cm^3	4.17	0.70
	0.0013 g/cm^3	4.13	4.43
	0.013 g/cm^3	1.10	0.77
Adult insects	0 g/cm^3	0.53	0.43
	0.00013 g/cm^3	0.63	1.53
	0.0013 g/cm^3	1.07	3.37
	0.013 g/cm^3	0.33	1.37

The number of nematodes in the insect's body is an evidence of its attractiveness as a food base and indicates nematodes' ability to infect the host. As an effect of the performed studies one may conclude that high mortality, extensiveness and intensity of infection of *A. diaperinus* larvae point to attractiveness of this growth stage for nematodes.

The study confirmed that it is possible to simultaneously apply biological and chemical control means which, when used at the recommended dose or ten times lower dose, increases the mortality of larvae, pupae and adult insects.

Conclusions

1. Mortality of the invasive larvae of *H. bacteriophora* exposed to Baycidal WP 25 depended on the concentration of solutions and on exposure time.
2. Mortality and extensiveness of infection by Baycidal WP 25 treated nematodes and control nematodes differed among various growth stages of the lesser mealworm. Larvae were most sensitive to nematodes.
3. Intensity of infection by nematodes was the highest in the larvae of the beetle.
4. Hermaphrodites dominated in the population structure of the parasitic generation in most larvae and pupae of *A. diaperinus* and L4 larvae dominated in adult insects.

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ŚMIERTELNOŚĆ I WŁAŚCIWOŚCI PATOGENNE *Heterorhabditis bacteriophora* (POINAR 1976) POCHODZĄCYCH Z BIOPREPARATU NEMATOP PO KONTAKCIE Z INSEKTYCYDEM

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Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Abstrakt: W warunkach laboratoryjnych badano wpływ insektycydu Baycidal WP 25 na śmiertelność nicieni entomopatogennych *Heterorhabditis bacteriophora* pochodzących z biopreparatu Nematop. Larwy inwazyjno-przetrwalnikowe (IJs) umieszczono w roztworach wodnych zawierających różne stężenia Baycidal WP 25. Dawka 0,0013 g/cm^3 środka (dawka zalecana przez producenta) wpływała niekorzystnie na żywotność larw. Stwierdzono również, że śmiertelność ich jest najniższa, przy najwyższej ilości substancji owadobójczej rozpuszczonej w wodzie. Zbadano również wpływ różnych stężeń Baycidal WP 25 na patogenność nicieni.

Słowa kluczowe: nicienie entomopatogenne, *Heterorhabditis bacteriophora*, Nematop, insektycyd, Baycidal WP 25, *Alphitobius diaperinus*

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 5–6 2011

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IMPACT OF MULTI-WALLED CARBON NANOTUBES ON VIABILITY AND PATHOGENICITY OF ENTHOMOPATHOGENIC NEMATODES

WPŁYW WIELOŚCIENNYCH NANORUREK WĘGLOWYCH NA ŻYWOTNOŚĆ I PATOGENNOŚĆ OWADOBÓJCZYCH NICIENI

Abstract: The investigations aimed at recognizing the impact of multi-walled carbon nanotubes on the activity of enthomopathogenic nematodes under laboratory conditions. Two kinds of nanotubes were applied to treat infective juveniles of two enthomopathogenic nematode species. The obtained results allowed for a conclusion that multi-walled carbon nanotubes do not cause mortality of the analysed nematode infective juveniles (IJs). However, they partially reduce their activity towards test insects.

Keywords: carbon nanotubes, enthomopathogenic nematodes

Nanotechnology is a field of science allowing for manufacturing and use of the structure sized between 1 and 100 nm. Nanotechnology makes possible manufacturing products and objects atom after atom, molecule after molecule without any wastes [1, 2]. One of the nanotechnology products are carbon nanotubes. Research has been conducted for many years to determine the properties of carbon nanotubes [3]. They are known to possess high mechanical resistance, conductivity of metallic or semiconductor type dependent on the kind of nanotube, low electrical resistance and excellent thermal conductivity, many times higher than copper [2, 4].

There are *single-walled carbon nanotubes* (SWCNT) and *multi-walled carbon nanotubes* (MWCNT). They differ in their shapes, length and properties [5, 6].

Carbon nanotubes are used in various fields of science. Research conducted by scientists from Mexico [7] revealed that single-walled carbon nanotubes may be used

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for detecting and removal of disease agents in contaminated potable waters. They also find numerous applications in medicine. Nanotubes are empty inside and once opened these empty channels provide a possibility to use them as storage for various substances (eg some medicines or aromas) protecting them against the unfavourable effect of external factors [1, 2].

Recently nanotechnology has been applied also in food technology, eg for crushing solid substances into nanoparticles or encapsulation of some nutrients, reserves or dyes which contributes to their release according to needs or after a determined period of time [8].

There are presumptions that nanotechnology may be also used in agriculture for crop protection. It has been known that some properties of nanoproducts allow for their use to fight dangerous pathogenic microorganisms [3]. Pike-Biegunski [9] stated that crystalline structure of nonionic nanoparticle preparations and their large active surface make them most efficient in controlling pathogens such as: fungi, bacteria and viruses, because they do not produce typical defensive mechanisms. Research conducted so far has revealed that copper products manufactured by nanotechnology have been already successfully used in agriculture for controlling fungi infecting numerous crops, such as potatoes, tomatoes or fruit [2]. However, it is still unknown whether nanoproduct application in agriculture may not contribute to reducing the numbers of organisms which are not the object of control (beneficial organisms, such as enthomopathogenic nematodes). Enthomopathogenic nematodes occur numerously in the unpolluted environment and are most sensitive to pollutants [10]. For this reason they may be used as indicator organisms. Nematodes also play an important role in biological pest control, therefore a growing number of preparations containing these organisms are created [11].

Rapidly developing technologies and potential which nanotechnology provides make necessary conducting further research enabling more precise identification of nanotube activity in the agricultural environment.

Material and methods

The analyses were conducted under laboratory conditions at the Department of Agricultural Environment Protection of the University of Agriculture in Krakow.

The initial stage of the experiment focused on the effect of two kinds of multi-walled carbon nanotubes (MWCNT) and carboxylated multi-walled carbon nanotubes (MWCNT(COOH)) on viability and behaviour of nematode infective juveniles. 1 cm^3 of multi-walled carbon nanotubes was added to 1.5 cm^3 glass vessels. Subsequently about 30 infective juveniles of *Steinernema feltiae* enthomopathogenic nematodes originating from Owinema®, Nemasys and Nemaplus, and nematodes of *Heterorhabditis bacteriophora* species originating from Nematop preparation were added to each vessel. Nematodes placed in glass vessels with distilled water provided the control. The nematodes were observed under a magnifying glass for five subsequent days since the experiment outset in order to determine their viability and their motion activity in the analysed solutions. Motion activity of the nematode infective juveniles was determined on a 6-degree scale. After 5 days of observations nematode activity was checked against *Tenebrio molitor* L. test insects. Therefore, the nematodes were moved to filter paper and after rinsing with distilled water and draining they were moved to moist three-layer filter papers placed in plastic Petri dishes. 10 *Tenebrio molitor* test insect larvae were put in each dish prepared in this way. For the following 7 days mortality of the test insect larvae was observed. The results were analysed statistically using the Statistica Programme, ANOVA analysis was conducted and Neuman-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

The first stage of the experiment aimed to check vitality of nematode larvae under the influence of applied two kinds of multi-walled carbon nanotubes. It was observed that nematodes kept in the vessels with nanotubes retained a 100 % vitality after 5 days, the same as in the control (Table 1). Moreover, no significant differences in nematode larvae behaviour was spotted under the influence of nanotubes, and their motion activity was approximate to the larvae in the control.

Table 1

Vitality of *S. feltiae* and *H. bacteriophora* infective juveniles (IJs) originating from Owninema, Nemasys, Nemaplus and Nematop preparations kept in solutions containing multi-walled carbon nanotubes

Combination	IJs viability after 96-hour contact with nanotubes [%]			
	Kind of IJs			
	Owinema	Nemasys	Nemaplus	Nematop
Control	100	100	100	100
MWCNT	100	100	100	100
MWCNT(COOH)	100	100	100	100

The subsequent stage of the research comprised determining the activity of investigated nematodes treated with various kinds of multi-walled carbon nanotubes towards the test insect larvae. Already after 24 hours the nematodes kept in the control caused significantly higher mortality of the test insect larvae in comparison with the nematodes kept in the solution containing nanotubes. The observations were conducted for the 7 subsequent days. Mortality of the test insects for individual nematodes during the period was presented in Fig. 1.

The average life span was calculated for IJ-treated test insects previously kept in the analyzed solutions containing multi-walled carbon nanotubes (Fig. 2). Despite that fact that multi-walled carbon nanotubes contributed to a delayed IJs activity towards the test insect larvae, statistical analysis did not reveal any significant differences in the life span of *T. molitor* larvae between the applied combinations.

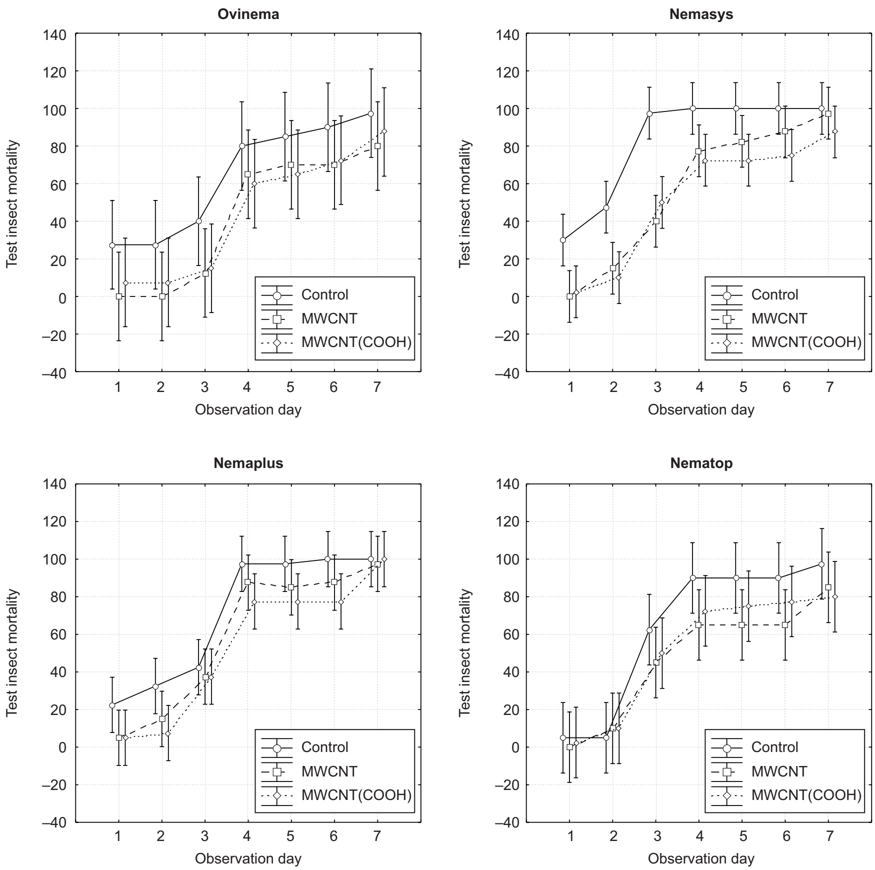

Fig. 1. Mortality of *T. molitor* L. larvae for 7 days under the influence of *S. feltiae* and *H. bacteriophora* infective juveniles (IJs) activity, originating from Owinema, Namasys, Nemaplus and Nematop biopreparations, kept in solutions containing multi-walled carbon nanotubes

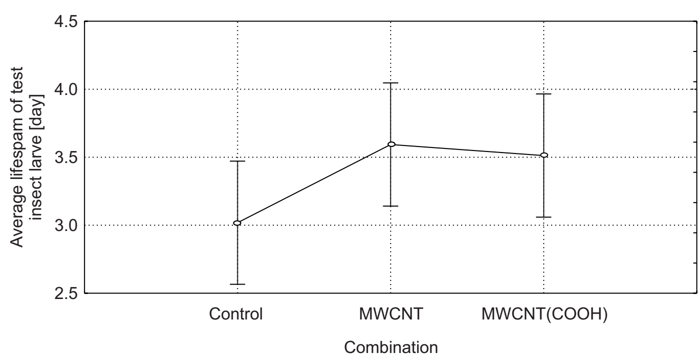

Fig. 2. Lifespan of *T.molitor* L. larvae caused by activity of *S. feltiae* and *H. bacteriophora* nematode infective juveniles (IJs), originating from Owinema, Namasys, Nemaplus and Nematop biopreparations, kept in solutions containing multi-walled carbon nanotubes

Discussion

Enthomopathogenic nematodes are organisms commonly present in the unpolluted natural environment [10]. Heavy metal accumulation in the environment where the nematodes live contributes to limiting their activity and sometimes also reduces their numbers. Wang et al [10] revealed in their studies that some nanocompounds, such as Nano-ZnO, Nano-Al_2O_3 or Nano-TiO_2 are instrumental in diminishing nematode body length, limiting the number of eggs laid in test insects and considerably decrease nematode larvae reproduction. The present Authors obtained similar results in their own experiment, where the analyzed multi-walled carbon nanotubes played a part in inhibiting the activity of investigated IJs towards *T. molitor* test insects. The research conducted by Gorczyca et al [3] demonstrated that carbon nanotubes significanty stimulate surface growth of *Paecilomyces fumosoroseus* mycelium but at the same time cause a reduction in its sporulation in comparison with the control samples. Nanotubes also pose a hazard for fish in the aquatic environment. Investigations carried out by American scientists [12] revealed that carbon nanotubes supplied to the aquatic environment in which rainbow trout existed irritated their branchiae and caused changes in their brains. Research conducted by Muller et al [13] on rats demonstrated that nanotubes caused considerable damage to these rodents' lungs. Moreover, currently American scientists have warned against some nanoparticle products. The risk is associated with the size of the particles which, inhaled by humans, may penetrate to various cells in human organism and accumulate there causing various illnesses. It has been demonstrated that nanotubes may damage lungs whereas their structure and size make impossible their complete removal from the organism. There are reports in literature also pointing to a toxic effect of such nanoparticles as: titanium, cobalt, iron, wolfram or silver [1].

Despite the fact that nanotubes seem to pose a considerable hazard to the surrounding environment, they find applications in more modern fields of science. They are most useful in medicine eg for combating neoplastic diseases.

The observable technological progress making use of nanotechnology makes some people optimistic, while others remain anxions. The opinions on nanoproducts are divided, which may be settled by conducting more extensive research towards a better understanding [14]. Therefore further investigations in this field seem both necessary and justified.

Conclusions

1. Multi-walled carbon nanotubes (MWNCT and MWCNT(COOH)) are non-toxic for the infective juveniles of enthomopathogenic nematodes of *Steinernema feltiae* (Owinema, Namasys, Nemaplus) and *Heterorhabditis bacteriphora* (Namatop) species.

2. Multi-walled carbon nanotubes (MWNCT and MWCNT(COOH)) are a factor in limiting the activity of infective juvenile larvae of enthomopathogenic nematodes of *Steinernema feltiae* (Owinema, Namasys, Nemaplus) and *Heterorhabditis bacteriphora* (Namatop) species.

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WPŁYW WIELOŚCIENNYCH NANORUREK WĘGLOWYCH NA ŻYWOTNOŚĆ I PATOGENNOŚĆ OWADOBÓJCZYCH NICIENI

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Abstrakt: Celem przeprowadzonych badań było poznanie wpływu wielościennych nanorurek węglowych na aktywność nicieni owadobójczych w warunkach laboratoryjnych. W badaniach zastosowano dwa rodzaje wielościennych nanorurek węglowych, którymi traktowano larwy inwazyjne dwóch gatunków nicieni owadobójczych. Uzyskane wyniki pozwoliły stwierdzić, że wielościenne nanorurki węglowe nie powodują śmiertelności badanych larw inwazyjnych (IJs) nicieni. Powodują jednak częściowe ograniczenie ich aktywności względem owadów testowych.

Słowa kluczowe: nanorurki węglowe, nicienie owadobójcze

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INFLUENCE OF CADMIUM ON PHYSIOLOGICAL PARAMETERS OF CLONE JORR OF BASKET WILLOW (*Salix viminalis* L.) FROM AQUATIC CULTURES

WPŁYW KADMU NA PARAMETRY FIZJOLOGICZNE KLONU JORR WIERZBY WICIOWEJ (*Salix viminalis* L.) Z KULTUR WODNYCH

Abstract: In the experiment the effect of differentiated doses of cadmium (0; 1.4; 28; 280 mg · dm^{-3}), applied in the form of $CdCl_2$ on selected physiological parameters of clone Jorr *Salix viminalis*, cultivated in water cultures with Hoagland's medium was determined. The statistical analysis of the results showed a significant influence of cadmium on the examined physiological parameters of clone Jorr. The addition of $CdCl_2$ to the medium in a dose of 280 mg · dm^{-3} caused a decrease in intensity of CO_2 assimilation of the examined clone by 70 % and transpiration by nearly 83 % in relation to the intensity of these processes in control leaves. A positive significant correlation between assimilation of CO_2 and transpiration was recorded. At all the dates of studies decreased contents of both chlorophyll and carotenoids in leaves were obtained after the application of three doses of cadmium chloride. The increase in the concentration of cadmium salt caused a decrease in the relative water content index (RWC) and an increase in the water saturation deficit (WSD) in the leaves of clone Jorr.

Keywords: *Salix viminalis*, cadmium, CO_2 assimilation, transpiration, assimilation dyes, water balance

The increase in the level of cadmium in the environment results from economic and industrial activity of human beings. The characteristic feature of this chemical element is the largest value of concentration index both in soil and in plant, as compared with other metals [1]. This phenomenon is attributed to the geochemical nature of cadmium, determining its large mobility in environment and easy uptake by plants [1–4]. The plants reaction to stress caused by an excessive amount of cadmium in the environment is reflected, among other things, by changes undergoing in the assimilation apparatus

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and physiological processes [3, 5–7]. The resistance of plants to toxic properties of cadmium is very differentiated and it depends not only on genetic properties of plants but also physiological ones.

For the reclamation of areas anthropogenically degraded plants are more and more frequently used [8–9]. Basket willow (*Salix viminalis* L.) and particularly its hybrid forms can play here a significant role, as due to its capability of removing impurities hard to degrade biologically, it is commonly used in protection and reclamation of soils [10–12]. To evaluate usefulness of plants for the reclamation of degraded areas a lot of anatomical and physiological features of plants are used.

The aim of the studies was to determine the physiological reaction of clone Jorr of basket willow (*Salix viminalis* L.) under the conditions of a medium contaminated with cadmium and to define usefulness of this form for bringing anthropogenically degraded areas into cultivation.

Material and methods

Material for the studies was clone Jorr of basket willow (*Salix viminalis* L.) [13]. Willow cuttings used in the experiment were taken from the plantation of the Department of Physiology of Plants, Westpomeranian Technological University in Szczecin. Whereas maternal material was from a plantation in Denmark, possessing a health certificate. The experiment was carried out in laboratory conditions in 2007–2008. During the period from April to June basket willow breeding was carried out in water cultures (of volume 1 dm^3) filled with a 1.5-fold concentrated full Hoagland's medium of pH = 5.8 and with appropriate doses of cadmium. Cadmium was introduced to the medium in the form of $CdCl_2$. In the experiment, set in 3 replications, the following combinations were taken into consideration: 1 – control (a full medium according to Hoagland); 2 – a full medium + I concentration Cd (1.4 mg $\cdot$ dm^{-3}); 3 – a full medium + II concentration Cd (28 mg $\cdot$ dm^{-3}); 4 – a full medium + III concentration Cd (280 mg $\cdot$ dm^{-3}). During the experiment the composition of individual media was changed every 5 days in order to maintain a stable level. Each set water culture contained 4 willow cuttings of 22 cm in length. After the cuttings had rooted and the shoots had formed, differentiated doses of $CdCl_2$ were added according to the experimental combination. The determination of physiological parameters was carried out on three dates: on the 26th (1st date), 36th (2nd date) and 46th (3rd date) day after the setting of the experiment. The content of assimilation dyes (chlorophyll *a*, *b*, total and carotenoids) leaves was determined by means of the Lichtenthaler and Welburn method [14]. Water balance was defined by the RWC index (*relative water content*) and WSD (*water saturation deficit*) [15]. Intensity of photosynthesis and transpiration was measured (repeating the measurements four times) only in 2008 using a mobile gas analyzer TPS-2 manufactured by PP Systems (UK), at stable lighting of 2053 μmol $\cdot$ m^{-2} $\cdot$ s^{-1}. On the basis of the obtained results of intensity of assimilation and transpiration the photosynthetic efficiency of water use was calculated (ω_F). The obtained results were worked out by means of a two factor variance method using Tukey's test at the level of significance $LSD_{0.05}$. Using the coefficient of correlation (r) the relation between intensity of CO_2 assimilation and intensity of transpiration was presented.

Results and discussion

Increasing doses of cadmium in the medium significantly inhibited intensity of CO_2 assimilation and transpiration in clone Jorr basket willow. A negative effect of the length of the period of the influence of an increased concentration of cadmium in the medium on intensity of these processes was also observed. The largest decrease in intensity of the examined physiological processes was observed when the maximum dose of cadmium was applied at all the dates of studies. Intensity of the photosynthesis at a dose of 280 mg · dm^{-3} decreased by 70 %, while that of transpiration by nearly 83 % as compared with the control (Table 1).

Table 1

Intensity of CO_2 assimilation and transpiration and water use photosynthetic efficiency (ω_f) of clone Jorr

CO₂ assimilation [μmol · m^{-2} · s^{-1}]				
Date of studies	Dose $CdCl_2$ [mg · dm^{-3}]			
	0	1.4	28	280
I	2.75	1.65	1.33	0.90
II	2.65	1.40	1.10	0.75
III	1.88	0.97	0.82	0.50
The average	2.43	1.34	1.08	0.72
$LSD_{0,05}$ for: dose – 0.98; date – n.s.				
Transpiration [mmol · m^{-2} · s^{-1}]				
I	1.27	0.87	0.48	0.22
II	1.11	0.59	0.36	0.19
III	0.74	0.48	0.16	0.14
The average	1.04	0.65	0.33	0.18
$LSD_{0,05}$ for: dose – 0.36; date – 0.19				
Water use photosyntetic efficiency [ω_f]				
I	2.16	1.89	2.77	4.09
II	2.39	2.37	3.05	3.95
III	2.54	2.02	5.12	3.57
The average	2.36	2.09	3.64	3.87

Photosynthetic effectiveness of water use is often a decisive indicator of productivity of plants under stressful conditions [16, 17]. The calculated index was differentiated depending on applied doses and dates of studies. A decrease in the effectiveness of this parameter by 11.5 % was recorded when a dose of 1.4 mg · dm^{-3} was applied, as compared with the control plants. Whereas the remaining doses caused a 1.5 fold increase in this index, as compared with the control. A high value of this parameter results first of all from low intensity of transpiration. Similar reactions of plants to the

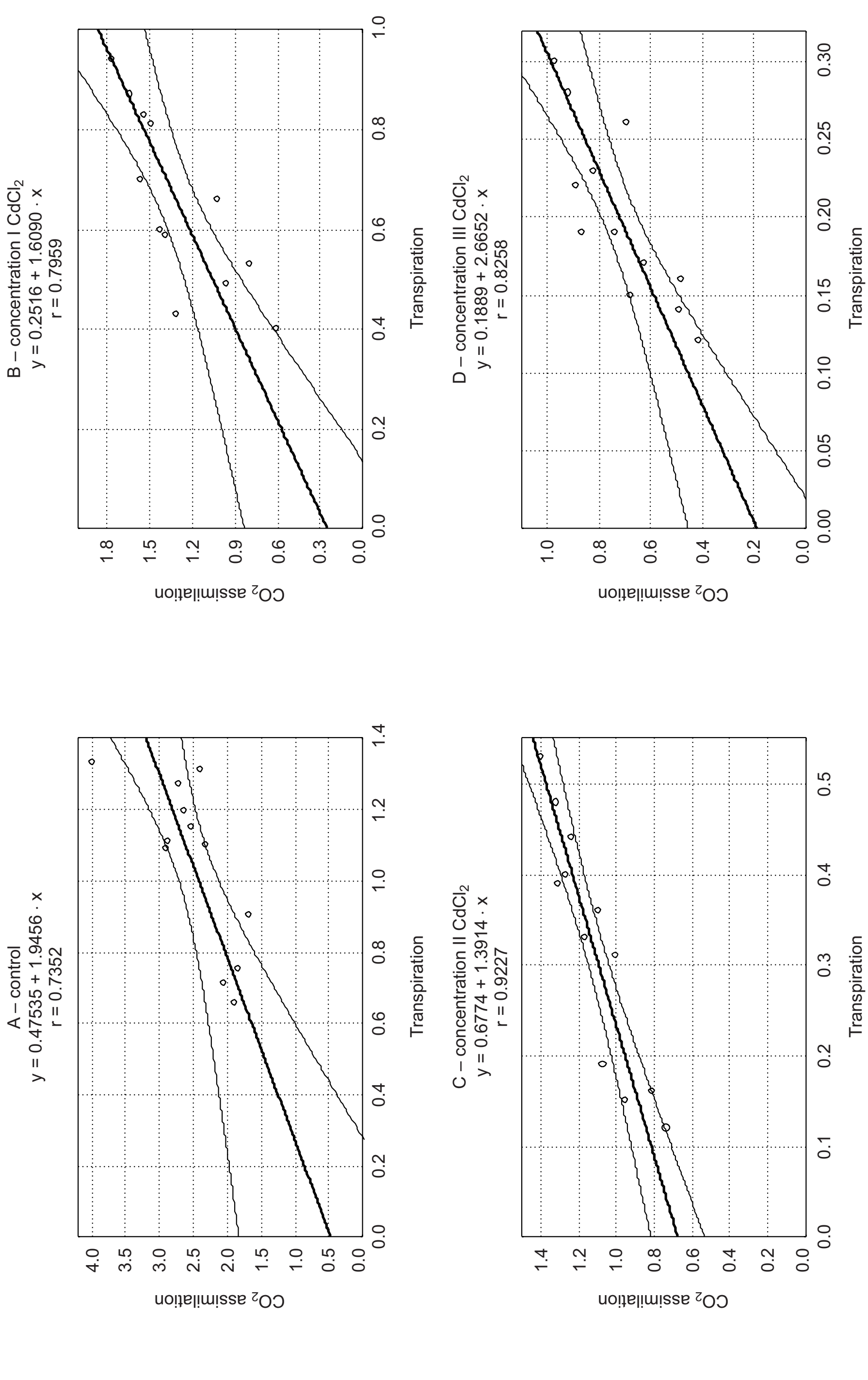

Fig. 1. Dependence of intensity of assimilation of CO_2 [μmol · m^{-2} · s^{-1}] on transpiration [mmol · m^{-2} · s^{-1}] in clone Jorr

effects of heavy metals were obtained by Malinowska and Smolik [18], Smolik and Malinowska [19], Jasiewicz et al [20]. Malinowska [21] recorded in her studies a negative significant correlation between intensity of photosynthesis and the content of cadmium in leaves of Norway maple. A decrease in intensity of photosynthesis and transpiration can be related to disturbances in the functioning of the photosynthetic apparatus of plants, a decrease in turgor, inhibition of transport of electrons in the process of photosynthesis and a decrease in activity carboxylase RuBP [5, 22, 23]. On the basis of the value of the correlation coefficient significant relationships were observed between assimilation and transpiration of clone Jorr, both under the control and stressful conditions (Fig. 1ABCD). The relationship between these parameters increased with an increase in the concentration of cadmium chloride in the medium. The highest value of the correlation coefficient: r = 0.9227* was observed at concentration II in the medium (Fig. 1C). High values of the correlation coefficient prove a close relationship between the examined features.

The content of assimilation dyes in plants is a characteristic feature of species and varieties. The influence of many environmental and anthropogenic factors considerably modifies their amount in plants [5, 6, 24, 25]. The application of a dose of cadmium significantly decreased their content in clone Jorr. At all the dates of studies decreased amounts of both chlorophyll and carotenoids were obtained in leaves after three doses of cadmium chloride had been used. An average amount of chlorophyll *a* + *b* in leaves of control plants was about 3.50 mg $\cdot$ g^{-1} f.m. The highest applied concentration – 280 mg $\cdot$ dm^{-3}, caused in 2007 a decrease in concentration of total chlorophyll by 50 % on the 1st date and by 75 % on the 2nd and 3rd dates of studies, whereas in 2008, by 80 % on all the dates, as compared with its content in the control leaves (Table 2).

Table 2

Content of assimilation dyes [mg $\cdot$ g^{-1} f.m.] in leaves of clone Jorr

Dose $CdCl_2$ [mg $\cdot$ dm^{-3}]	2007			2008		
	I	II	III	I	II	III
Content of chlorophyll *a* [mg $\cdot$ g^{-1} f.m.]						
0	3.43	3.02	1.92	2.59	2.40	2.22
1.4	2.49	1.52	1.73	0.74	0.60	0.46
28	1.75	0.95	0.65	0.73	0.58	0.43
280	1.63	0.73	0.43	0.48	0.37	0.27
$LSD_{0.05}$ for:	dose – 0.45; date – n.s.			dose – 0.85; date – n.s.		
Content of chlorophyll *b* [mg $\cdot$ g^{-1} f.m.]						
0	0.62	1.01	0.64	1.09	1.01	0.92
1.4	0.48	0.53	0.55	0.31	0.26	0.21
28	0.51	0.28	0.32	0.33	0.24	0.19
280	0.42	0.25	0.22	0.21	0.19	0.16
$LSD_{0.05}$ for:	dose – 0.24; date – n.s.			dose – 0.38; date – n.s.		

Table 2 contd.

Dose $CdCl_2$ [mg · dm^{-3}]	2007			2008		
	I	II	III	I	II	III
Content of total chlorophyll [mg · g^{-1} f.m.]						
0	4.05	4.03	2.56	3.68	3.41	3.14
1.4	2.97	2.05	2.28	1.05	0.86	0.67
28	2.26	1.23	0.97	1.06	0.82	0.62
280	2.05	0.98	0.65	0.69	0.56	0.43
$LSD_{0.05}$ for:	dose – 0.67; date – n.s.			dose – 1.22; date – n.s.		
Content of carotenoids [mg · g^{-1} f.m.]						
0	0.89	1.22	0.85	1.39	1.38	1.29
1.4	0.58	0.68	0.77	0.38	0.27	0.30
28	0.66	0.44	0.36	0.39	0.24	0.22
280	0.48	0.30	0.17	0.27	0.25	0.20
$LSD_{0.05}$ for:	dose – 0.19; date – n.s.			dose – 0.68; date – n.s.		

The addition of cadmium salt to the medium also caused a decrease in the content of carotenoids. The largest decrease of this dye was observed on the 46th day of the experiment and at the highest dose applied – by 80 % in 2007 and by 84.5 % in 2008 (Table 2). The inhibition of chlorophyll and carotenoids synthesis is a reflection of the activity of different heavy metals [5, 18, 20, 26]. Chen and Kreeb [6] recorded a more than twofold decrease in chlorophyll in maize under the influence of heavy metals, as compared with the control. Lukasik et al [24] and Malinowska [27] observed in their studies a negative significant correlation between the content of cadmium and chlorophyll *a* + *b* in leaves.

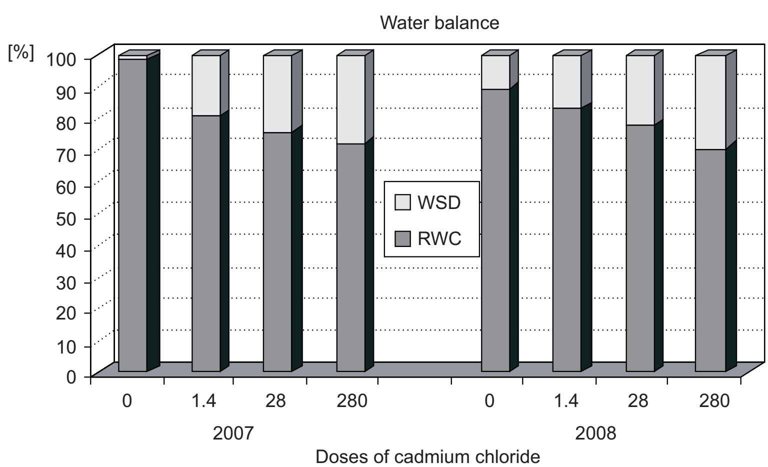

Fig. 2. Water indices [%] of basket willow – of clone Jorr in relation to a dose of cadmium chloride

An indicator of the changes in water balance in a plant are, among other things, RWC and WSD. The increasing doses of cadmium salt caused an increase in the content of the water in leaves of clone Jorr in both years of studies. The largest decrease in the index of relative water content (by 27 % in 2007 and by 21 % in 2008) was noticed after the application of the highest concentration of cadmium chloride in relation to the control plants (Fig. 2). The observed changes in intensity of the examined physiological parameters can be, under unfavourable conditions, the result of both stress and repair mechanisms [28]. The obtained results of the examined physiological parameters can be useful for evaluation of the resistance of clone Jorr to stress evoked by cadmium and the usefulness for reclamation of anthropogenically degraded areas.

Conclusions

1. The applied concentrations of cadmium chloride decreased significantly the intensity of CO_2 assimilation and transpiration and the content of assimilation dyes in leaves of clone Jorr.

2. A significant correlation between assimilation of CO_2 and transpiration in the studied form of willow was observed The value of the correlation coefficient was the highest at the dose of 28 mg Cd $\cdot$ dm^{-3} in the medium.

3. The increase in the concentration of salt caused a decrease in the index of the relative water content and an increase in the water saturation deficit in leaves of clone Jorr.

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WPŁYW KADMU NA PARAMETRY FIZJOLOGICZNE KLONU JORR WIERZBY WICIOWEJ (*Salix viminalis* L.) Z KULTUR WODNYCH

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Abstract: W przeprowadzonym doświadczeniu określano wpływ zróżnicowanych dawek kadmu (0; 1,4; 28; 280 mg · dm^{-3}), zastosowanych w formie $CdCl_2$ na wybrane parametry fizjologiczne klonu Jorr *Salix viminalis* L., uprawianej w kulturach wodnych z pożywką Hoaglanda.

Analiza statystyczna wyników wykazała istotny wpływ kadmu na badane parametry fizjologiczne klonu Jorr. Dodatek do pożywki $CdCl_2$ w dawce 280 mg · dm^{-3} spowodował obniżenie intensywności asymilacji CO_2 badanego klonu o 70 %, a transpiracji o prawie 83 % w stosunku do intensywności tych procesów w liściach kontrolnych. Stwierdzono statystycznie istotną dodatnią korelację między asymilacją CO_2 a transpiracją. We wszystkich terminach badań uzyskano obniżone zawartości zarówno chlorofilu, jak i karotenoidów w liściach po zastosowaniu trzech dawek chlorku kadmu. Wzrost stężenia soli kadmu spowodował spadek wskaźnika *względnej zawartości wody* (RWC) oraz wzrost *deficytu wysycenia wodą* (WSD) w liściach klonu Jorr.

Słowa kluczowe: *Salix viminalis*, kadm, asymilacja CO_2, transpiracja, barwniki asymilacyjne, bilans wodny.

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CADMIUM IN SOILS OF THE OJCOW NATIONAL PARK

KADM W GLEBACH OJCOWSKIEGO PARKU NARODOWEGO

Abstract: The aim of the paper was to investigate cadmium contamination of soils of the Ojcow National Park and to evaluate influence of location, soil type and soil properties on contamination degree with this element. Investigations was conducted on soil samples from 24 profiles represented main types of the park soil cover: rendzinas (13 profiles), lessive soils (8 profiles), brown soils proper (1 profile), pseudogley soils (1 profile) and river alluvial soils (1 profile).Cadmium content ranged from 0.4 to 12.8 mg $\cdot$ kg^{-1} in surface horizons of investigated soils. On the base of limit values proposed by IUNG and defined in Decree of the Minister of Environment of 9th September, 2002 on soil quality standards and earth quality standards, was stated that almost all soils (except 2 profiles of lessive soils) of the Ojcow National Park are polluted with cadmium. The highest cadmium concentration was analyzed in soils located close to the Czajowice village and the town of Skala. The higher content of analyzed element was measured in rendzinas than in lessive soils. In opposite was higher accumulation index in luvisols than in rendzinas. On the base of statistical analyze were state that soil reaction and below 0.02 mm fraction content are influenced on cadmium concentration in investigated soils.

Keywords: cadmium, soil, pollution, the Ojcow National Park

Geographical location of the Ojcow National Park in within the large industrial centres operation range (Upper Silesia Industrial Region, Olkusz, Jaworzno and Trzebinia-Siersza), considerable number of windless periods and small wind velocity favour persistence of air pollutants flowing from the west, north-west and south-west [1].

According to Grodzinska [2], who investigated contamination of national parks in Poland in the eighties of the previous century, the Ojcow National Park was counted to the group of parks seriously polluted with heavy metals. Moreover, Grodzinska demonstrated that contamination noted in the Ojcow National Park was the highest among the analyzed parks.

Presented investigations focused on the assessment of cadmium pollution in the Ojcow National Park and determining the influence of its location, type and properties of soil on the degree of pollution with this element.

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Material and methods

Soil material was collected from 24 soil profiles located in the area of the Ojcow National Park (Fig. 1) and representing main typological units of the park soil cover: rendzinas (13 profiles); lessive soils (8 profiles), brown soils (1 profile), pseudogley soils (1 profile) and alluvial soils (profile) [3, 4].

Rendzinas developed from Jurassic limestones, whereas brown soils, soil lessives and pseudogley soils formed from loesses lying on limestones, and river alluvial soils from river alluvia. The soil profiles were situated in the forested areas, only the alluvial soils (profile 13) was a grassland.

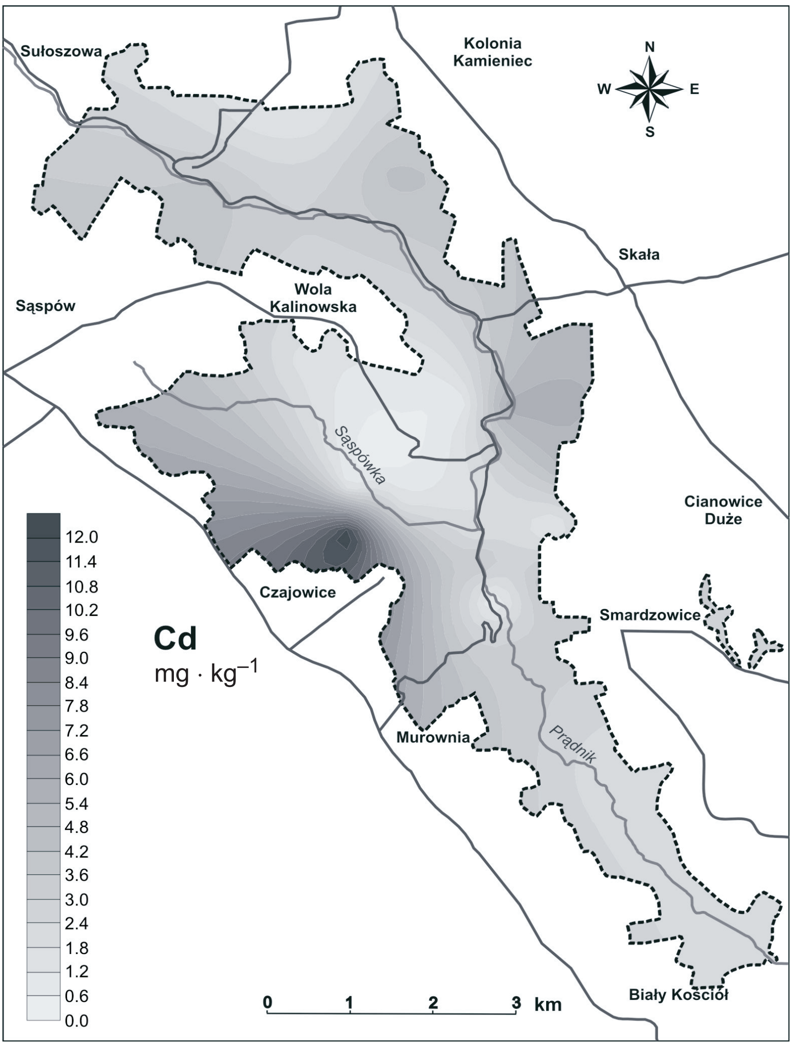

Fig. 1. Cadmium content in surface horizons of the Ojców National Park soils

The soil material was dried at room temperature and sifted through a sieve with 1 mm mesh. Subsequently basic physicochemical properties were determined:
– pH in distilled water with potentiometric method,
– total nitrogen with Kjeldahl method on Kjeltec apparatus (Tecator),
– organic carbon with Tiurin method in Oleksynowa's modification,
– granulometric composition with Casagrande method modified by Proszynski,
– total cadmium content with AAS method using acetylene-air flame for atomization (Philips PU 9100x apparatus) following previous soil solution in a mixture of concentrated nitric(V) and chloric(VII) acids in 2:1 ratio [5].

The obtained results were subjected to statistical analysis using Statistica 6.1 programme; simple correlation coefficients were computed and their significance was determined by t-Student test. Cadmium accumulation indices were also computed as a ratio of the element content in surface and bottom horizons of the profile. Surfer 8.0 software was used to create maps of cadmium occurrence in the park area.

Results and discussion

Cadmium content in surface horizons of the analyzed soils ranged from 0.4 to 12.8 $mg \cdot kg^{-1}$ (Table 1). In most cases it is a definitely higher content than mean cadmium concentrations in the soils of Poland, ie 0.2–2 $mg \cdot kg^{-1}$ [6]. Such high contents are due to the element deposition from the Olkusz region, which is the colour metal processing centre [7]. The results of investigations on the Ojcow National Park (ONP) soil pollution are identical with the analysis of heavy metal concentrations in moss tissues in the area of Poland conducted by Grodzinska [8] in the late nineties of the previous century. The highest cadmium contents (over 1.5 $mg \cdot kg^{-1}$) were assessed in plant samples collected in the Ojcow National Park.

Table 1

Cadmium content in surface horizons of the Ojców National Park soils

Soil type	Horizon	Range of cadmium content (average) [$mg \cdot kg^{-1}$]
Rendzinas	O	1.0–1,1 (1.11)
	A	0.4–3.0 (1.18)
Lessive soils	O	12.8
	A	1.7–7.0 (3.72)
Brown soil	A	4.4
Pseudogley soil	O	1.3
River alluvial soil	A	1.9

The highest cadmium content, on average 4.42 $mg \cdot kg^{-1}$ (ranging from 1.7–12.8 $mg \cdot kg^{-1}$) was characteristic for surface horizons of rendzinas, slightly lower quantities of this element were found in lessive soils, respectively 1.15 mg · kg^{-1} (0.4––3.0 mg · kg^{-1}) (Table 1). The higher content of the analyzed element in rendzinas results from its elevated natural content in Jurassic limestones, which in the north-western part of the Malopolska region are in contact with Trias rocks constituting the source of colour metal ores [7].

In compliance with the Decree of the Minister of the Natural Environment on the soil quality standards and earth quality standards, the soils of national parks are classified to group A soils, where limit numbers for toxic substances content are especially strict [9]. Cadmium content in the group A, above which the soils are considered as polluted, is 1 mg · kg^{-1}. On the basis of the above – mentioned regulation, as many as 21 profiles of the analyzed Ojcow National Park soils should be regarded as polluted with cadmium. Only 3 lessive soils profiles were characterized by the content lower than 1 mg · kg^{-1}, therefore may be counted among the unpolluted soils.

When limit numbers for heavy metal content in arable soils as suggested by IUNG were applied, only 3 soils lessives profiles could be counted among the soils with natural cadmium concentrations [11]. 10 profiles (including 4 soils lessives profiles, 4 rendzinas profiles, brown soil and alluvial soil) were classified to the soils with elevated Cd concentrations (I category). 10 profiles of the tested soils, in which 9 were rendzinas were counted among weakly polluted soils (II category) and medium polluted (III category).

Analysis of the spatial distribution of Cd in the ONP area shows that the analyzed element accumulation was the highest in the park soils collected in the area of Czajowice and Skala villages (Fig. 1). Higher concentrations were assessed in the profiles collected in the hilltop parts of the park than in the Pradnik River or Saspowka River Valley. More exposed surface features of the park are more liable to deposition of pollutants flowing from the west.

Accumulation index (AI) computed for the analyzed soils was between 0.2 and 15.9 (Fig. 2). Mean accumulation index was higher for soils lessives (6.07) than for rendzinas (3.19) (Fig. 2). Soils lessives characterized by a relatively low Cd content in their surface horizons, were underlain by carbonate rocks in which higher heavy metal

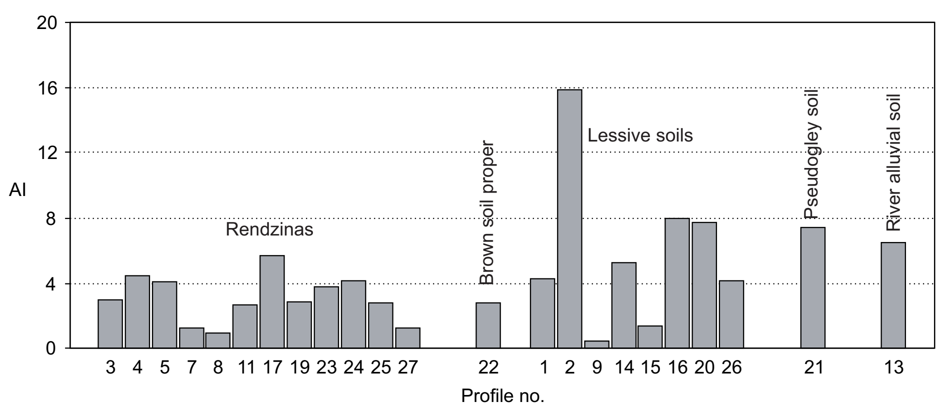

Fig. 2. Accumulation index (AI) calculated for investigated soils

concentrations occur [7]. Low values of AI in rendzinas result both from metal release also in the surface horizons and high permeability of these soils' profiles [12].

On the basis of limit numbers referring to AI suggested by Southerland [13], 5 profiles were counted among the soils with minimum accumulation, 11 to soils with moderate accumulation and 7 to soils with considerable metal accumulation (Table 2). As many as 9 profiles of the analysed rendzinas revealed a moderate or considerable heavy metal accumulation.

Table 2

Classification of cadmium accumulation degree in soils of the Ojców National Park on the base of AI level

Cadmium accumulation degree	Soil type			Total
	Rendzinas	Lessive soils	Other	
Minimal	3	2	0	5
Moderate	8	2	1	11
High	1	4	2	7

On the basis of the conducted statistical analysis it was found that cadmium content in the investigated soils depended mainly on the soil pH (0.56**) and clay fraction content (< 0.02 mm) (0.48**) (Table 3). It corroborates the results of research on cadmium contamination in forest soils of France [12].

Table 3

Dependence of cadmium content and chosen soil properties

Soil properties	Linear correlation coefficients		
	Rendzinas (14 profiles)	Lessive soils (8 profiles)	Total (24 profiles)
pH_{H_2O}	–0.02	0.94***	0.56**
pH_{KCl}	–0.01	0.85**	0.56**
% C_{org}	0.90***	–0.21	0.28
% N	0.87***	–0.39	0.52
C/N	0.75**	0.26	0.03
% fraction < 0.02	0.08	–0.16	0.48**
% fraction < 0.002	0.90***	0.19	0.35

* significance level 0.05; ** significance level 0.01; *** significance level 0.001.

Conclusions

1. A majority of analysed soils of the Ojcow National Park was considered as polluted with cadmium. It was assessed that the analyzed element accumulation in the investigated soils was moderate or considerable.

2. Accumulation of the analysed element was the highest in the hilltop parts of the park situated in its northern and western regions.

3. Higher cadmium content was assessed in the surface horizons of rendzinas than in lessive soils, whereas the average accumulation index values were the opposite.

4. Cadmium concentrations in the analyzed soils depended mainly on soil reaction and clay fraction content (< 0.02 mm).

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KADM W GLEBACH OJCOWSKIEGO PARKU NARODOWEGO

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Abstrakt: Celem badań było określenie zanieczyszczenia kadmem gleb Ojcowskiego Parku Narodowego i określenie wpływu położenia, typu i właściwości gleb na stopień zanieczyszczenia tym pierwiastkiem. Badania zostały przeprowadzone na próbkach glebowych pochodzących z 24 profilów glebowych reprezentujących główne jednostki typologiczne pokrywy glebowej parku: rędziny (13 profilów), gleby płowe (8 profilów), gleby brunatne właściwe (1 profil), gleby opadowo-glejowe (1 profil) i mady (1 profil). Zawartość kadmu w poziomach powierzchniowych badanych gleb wynosiła od 0,4 do 12,8 $mg \cdot kg^{-1}$. Na podstawie porównania z normami zaproponowanymi przez IUNG oraz zdefiniowanymi w Rozporządzeniu Ministra Środowiska z 2002 w sprawie standardów jakości gleby oraz standardów jakości ziemi, stwierdzono, że prawie wszystkie (oprócz 2 profilów gleb płowych) badane gleby (24 profile) Ojcowskiego Parku Narodowego należy uznać za zanieczyszczone kadmem. Zawartość kadmu była największa w glebach parku pobranych w rejonie Czajowic oraz Skały. W rędzinach oznaczono wyższą zawartość badanego pierwiastka niż w glebach płowych. Z kolei obliczony wskaźnik akumulacji był większy dla gleb płowych niż dla rędzin. Według przeprowadzonej analizy statystycznej stwierdzono, że zawartość kadmu w badanych glebach zależała od odczynu i zawartości frakcji < 0,02 mm.

Słowa kluczowe: kadm, gleby, zanieczyszczenie, Ojcowski Park Narodowy

ECOLOGICAL CHEMISTRY AND ENGINEERING A

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INFLUENCE OF A POLYMER SUPERSORBENT ON SELECTED PHYSIOLOGICAL FEATURES OF STRAWBERRY

WPŁYW SUPERSORBENTU POLIMEROWEGO NA WYBRANE CECHY FIZJOLOGICZNE TRUSKAWKI

Abstract: The aim of the studies carried over 2007–2008 was to evaluate the physiological reaction of strawberry var. 'Elsanta' to the addition of AgroHydrogel to the medium. In the vegetation hall of the West Pomeranian University of Technology, a vegetation pot experiment in the system of complete randomization in four replications was carried out. The experimental factor was the addition of AgroHydrogel to the medium. Two doses were used (15 and 30 g per Kick's vessel) against the control, ie the medium without gel. During the vegetation season of plants, the content of assimilation pigments (chlorophyll *a*, *b*, total and carotenoids) in leaves was determined three times. Parameters of water balance – the index of relative water content and the water saturation deficit in tissues of leaves and the area of the leaves were also defined.

Keywords: strawberry, AgroHydrogel, assimilation pigments, water balance, leaf area

A factor affecting, to a significant degree, the yields of berry plants, among them strawberries, is water deficit. A characteristic feature of this species is its large sensitivity to drought and this results in decreasing the quantity and quality of crops [1]. Water and air properties of soil can be improved by using preparations that increase its water volume. These are so called hydrogels or supersorbents [2, 3]. These compounds are polymers capable of storing gravitational water, limiting the level of free water in favour of water accessible to the root system of plants. A positive feature of sorbents is also their capability of maintaining optimum water and air conditions of the medium, even at its strong compaction [4]. They can also enrich the soil with mineral components and be favourable to its rational relations [5].The available literature does not present explicit conclusions as to the effect of hydrogels on physiological features of plants, deciding about their productivity.

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The aim of the studies was to assess the effect of polymer supersorbent called AgroHydrogel, added to the medium, on selected physiological features of strawberry (*Fragaria ananassa* Duch) var. 'Elsanta'.

Material and methods

In 2007–2008 a vegetation experiment in the system of complete randomization was carried out in four replications in the vegetation hall of the West Pomeranian University of Technology in Szczecin.

The objective of the research was strawberry var. 'Elsanta'. The first experimental factor was the addtion of polymer supersorbent AgroHydrogel to the medium. Two levels were used: 1.8 and 3.6 g $\cdot$ dm^{-3}, ie 15 and 30 g per Kick's container, against the control that was the medium with no gel added. The 10 dm^3 capacity pots were filled with 8 dm^3 of soil material. Before the pots were filled, hydrogel had been added to the medium and then all was mixed thoroughly. Table 1 shows the characteristics of the soil material.

Table 1

The properties of soil

pH		Percent of clay fraction [%]	S_o [g/cm^3]	Pkw [%]	Pkv [%]	Wtw [%]	Wtv [%]
H_2O	KCl						
6.58	5.97	21	1.24	24.7	34.0	32.9	38.0

S_o – bulk density, Pkw – capillary weight, Pkv – capillary volume, Wtw – total water capacity in investigated soil – weight, Wtv – total water capacity in investigated soil – volume.

Mineral fertilizing was in the doses of 50, 80 and 100 kg NPK $\cdot$ ha^{-1}. Fertilizing with potassium, phosphorus and half a dose of nitrogen was used before planting.The soil was top dressed with half a dose of nitrogen before the blooming of plants.

In the second decade of April 2007 seedlings were placed in containers: 1 item per pot. The experiment was carried out in a roofed place. The plants wintered in an unheated greenhouse. The soil mixture was measured using contact soil tensometers. The plants were watered by 0.5 dm^3/pot, when the tensometer, which was fixed in the medium with 15 g of gel per pot, showed 450 hPa.

In both years of studies, three times during plants vegetation: in the second decade of May (blooming phase) – date I, June (fruiting phase) – date II and July (post fruiting phase) – date III, the content of assimilation pigments (chlorophyll *a*, *b*, total, carotenoids) was determined and the water balance parameters – the index of *relative water content* (RWC) and the *water saturation deficit* (WSD) of leaves tissues, were defined [6]. The research material was taken from three representative plants (healthy, full-grown leaves were selected) from each experimental variant. To determine the content of assimilation pigments the Lichtenthaler and Wellburn's method was used [7]. According to Arnon et al [8] the content of chlorophyll and carotenoids were calculated. After fruit picking (second decade of July), the total surface of leaf laminas of individual plants, the assimilation surface of individual plants and the number of leaves on a plant (both features were determined in three replications) were also defined. The area of leaf laminas were determined by the DIAS computer apparatus.

In order to compare experimental objectives as to the results of the content of assimilation pigments, the two factor analysis of variance was used (the first experimental factor – the quantity of the dose of AgroHydrogel, the second factor – the term of the measurement). While for the results of assimilation surface and the number of leaves, the one factor analysis was carried out. The significance of differences between the averages was defined by means of Duncan's test at a level of significance $\alpha = 0.05$. Due to homogeneity of the error variance, synthesis of the results of two year research was carried out [9].

Results and discussion

The addition of AgroHydrogel in a dose of 15 g per pot did not effect significantly the content of chlorophyll *a*, *b* and total in leaves of the examined variety of strawberry. No significant effects of 30 g dose of supersorbent on these physiological features were observed, either. Only in the case of chlorophyll *b*, its slightly larger content was noticed in the leaves of strawberry growing in the medium with 30 g of supersorbent added, than in the plants of the remaining experimental variants. It amounted to 0.553 $mg \cdot g^{-1}$ of fresh matter. As to the concentration of carotenoids, despite the fact that the statistical analysis did not show any significance of differences, a slightly smaller amount of these pigments (0.712 $mg \cdot g^{-1}$ of fresh matter) was observed in leaves of control plants, than in plants growing in the medium with AgroHydrogel added (Table 2).

Table 2

Content of chlorophyll *a*, *b*, *a* + *b* and carotenoids in leaves of strawberry variety 'Elsanta' [$mg \cdot g^{-1}$ fresh matter]

Dose of AgroHydrogel	Date of measurement			Mean
	I	II	III	
		Chlorophyll *a*		
Control	1.240 ab*	1.487 b	1.068 a	1.265 a
15 g per pot	1.230 ab	1.266 ab	1.266 ab	1.254 a
30 g per pot	1.405 b	1.195 ab	1.088 a	1.229 a
Mean	1.292 ab	1.316 b	1.141 a	
		Chlorophyll *b*		
Control	0.469 ab	0.649 c	0.450 a	0.523 a
15 g per pot	0.460 a	0.540 abc	0.540 abc	0.513 a
30 g per pot	0.542 abc	0.503 abc	0.614 bc	0.553 a
Mean	0.490 a	0.564 a	0.535 a	

Table 2 contd.

Dose of AgroHydrogel	Date of measurement			Mean
	I	II	III	
Chlorophyll *a* + *b*				
Control	1.709 ab	2.136 c	1.518 a	1.787 a
15 g per pot	1.690 ab	1.806 abc	1.806 abc	1.767 a
30 g per pot	1.947 bc	1.806 ab	1.702 ab	1.782 a
Mean	1.782 a	1.880 a	1.675 a	
Carotenoids				
Control	0.729 abc	0.802 bcd	0.605 a	0.712 a
15 g per pot	0.818 cd	0.687 abc	0.687 abc	0.731 a
30 g per pot	0.931 d	0.645 abc	0.617 ab	0.731 a
Mean	0.826 b	0.712 a	0.636 a	

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

The term of leaves gathering did not have any significant effect on the content of chlorophyll *b* and total. In the case of chlorophyll *a*, a significant decrease in its content was observed in leaves of plants gathered after fruiting, as compared with the amount during fruiting. The significantly largest amount of carotenoids (0.826 mg $\cdot$ g^{-1} of fresh matter) was noticed in the phase of blooming (Table 2).

The largest index of relative water content (RWC), and thus the smallest index of water saturation deficit (WSD), was observed in all the experimental variants after strawberry fruiting, whereas the smallest during the phase of blooming.

The 15 g dose of AgroHydrogel per pot did not cause considerable changes in the water balance of the studied plant. Only during the phase of fruiting, a slightly larger index of water saturation (89.67 %) was observed in plants with this dose of supersorbent than in control plants (88.08 %) – Fig. 1. Whereas the application of larger dose of AgroHydrogel (30 g per pot), in all the analysed growth phases of strawberry, had an unfavourable effect on the parameters of water balance, causing an increase in the index of water saturation deficit WSD, and thus a decrease in RWC. It was particularly distinct during the phase of blooming, when the index of water saturation deficit at this dose of AgroHydrogel was by 8.07 % larger than at the dose of 15 g per pot (Fig. 2). Such a relationship can prove that the 30 g dose of supersorbent could cause disturbances in water-air relations in the medium and that resulted in unfavourable parameters of water balance. Similar results of the research on the effect of hydrogel on the growth of tobacco were obtained by Kowalczyk-Jusko and Koscik [3].

No significant effect of the addition of AgroHydrogel on the total assimilation area of the leaves of examined strawberry variety was observed. However, the 30 g dose of supersorbent caused a significant decrease in the number of leaves on an individual

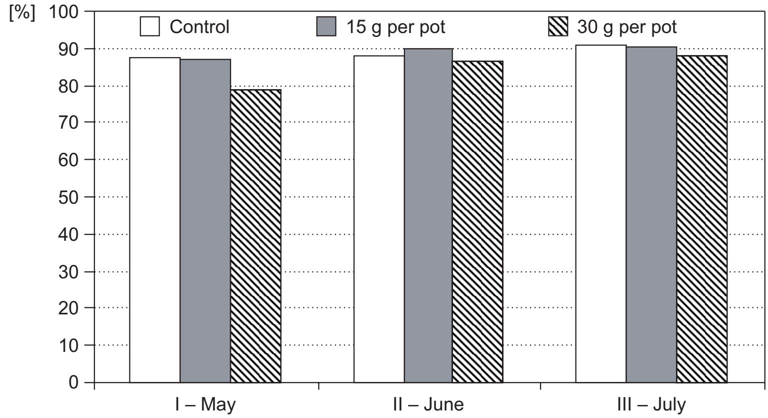

Fig. 1. Index of relative water content (RWC) in leaves of strawberry variety 'Elsanta'

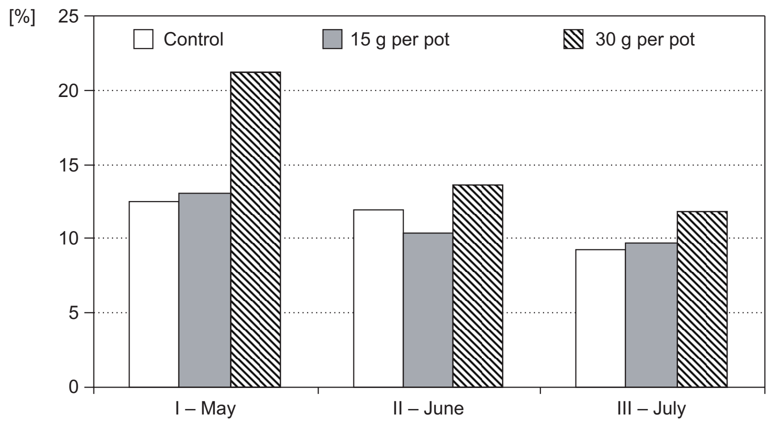

Fig. 2. Index of water saturation deficit (WSD) in leaves of strawberry variety 'Elsanta'

plant (27.5 pieces) and at the same time, a significant increase in the quantity of assimilation area of an individual leaf (55.8 dm^2) (Table 3).

Table 3

Area and number of the leaves of strawberry variety 'Elsanta'

Dose of AgroHydrogel	Quantity of the leaves	Area of the leaves [$cm^2 \cdot plant^{-1}$]	Area of the leaf [cm^2]
Control	38.5 a	1653 a	42.9 a
15 g per pot	33.5 a	1658 a	49.3 a
30 g per pot	27.5 a	1412 a	55.8 a
Mean	33.2	1574	49.3

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

According to Makowska [10], the application of larger doses of hydrogel leads to a stronger binding of water by a large amount of hydrogel particles and that can have an unfavourable effect on the growth of plants. Thus, it seems very important to choose appropriate quantities of polymer supersorbent, depending on its type and on physical and chemical properties of the medium and the needs of species and varieties of the plant.

Conclusions

1. No significant effect was observed of the applied supersorbent on the content of assimilation pigments in leaves of strawberry var. 'Elsanta'.
2. The largest amount of chlorophyll *a* was characteristic of the leaves of the investigated variety during the fruiting phase, whereas the largest content of carotenoids was characteristic of the blooming phase.
3. The 15 g dose of AgroHydrogel per pot did not affect the parameters of water balance of strawberry. While the application of 30 g of this supersorbent resulted in an increase in the index of water saturation deficit WSD.
4. No significant influence was shown of AgroHydrogel on the quantity of the total assimilation area of strawberry leaves.
5. The addition of 30 g of supersorbent per pot had a significant effect on a decrease in the number of leaves on a plant and at the same time on an increase in the area of an individual leaf.

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WPŁYW SUPERSORBENTU POLIMEROWEGO NA WYBRANE CECHY FIZJOLOGICZNE TRUSKAWKI

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Abstrakt: Celem badań przeprowadzonych w latach 2007–2008 była ocena reakcji fizjologicznej truskawki odmiany 'Elsanta' na dodatek AgroHydrogelu do podłoża. W hali wegetacyjnej Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie przeprowadzono wazonowe doświadczenie wegetacyjne, w układzie kompletnej randomizacji, w czterech powtórzeniach. Czynnikiem doświadczalnym był dodatek

AgroHydrogelu do podłoża – zastosowano dwa poziomy (15 i 30 g na pojemnik Kicka) na tle kontroli – podłoże bez dodatku żelu. Trzykrotnie w czasie sezonu wegetacyjnego roślin określono zawartość barwników asymilacyjnych (chlorofilu *a*, *b*, całkowitego oraz karotenoidów) w liściach. Określono także parametry bilansu wodnego – wskaźniki względnej zawartości wody i deficytu wysycenia wodą tkanek liści.

Słowa kluczowe: truskawka, AgroHydrogel, barwniki asymilacyjne, bilans wodny, powierzchnia liści

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BIOINDICATION OF HEAVY METALS POLLUTION IN THE TOWNS: BEDZIN AND CZELADZ

BIONDYKACJA ZANIECZYSZCZENIA METALAMI CIĘŻKIMI BĘDZINA I CZELADZI

Abstract: The analysis of heavy metals in plant leaves is suggested as a method for the identification of polluted areas. We determinted the following heavy metals: Zn, Pb and Cd concentrations in birch *Betula pendula* leaves, a common species found in urban parks and postindustrial areas in Poland. Samples of soil and birch leaves were collected in September 2007 from more and less polluted sites in Bedzin and Czeladz (Silesian province, southern Poland, 8 stands for each town). Concentrations of Zn, Pb and Cd in leaves and the upper layer of soil (0–10 cm) were determined. The heavy metal pollution of the investigated cities was on a comparable level. The obtained Cd and Pb concentrations in leaves were below the toxic range. Only Zn concentrations in some sites were higher than the level considered as toxic. Pb and Cd levels determined in the investigations exceed acceptable metal concentrations for soil in more stands in Bedzin.

Keywords: heavy metals, *Betula pendula* Roth., pollution

Foliage analysis has been used as a valid indicator of air pollution and in order to identify and possible polluted areas classification according to their pollution level [1–4]. Some investigations were performed on trees (common birch, *Betula pendula* Roth, Scots Pine, *Pinus sylvestris* L., Black Locust, *Robinia pseudoacacia* L., Sycamore Maple, *Acer pseudoplatanus* L., European Beech, *Fagus sylvatica* L., Norway Spruce, *Picea abies* (L.) Karst) and in the leaves of shrubs (Common Snowberry, *Symphoricarpos albus* (L.) Blake, Common Privet, *Ligustrum vulgare* L., White Syringa, *Philadelphus coronarius* L.) [5] in the Silesian towns, in Krakow, in municipal parks, in nature reserves and in the vicinity of industrial plants, to assess the heavy metal pollution level [4, 6–8]. Also the soil pollution with heavy metals such Zn, Cd, Pb, Cu and Cr was a concern. The soil contamination with metals results from local sources such as: industry, waste incineration, combustion of fossil fuels and traffic [9].

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Common birch*, Betula pendula* Roth. is a pioneering species on dumps and other postindustrial areas [9, 10]. It is a common plant in the cities too. It is postulated to plant it in a town green areas such as parks and squares and in larger distances from traffic routes [11, 12]. The investigations were performed in Czeladz and Bedzin, which are the oldest towns in the Zaglebie Dabrowskie region (Silesian province, southern part of Poland) [13]. The town Czeladz is exposed to low emission, ambient concentration from neighbouring towns and in Bedzin from emitters such as the power plant "Lagisza", heat and power plant, non-ferrous plant and others [14]. The aim of this study was to assess contamination of Bedzin and Czeladz by measuring zinc, lead and cadmium bioaccumulation in birch *Betula pendula* Roth. leaves and their content in soil samples from the upper layer (0–10 cm).

Material and methods

The *Betula pendula* Roth. leaves and soil samples from the upper layer (0–10 cm) were collected in September 2007. The birch (*Betula pendula* Roth.) leaves were collected randomly from ten trees situated in parks, plantings next to roads and in the vicinity of emitters. In each town 8 investigation sites were established. These sites exhibited varied heavy metal contamination level.

The air dried soil samples, sieved on a 1mm sieve was used to determine pH values in H_2O. 10 g soil samples were extracted with 10 % HNO_3. The concentration of metals Zn, Cd and Pb was measured with the flame Atomic Absorption Spectrometry (AAS) method [15]. Soil pH was measured in water (1: 2.5 soil:water ratio) using a pH meter, and organic matter content (expressed in %) was estimated by Ostrowska's methods [15]. In order to determine the heavy metal concentration, the plant material was washed in tap and distilled water, dried in 105 ^{o}C to constant weight and ground to fine powder, then mineralized and dissolved in 10 % HNO_3. After filtration Zn, Pb, and Cd content was measured using the flame Atomic Absorption Spectrometry (AAS) [mg/kg d.m.] [15, 16]. The quality of the analytical procedure was controlled by using samples of the reference material in each series of analysis. (Certified Reference material CTA-OTL-1 Oriental Tobacco Leaves) All plant and soil samples were studied in six replications. The data were processed using software Statistica to compute significant statistical differences between samples ($p < 0.05$) according to Tukey's multiple range test and to compute Pearson's correlation coefficients. Figures 1–6 were drawn with the use of Surfer 8 program.

Results and discussion

Zn concentration in the upper layer soil sample was higher in Czeladz than in Bedzin It ranged from 60.5 (power station "Lagisza") to 582.8 mg/kg (the park Zamkowa hill) in Bedzin and from 95.5 (Wiosenna (Staw) to 945.95 mg/kg (park Grabek) in Czeladz. Pb and Cd content in soil was on a comparable level in both towns. Pb content in soil sample varied from 61.5 (power plant) to 219.7 mg/kg (Sw. Dorota hill) in Bedzin and as 30 Wiosenna (Staw) to 283.2 mg/kg (Borzecha hill) in Czeladz. The highest

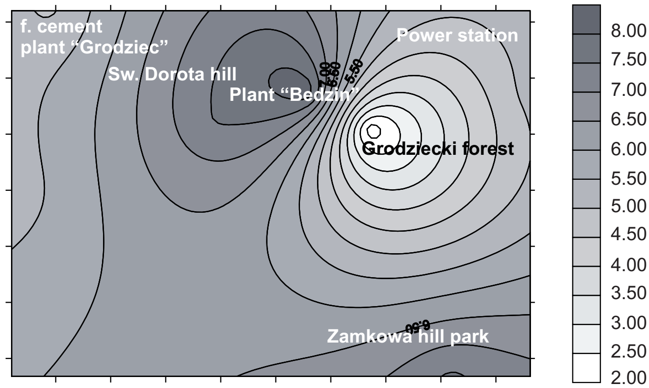

Fig. 1. Pb contamination in Bedzin based on metal content in birch leaves [mg/kg d.m.]

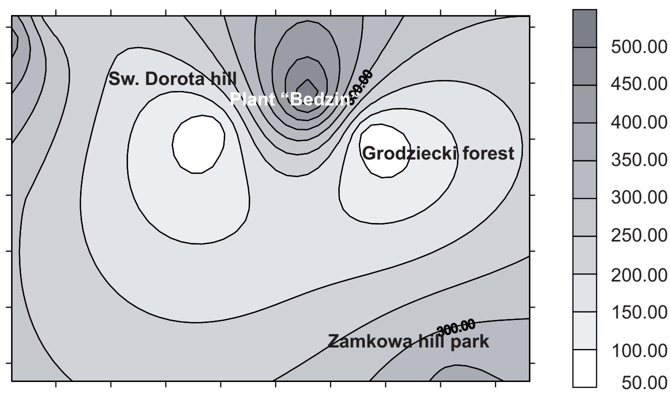

Fig. 2. Zn contamination in Bedzin based on metal content in birch leaves [mg/kg d.m.]

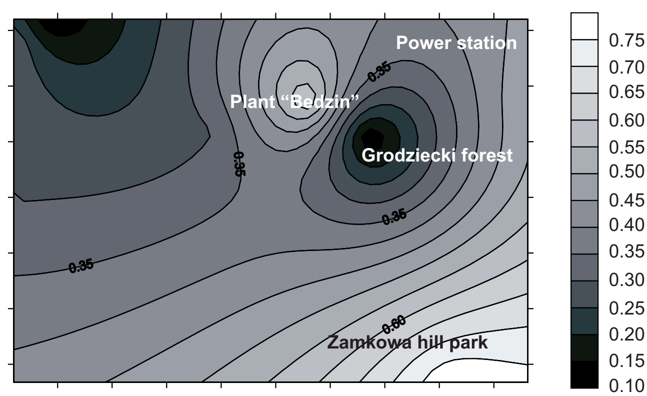

Fig. 3. Cd contamination in Bedzin based on metal content in birch leaves [mg/kg d.m.]

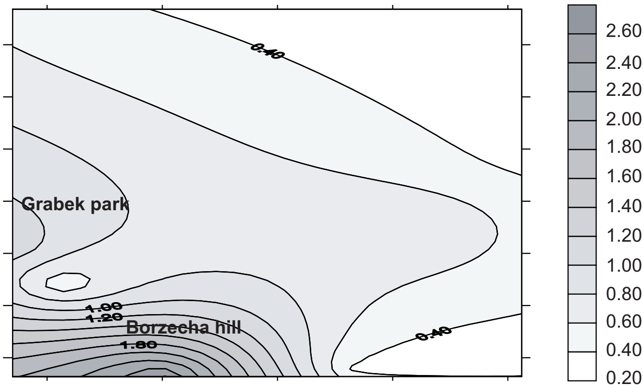

Fig. 4. Cd contamination in Czeladz based on metal content in leaves of birch [mg/kg d.m.]

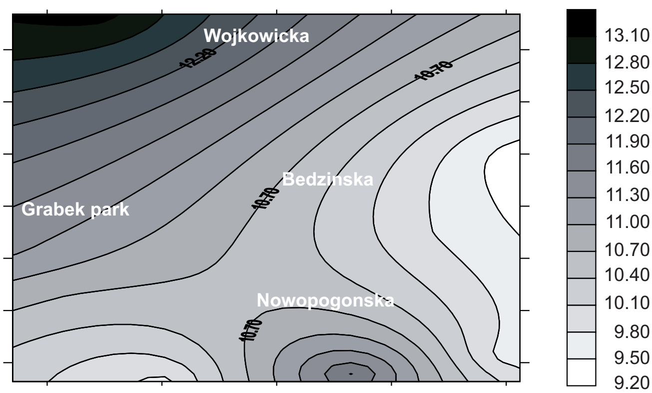

Fig. 5. Pb contamination in Czeladz based on metal content in leaves of birch [mg/kg d.m.]

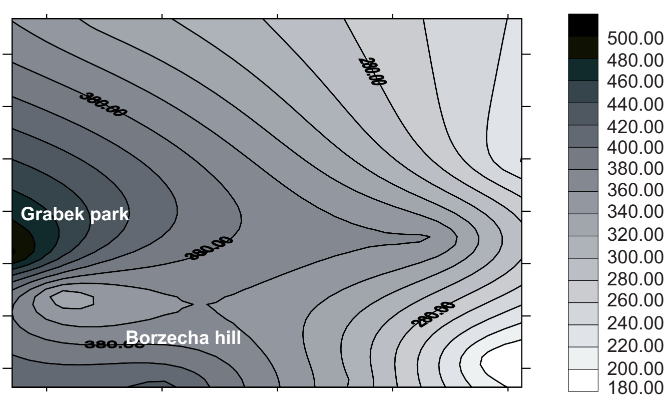

Fig. 6. Zn contamination in Czeladz based on metal content in leaves of birch [mg/kg d.m.]

concentration of Cd was found in Sw. Dorota hill (21.3 mg/kg) and the lowest 4.4 mg/kg (former cement plant) in Bedzin. Cd concentration in soil samples in Czeladz ranged from 14.1 (park Grabek) to 1.55 mg/kg Wiosenna (Staw) (Table 1, 2) [16, 17].

Table 1

Zn, Pb and Cd content, organic matter content and pH value in soil samples (upper layer) in Bedzin

	Zn	Pb	Cd	Organic matter content	pH value
Former mine Grodziec	184.1 ± 21 a	180.7 ± 17 a	11.9 ± 0.9 a	9.1 ±0.2 a	7.6 ± 0.02 a
Sw. Dorota hill	825.3 ± 32 b	219.7 ± 12 b	21.3 ± 0.3 b	4.1 ± 0.2 b	7.7 ± 0.03 b
Grodziecki forest	128.3 ± 26 c	179.9 ± 15 a	9.4 ± 0.5 c	3.6 ± 0.04 c	7.2 ± 0.04 c
Rozkowka park	149.2 ± 31 c	166.2 ± 16 ad	5.8 ± 0.9 d	7.1 ± 0.02 d	7.3 ± 0.01 c
Plant “Bedzin”	181.3 ± 36 a	181.6 ± 13 a	6.8 ± 0.8 de	4.8 ± 0.3 e	7.3 ± 0.05 c
Power plant “Lagisza”	60.5 ± 29 d	61.5 ± 12 c	12.4 ± 1 a	3 ± 0.09 f	6.8 ± 0.02 d
Former cement plant “Grodziec”	164.3 ± 21 b	149.2 ± 14 d	4.4 ± 1.5 d	6.4 ± 0.02 g	7.5 ± 0.01 a
Zamkowa hill park	582.8 ± 30 e	198.6 ± 17 a	4.6 ± 0.9 d	7 ± 0.04 d	7.3 ± 0.04 c

Values with the same letter are statistically the same for $p < 0.05$.

Table 2

Zn, Pb, Cd content, organic matter content and pH value in soil samples (upper layer) in Czeladz

	Zn	Pb	Cd	Organic matter content	pH value
Wojkowicka	257.4 ± 0.12a	87.14 ± 6.25 a	3.78 ± 0.1a	4.4 ± 0.1 a	7.43 ± 0.03 a
Bedzinska	510.6 ± 1.6b	181.2 ± 5.51 b	6.6 ± 1.6 b	9.03 ± 1.02 b	7.56 ± 0.1ab
Park Kosciuszki near Katowicka street	353.95 ± 0.12 c	91.76 ± 18.51a	3.85 ± 0.12 a	5.45 ± 0.13 c	7.3 ± 0.05 a
Park Grabek	945.95 ± 0.16 d	280.2 ± 6.08 c	14.1 ± 0.16 c	7.65 ± 0.7 b	6.61 ± 0.1 c
Borzecha hill	731.55 ± 0.10 e	283.2 ± 5.38 c	8.56 ± 0.11 d	7.97 ± 0.22 b	7.03 ± 0.11 d
(Wiosenna) Staw	96.5 ± 0.17 f	30.025 ± 6.9 d	1.55 ± 0.17 e	3.12 ± 0.51d	7.26 ± 0.02 e
Nowopogonska	642.05 ± 0.32 g	119.85 ± 0.2 e	5.29 ± 0.32 b	5.31 ± 0.3 c	7.4 ± 0.1 ab
Park near Mickiewicza street	299.45 ± 0.04 h	86.88 ± 2.05 a	3.81 ± 0.043 a	3.06 ± 0.44 d	7.7 ± 0.1 b

Values with the same letter are statistically the same for $p < 0.05$.

The surface accumulation of heavy metals is characteristic of aerial deposition of metal particulates [7]. Heavy metal concentrations in the upper layer of soil which were much higher than the results obtained in this study were found in the soil samples from Piekary Slaskie (6–143 mg/kg Cd, 378–2465 mg/kg Pb, 2730–8725 mg/kg Zn). The loads of metals in the soils in Piekary Slaskie have increased during some decades because of the pollution from the mine and the lead zinc smelter. The obtained concentrations of Zn, Cd and Pb in the top soil were higher or on a comparable level to the content, which were found around steelworks KH "Katowice" in Dabrowa Gornicza in the vicinity of the investigated cities (Zn 40–120 mg/kg, 2–3 mg/kg Cd, 38–62 mg/kg Pb) [5, 7]. Wyzgolik noted similar or higher soil contamination results in Dabrowa Gornicza for 100–1000 Zn mg/kg [18]. The results of the metal concentration in the nature reserves "Las Murckowski" and "Ochojec" in Katowice were similar to the results obtained in the previous study (144–230 Zn, 115–150 Pb, 5,5–10 Cd mg/kg) [8]. Zn content in soil upper layer in most investigated stands in Czeladz and Zamkowa hill park and Sw. Dorota hill in Bedzin were higher than the level considered as permitted (300 mg/kg). Pb and Cd concentrations determined in our investigations exceed the acceptable metal concentrations for soil (100 mg/kg Pb, 4 mg/kg Cd) in most stands in Bedzin and in a half of the investigated stands in Czeladz [19, 20]. The soil pH in the studied cities were above 7 (see Table 1, 2). The sorption of Pb, Cd and Zn increases with increasing alkaline value and pH > 6.5 influenced the immobilizing action of Pb and Cd on the forms weakly dissolvable [7]. At pH value about 7 the influence of pH on heavy metals binding by organic matter is very strong [21].

The content of heavy metals in the leaves of birch was presented by means of isolines in the Figures 1–6. The Zn content noted in birch, *Betula pendula* Roth leaves ranged from 49,4–556 mg/kg d.m. in Bedzin and 178–506.75 mg/kg d.m. in Czeladz (Fig. 1–6). The Pb content in birch leaves ranged from 4.9–7.6 in Bedzin and 9.7–13.21 mg/kg d.m. in Czeladz. The Cd content in birch leaves was below 0.5 in most investigated stands in Bedzin and Czeladz (Fig. 1–6) [16, 17]. This study showed that the Zn concentrations noted for birch leaves were in the range (100–400 mg/kg d.m.) considered as toxic [22]. The Pb and Cd concentrations observed for birch leaves did not exceed toxical levels for plant tissues (5–30 mg/kg d.m. Cd, 30–300 Pb). Lead, cadmium and zinc concentration in birch leaves was the highest in the places in the vicinity of the emitter (non-ferrous plant "Bedzin") 556.3 mg/kg d.m. Zn, 6.2 mg/kg d.m. Pb, 0.35 mg/kg d.m. Cd or in the places which are near the roads – Zamkowa hill park in Bedzin 371 mg/kg d.m. Zn, 7.6 mg/kg Pb, 0.8 mg/kg Cd and in Czeladz Wojkowicka street (332.9 Zn, 13.21 mg kg d.m. Pb, 0.43 mg/kg d.m. Cd, Nowopogonska street 178.7 mg/kg d.m Zn, 9.7 Pb, 0.35 mg/kg d.m. Cd). Elevated concentration of Zn (506.75 park Grabek in Czeladz and 435.2 mg kg d.m. former coal mine "Grodziec" in Bedzin) was observed in the vicinity of former coalmines. Reiman et al investigated Cd, Zn and Ni in the leaves of birch (the contents were 0.13, 88.9, 109 respectively). Baycu et al in leaves of the investigated trees in Istambul noted Cd concentration between 0–1.33, Zn concentration 17.47–592.6 and Pb 3.99–34.4 mg/kg d.m. [23]. Lukasik et al [6] observed that Pb concentrations ranged as 13 in Sycamore Maple (*Acer pseudoplatanus* L.) to 53 mg/kg d.m. in Black Locust (*Robinia pseudoacacia* L.), Zn concentration ranged from 141 to 275 mg/kg d.m. and Cd concentrations were below 1 mg/kg d.m. in the tree leaves in Piekary Slaskie. Zn contents in birch (*B. pendula* Roth.) leaves in this study were on a comparable level to the results for this plant leaves in the vicinity of steelwork HK "Huta Katowice" [5, 7].

Heavy metals may be accumulated by plants from the air and soil. Our study confirmed the statement that leaf tissues may reflect the input of elements near emission sources because plants interact with their local environment [23]. As reported by Ross, aerial sources of metals can contribute > 90 % of Pb, > 80 % Zn present in the leaves of various plants [24]. A positive (not statistically significant) correlation between Zn, Cd and Pb content in soil and in leaves was found in Bedzin and Czeladz.

Conclusions

Biological responses (eg heavy metal bioaccumulation) allow for estimating the level of pollutants and their impact on biological receptors. It was shown that Pb in the plants and soil samples tends to increase as traffic density increases (eg Zamkowa hill park, Sw. Dorota hill; Wojkowicka, Nowopogonska streets). It was suggested that the concentrations of the investigated metals in plants and soils in the industrial regions depend on the traffic and on the pollutants coming from neighbouring industrial sources (eg non-ferrous plant ”Bedzin”, power station “Lagisza”, former mine, cement plant “Grodziec”).

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BIONDYKACJA ZANIECZYSZCZENIA METALAMI CIĘŻKIMI BĘDZINA I CZELADZI

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Abstrakt: Jako jedną z metod identyfikacji zanieczyszczonych powierzchni podaje się analizę koncentracji metali ciężkich w liściach roślin. Oznaczano zawartość metali ciężkich Zn, Pb i Cd w liściach brzozy brodawkowatej *Betula pendula* Roth. pospolitego gatunku występującego w parkach i terenach poprzemysłowych w Polsce. Próbki gleby i liści brzozy pobierano we wrześniu 2007 r. z mniej i bardziej zanieczyszczonych miejsc miasta Czeladzi i Bedzina (województwo śląskie, Polska południowa, 8 stanowisk dla każdego miasta). Mierzono zawartość Zn, Pb, Cd w liściach oraz wierzchniej warstwie gleby (0–10 cm). Stwierdzono podobne zanieczyszczenie Zn, Pb i Cd dla badanych miast. Zawartość Cd i Pb w liściach brzozy była poniżej zakresu stężeń uznawanego za toksyczny dla roślin. Tylko koncentracje Zn na kilku stanowiskach były większe od uznawanych za toksyczne. Dla większości stanowisk badawczych Bedzina stwierdzona w glebie zawartość Pb i Cd była powyżej stężeń uznanych za dopuszczalne.

Słowa kluczowe: metale ciężkie, *Betula pendula,* zanieczyszczenie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

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EFFECT OF SOWING DENSITY ON THE YIELD AND CHEMICAL COMPOSITION OF OAT GRAINS

WPŁYW GĘSTOŚCI SIEWU NA PLON I SKŁAD CHEMICZNY ZIARNA OWSA

Abstract: The research were aimed at determining the effect of sowing density on the yield and chemical composition of grain of two common oat forms. Two-factorial field experiment was set up on brown alluvial soils in 1999–2001 in southern Poland. The first experimental factor included 2 forms of oat: husked oat (cv. Dukat) and naked oats (2 genotypes POB-W-481, POB-W-492 and cv. Akt). The second factor included two levels of sowing density: 450 grains per 1 m^2 and 550 grains per 1 m^2. The analyzed forms differed significantly in their grain yields. The husked cv. Dukat (average yield for 3 years 3.62 $Mg \cdot ha^{-1}$) in all years of the experiment yielded 16.7–30 % higher when compared with cv. Akt (average yield for 3 years 2.97 $Mg \cdot ha^{-1}$) and the naked genotypes (average yield for 3 years POB-W-481 3.02 $Mg \cdot ha^{-1}$, POB-W-492 2.99 $Mg \cdot ha^{-1}$). Higher sowing density favorably increased the number of panicles formed per area unit (450 grains per m^2 – 319, 550 grains per m^2 – 333 panicles per m^2) and therefore gave higher grain yields (450 grains per m^2 – 3.09 $Mg\ h^{-1}$, 550 grains per m^2 – 3.21 $Mg \cdot ha^{-1}$). The compared cultivars differed considerably in their content of K, P, Ca and Mg. The naked forms had higher concentrations of K, P and Ca but lower of Mg. The analyzed sowing quantities did not affect significantly the content of macroelements in oat grain. However, in the first year of the trial significantly higher P and Mg contents were noted at lower sowing density.

Keywords: naked oats, husked oats, sowing density, grain yield, chemical composition

One of the main determinants of grain yield and a basic element of oat yield structure is sowing density [1, 2]. In addition, sowing density is the main factor determining the number of panicles per area unit [2, 3]. Technology of oat cultivation in the COBORU

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experiments assumes 500 germinating grains per area unit (m^2), irrespective of cultivar variety or habitat conditions. It results in elimination of too many experimental factors. However, in practice the sowing density should be adjusted to soil and climatic conditions, the sowing material used, the date of sowing and above all to the oat form and/or cultivar.

It has been assumed that in comparison with the other cereal species oat is particularly abundant in Ca, Fe, Zn and Mn, therefore it may be an important source of these minerals in human nutrition [4, 5].

The aim of the experiment was to assess grain yield and macroelement concentrations in grain of 2 oat forms: husked (cv. Dukat) and naked oats (2 genotypes POB-W-481, POB-W-492 and cv. Akt.), as affected by different sowing density.

Material and methods

The experiments were carried out over a 3-year period (1999–2001) using a split-plot design with 4 replications in Nawojowa near Nowy Sacz. The first experimental factor involved two forms of oat: husked cv. Dukat, and naked oats (genoptypes POB-W-481, POB-W-492 and cv. Akt). The second factor involved two sowing densities: lower 450 grains per m^2 and higher 550 grains per m^2. The forecrop for oat was winter wheat; after its harvesting first ploughing combined with harrowing was conducted, and subsequently winter ploughing in autumn. Soil loosening and harrowing were conducted in spring preceded by mineral fertilization. The soil abundance in P, K and Mg [mg/100 g] in the successive years of the experiment was as follows: 1999 – 30, 37, 15; 2000 – 18, 28.4, 11.9; 2001 – 6.5, 15.0, 11.5. Harrowing was made after sowing. Weed control was conducted at the tillering stage using Chwastox Turbo dosed at 2 dm^3/ha. The dates of sowing in the successive years differed greatly due to the weather conditions. (Table 1).

Table 1

Time of sowing of oat in the successive years of the experiment

Year	1999	2000	2001
Day and months	31 March	17 April	18 April

Prior to harvesting the number of grains per panicle and number of panicles per area unit were counted. Subsequently grain samples were collected, and the 1000 grain weight [g] was determined. Grain samples were analysed for macroelements (K, P, Ca, Mg, Na) using 989 Solar atomic absorption spectrophotometer (Unican). The data were subjected to ANOVA using STAT Skierniewice programme [6]. The significance of differences between means was detected using Tukey test at the level of $\alpha = 0.05$.

The course of weather condition in the successive years of the field experiment was presented in Figs. 1 and 2.

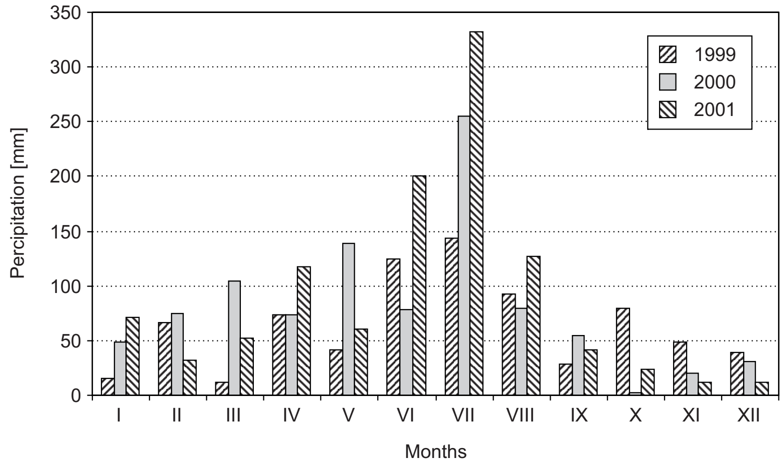

Fig. 1. Precipitations during the vegetation seasons 1999–2001

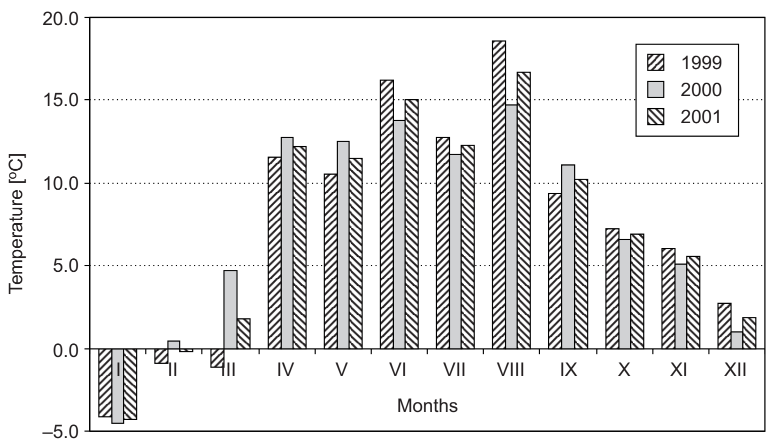

Fig. 2. Temperatures during the successive vegetation seasons 1999–2001

Results and discussion

The analyzed oat forms differed significantly in their grain yields. In all years of the experiment husked oat cv. Dukat gave 16.7 to 30 % higher yields than cv. Akt. and naked oat genotypes (Table 2). The course of the weather conditions was different in successive seasons. Notably, the first year of field experiments (1999) with lower precipitations and higher average air temperatures than in the years 2000 and 2001,

Table 2

Effect of years of the experiment and forms of oat on grain yield and its components

Years	Oat forms	Grain yield [Mg ha^{-1}]	Number of panicles per sq. meter	Number of grains per panicle	Mass of 1000 seeds [g]
1999	Dukat	4.02	356.4	34.5	28.6
	Akt	3.41	389.7	38.3	25.5
	POB-W-481	3.32	332.9	34.9	24.9
	POB-W-492	3.49	344.1	38.5	26.5
LSD for oat forms		0.36	35.4	3.3	2.8
2000	Dukat	3.64	363.4	34.6	39.7
	Akt	3.29	343.2	34.6	27.3
	POB-W-481	3.13	396.3	31.4	28.3
	POB-W-492	3.03	341.9	33.5	29.3
LSD for oat forms		0.30	56.1	ns	2.3
2001	Dukat	3.19	260.4	36.3	36.9
	Akt	2.21	276.5	36.9	27.7
	POB-W-481	2.65	270.3	37.7	25.9
	POB-W-492	2.45	235.8	36.6	27.6
LSD for oat forms		0.28	29.9	ns	3.7
Means for years 1999–2001	Dukat	3.62	326.7	35.1	35.1
	Akt	2.97	336.4	36.6	26.8
	POB-W-481	3.02	333.2	34.6	26.3
	POB-W-492	2.99	307.5	36.2	27.8
LSD for oat forms		0.16	23.5	1.9	1.4

n.s. – non significant

favorably affected the obtained yields (Figs. 1 and 2). From among the compared yield structure components, the number of panicles formed per area unit and *thousand grain weight* (TGW) proved statistically significant. High weight of a thousand grains of husked oat cv. Dukat had a significant influence on higher yields of this variety in all years of the experiment. Despite the largest number of panicles formed and number of grains per panicle, but very small TGW, cv. Akt gave the smallest yields among the compared cultivars and genotypes. Taking into account soil and adverse climatic conditions of the experimental site (Nawojowa), grain yields obtained in the present experiment can be considered good as compared with the yields commonly generated under sub-mountain conditions [7].

Greater sowing density (550 grains per m^2) favorably affected grain yields resulting in an increase in the number of panicles formed per area unit and subsequently greater grain yields (Table 3). On the other hand, different sowing densities had no effect on other components of yield structure: number of grains per panicle and TGW. Latest literature data concerning the influence of oat sowing density on grain yields are greatly diversified. Walens [8] who investigated the effect of nitrogen fertilization and sowing density on the amount and quality of husked and naked oats grain yield showed that increasing sowing density (400, 500 and 600 grains/m^2) had no effect on the yield of either husked cv. Deresz or naked cv. Akt. On the other hand, Dubis and Budzynski [9] demonstrated a significant increase in grain yield of cv. Akt as resulting from increased sowing density from 400 to 800 grains/m^2. Kozlowska-Ptaszynska et al [3] and Tobiasz-Salach and Bobrecka-Jamro [2], Leszczynska and Noworolnik [10] obtained similar results in studies on naked forms of oat. In case of husked forms, both Kozlowska-Ptaszynska et al [3] and Scigalska [11] state that traditional cultivars may produce satisfactory yields at a lower sowing density (ie 400 grains per m^2).

Table 3

Effect of years of the experiment and sowing density on grain yield its components

Years	Sowing density [grains per sq. meter]	Grain yield [Mg · ha^{-1}]	Number of panicles per sq. meter	Number of grains per panicle	Mass of 1000 seeds [g]
1999	450 550	3.52 3.60	347.4 364.4	37.3 35.8	26.0 26.7
LSD for sowing density		n.s.	n.s.	n.s.	n.s.
2000	450 550	3.26 3.28	352.3 370.1	33.9 33.1	31.1 31.1
LSD for sowing density		n.s.	n.s.	n.s.	n.s.
2001	450 550	2.50 2.75	256.9 264.6	36.7 37.0	29.5 29.5
LSD for sowing density		0.15**	n.s.	n.s.	n.s.
Means for years 1999–2001	450 550	3.09 3.21	318.9 333.0	36.0 35.3	28.9 29.1
LSD for sowing density		0.11	11.1	n.s.	n.s.

n.s. – non-significant.

The analyzed oat cultivars and genotypes differed significantly in their grain concentrations of K, P, Ca and Mg (Table 4). Naked oat forms had higher content of K, P and Ca but lower Mg in comparison with the husked forms; only in case of Na no statistically significant differences were found. From among the four compared cultivars and genotypes, the highest K and Ca concentrations were found in the naked cv. Akt. High concentrations of P, Mg, and Na were found in naked POB-W-481 genotypes.

The two sowing densities tested in the present experiment did not markedly affect the content of macroelements in oat grain, although in the first year of the investigations a significantly higher concentrations of P and Mg were registered at lower sowing quantity (Table 5). The content of macroelements in grain of the analyzed oat cultivars and genotypes is comparable to the results obtained by Pisulewska et al [5] in studies on chemical composition of cultivars and genotypes of common oats with yellow and brown-coloured husks and to the results presented by Witkowicz and Antonkiewicz [12] who analyzed the influence of five other agronomic factors on the content of mineral elements. The present results confirm the opinion that oat is a very good source of minerals and should be used in a far greater degree in human nutrition than has been used so far [4].

Table 4

The content of macroelements (g/kg d.m.) in different forms of oat in years 1999–2001

Years	Oat forms	Content				
		K	P	Ca	Mg	Na
1999	Dukat	2.473	3.01	0.677	0.826	0.018
	Akt	2.780	4.23	0.711	0.953	0.019
	POB-W-481	2.685	4.15	0.652	1.007	0.020
	POB-W-492	2.800	4.34	0.67.	0.974	0.017
LSD for oat forms		n.s.	0.07	0.050	0.039	n.s.
2000	Dukat	4.135	3.12	0.362	0.593	0.026
	Akt	4.125	3.85	0.331	0.682	0.024
	POB-W-481	4.103	4.04	0.279	0.703	0.024
	POB-W-492	4.100	4.03	0.288	0.681	0.026
LSD for oat forms		n.s.	0.54	n.s.	0.071	n.s.
2001	Dukat	3.618	3.36	0.759	0.871	0.026
	Akt	4.143	4.23	0.767	1.114	0.026
	POB-W-481	4.035	4.27	0.695	1.115	0.026
	POB-W-492	3.963	4.04	0.737	1.097	0.023
LSD for oat forms		0.514	0.92	0.066	0.038	n.s.
Means for years 1999–2001	Dukat	3.408	3.17	0.599	0.763	0.023
	Akt	3.683	4.11	0.603	0.916	0.023
	POB-W-481	3.608	4.15	0.542	0.941	0.023
	POB-W-492	3.621	4.15	0.565	0.917	0.022
LSD for oat forms		0.171	0.23	0.022	0.019	n.s.

n.s. – non-significant.

Table 5

Effect of years of the experiment and sowing density on content of macroelements in oat grain

Years	Sowing density [grains per sq. meter]	Content				
		K	P	Ca	Mg	Na
19993	450	2.720	4.00	0.686	0.948	0.019
	550	2.649	3.88	0.670	0.932	0.018
LSD for sowing density		n.s.	0.04	n.s.	0.012	n.s.
2000	450	4.134	3.77	0.320	0.654	0.025
	550	4.098	3.75	0.310	0.675	0.025
LSD for sowing density		n.s.	n.s.	n.s.	1.3	n.s.
2001	450	3.968	3.89	0.743	1.050	0.025
	550	3.911	4.06	0.736	1.047	0.025
LSD for sowing density		n.s.	n.s.	n.s.	n.s.	n.s.
Means for years 1999–2001	450	3.607	3.89	0.583	0.884	0.023
	550	3.553	3.90	0.572	0.885	0.023
LSD for sowing density		n.s.	n.s.	n.s.	n.s.	n.s.

n.s. – non-significant.

Conclusions

1. The studied forms of oat forms ie husked cv. Dukat, naked cv. Akt and two naked oat genotypes (POB-W-481 and POB-W-492) responded differently to the course of weather conditions in the successive years of the experiment. The husked cultivar gave the highest yields in the year with the greatest precipitations (2001), whereas the naked oat forms produced the highest yields in the season with the lowest precipitations (1999).
2. Of the two tested sowing densities, higher sowing density favorably affected the grain yields resulting from an increase in the number of panicles formed per area unit.
3. The tested naked forms of oat differed significantly in concentrations of K, P, Ca and Mg in grain in comparison with the husked form.
4. Different sowing densities had no effect on macroelement content in grain of the tested oat forms.

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WPŁYW GĘSTOŚCI SIEWU NA PLON I SKŁAD CHEMICZNY ZIARNA OWSA

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Abstrakt: Celem badań było określenie wpływu gęstości siewu na plon oraz skład chemiczny ziarna dwóch form owsa siewnego. Dwuczynnikowe doświadczenia polowe zakładano na madach rzecznych brunatnych w latach 1999–2001 w Polsce Południowej. Pierwszym czynnikiem badawczym były dwie formy owsa: oplewiona (odmiana Dukat) oraz nieoplewiona (2 rody POB-W-481, POB-W-492 i odmiana Akt). Czynnikiem drugim była zróżnicowana gęstość siewu: 450 ziaren na 1 m^2 i 550 ziaren na 1 m^2. Badane formy różniły się istotnie plonami ziarna. Oplewiona odmiana Dukat (średni plon z 3 lat 3,62 $Mg \cdot ha^{-1}$) we wszystkich latach prowadzenia doświadczeń plonowała wyżej o 16,7 – 30 % w porównaniu z odmianą Akt (średni plon z 3 lat 2,97 $Mg \cdot ha^{-1}$) i rodami nieoplewionymi (średni plon z 3 lat POB-W-481 3,02 $Mg \cdot ha^{-1}$, POB-W-492 2,99 $Mg \cdot ha^{-1}$). Większa gęstość siewu korzystnie wpłynęła na podniesienie liczby wiech wykształconych na jednostce powierzchni (450 ziaren na 1 m^2 – 319, 550 ziaren na 1 m^2 – 333 wiechy na 1 m^2) a tym samym na wyższe plony ziarna (450 ziaren na 1 m^2 – 3,09 $Mg \cdot h^{-1}$, 550 ziaren na 1 m^2 – 3,21 $Mg \cdot ha^{-1}$). Porównywane odmiany różniły się istotnie zawartością K, P, Ca i Mg. Formy nieoplewione miały wyższą zawartość K, P i Ca, ale niższą Mg. Badana gęstość siewu nie wpłynęła istotnie na zawartość makroskładników w ziarnie, chociaż w 1 roku badań stwierdzono istotnie większą zawartość P i Mg przy niższej ilości wysiewu.

Słowa kluczowe: owies nieoplewiony, owies oplewiony, plon ziarna, gęstość siewu, skład chemiczny

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RETRIEVAL IRRADIATED ENTOMOPAHTHOGENIC NEMATODES – *Steinernema feltiae* (Filipiev, 1934) FROM THE SOIL

ODZYSKIWANIE NAPROMIENIOWANYCH NICIENI ENTOMOPATOGENNYCH *Steinernema feltiae* (Filipiev, 1934) ZE ŚRODOWISKA GLEBOWEGO

Abstract: Infective juveniles *Steinernema feltiae* obtained in the field (Biała Podlaska) and from biopreparation Ovinema were used in the study. Gamma rays ie electromagnetic wave emitted by nuclei of excited radioactive atoms of ^{60}Co were used in experiments. The differences were revealed among applied doses and nematode strains response to irradiation. The same species may respond to a given factor in various ways. Confirmation of the phenomenon of radiation hormesis needs many repetitions and seems accidental. Changes in the nematode genotype under the effect of gamma radiation or of other mutagenic factors might be used in future production of biopreparations.

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

In the age of organic agriculture and nature protection chemical means of plant protection are used less commonly. One of the alternative insect pest control measures are entomopathogenic nematodes. Nematodes of the families *Steinernematidae* and *Heterorhabditidae* play important role in controlling the so-called soil pests which develop, at least partly, in the soil [1, 2]. The nematodes possess many excellent attributes of biological control factor. They are safe for the environment, have broad range of natural hosts, and are able to actively search, infect and kill the host insect. They are characterised by a high reproductive potential and produce dormant growth stages – an infective *dormant juvenile* (DJ) – which allow them to persist longer in the soil. Moreover, it is possible to multiply them in artificial media. They are safe for mammals and other non-target organisms [3].

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Increasing economic importance of entomopathogenic nematodes for agricultural practise creates a constant demand for new strains of high reproductive potential, endurance to handling during storage and transport and competitive (with chemical plant protection measures) efficiency in controlling harmful insects. Unfortunately, under specific field conditions commercial biopreparations are not effective.

Therefore, it seems reasonable to search for fast and effective method which would introduce changes of possibly narrow and easily controllable range into population. Ionising radiation is an abiotic factor which affects cell processes in nematodes and brings biological and chemical alterations within nematodes' tissue [4–6] Low doses of ionising radiation are known to bring positive effect and be safe. It was demonstrated that physiological responses of plants and animals to low radiation doses (*radiation hormesis*) are similar to the effects of many natural elements and chemical compounds which are nutrients at low concentrations but become toxic at higher concentrations.

The experiment was performed to check whether low doses of ionising radiation may improve pathogenic properties of nematodes and thus be used in biological plant protection and commercial production of nematodes.

Material and methods

Infective juveniles *Steinernema feltiae* obtained in the field (Biala Podlaska) and from the Ovinema biopreparation were used in the experiment. Cobalt bomb (RChM-γ-20) emitting γ radiation from ^{60}Co was a source of radiation. Three doses of radiation: 0.1, 0.05 and 0.01 kGy were applied. Non-radiated larvae of the nematode *S. feltiae* were used as a control. Experiment was carried out in 150 cm^3 pots with sterilised soil and 1000 L3 larvae per pot. Then two caterpillars of the greater wax moth *Galleria mellonella* were placed in every pot. Pots were checked after 48 hours. Infected individuals of *Galleria mellonella* were incubated for 24 hours in a SANYO chamber at 25 ^{o}C. Afterwards, insects were dissected to check whether nematodes were the reason of their death. The intensity of infection (the number of nematodes that penetrated insect's body) was also analysed to estimate the number of nematodes retrieved from the soil. The procedure was replicated three times every two days. The experiment was performed in nine pots in three repetitions.

Results and discussion

The highest percent of retrieved nematodes from every analysed variant was observed in the first sample, definitely lower in the second. Dissections of the third sample revealed only single adult individuals. In total from 22 % (control) to 27 % (0.01 kGy) nematodes *S. feltiae* Owinema were retrieved. *S. feltiae* from Biala Podlaska responded differently to gamma radiation. An increase in the applied dose negatively affected the percent of retrieved nematodes which varied from 38 % (0.05 and 0.1 kGy) to 56 % (control) (Fig. 2).

Experiments performed to test the effect of gamma radiation on active search for the host insect by nematode infective dormant juveniles not give obvious solution.

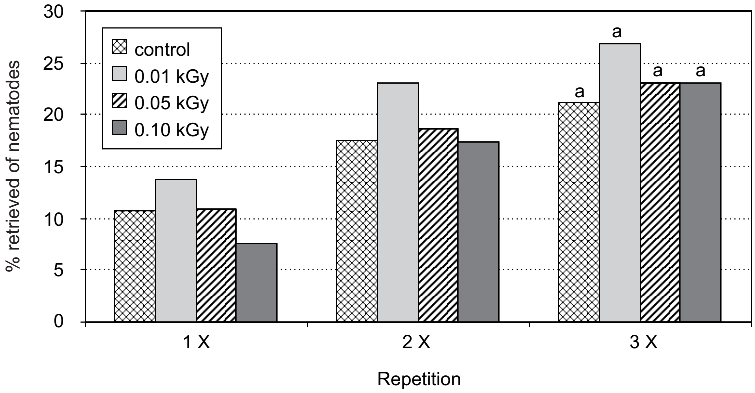

Fig. 1. Mean percent of *S. feltiae* Owinema retrieved from the soil after preliminary irradiation with different doses of ionising radiation (different letters denote significant differences at $p < 0.05$)

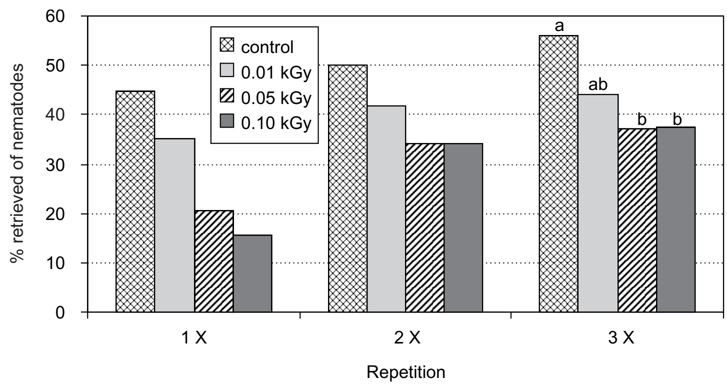

Fig. 2. Mean percent of *S. feltiae* Biała Podlaska retrieved from the soil after preliminary irradiation with different doses of ionising radiation (different letters denote significant differences at $p < 0.05$)

Strain-specific differences were found in the response of nematodes to ionising radiation. The larvae of *S. feltiae* obtained from the Ovinema biopreparation did not change their pathogenic properties. It even seems that the dose of 0.01 kGy exerted slightly positive effect. Resistance of this strain to abiotic factors is probably the result of its specialization, stability and long selection. The larvae of *S. feltiae* from Biala Podlaska responded quite differently. Applied doses negatively affected their invasiveness in the soil habitat. Two applied doses of gamma rays (0.1 and 0.05 kGy) caused changes significantly different from the control.

Positive effect of ionising radiation was hard to observe. The same species may respond to a given factor in various ways. Confirmation of the phenomenon of radiation hormesis needs many repetitions but the effect seems accidental. Changes in the nematode genotype under the effect of gamma radiation or of other mutagenic factors might be used in future production of biopreparations.

Conclusions

1. Nematodes *S. feltiae* from the Owinema biopreparation, are resistant to low doses of ionising radiation.

2. Nematodes *S. feltiae* obtained from the field (Biala Podlaska), change their pathogenic properties after exposure to low doses of ionising radiation.

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ODZYSKIWANIE NAPROMIENIOWANYCH NICIENI ENTOMOPATOGENNYCH *Steinernema feltiae* (Filipiev, 1934) ZE ŚRODOWISKA GLEBOWEGO

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Abstrakt: Przeprowadzono badania w celu odzyskania napromieniowanych nicieni entomopatogennych z gleby. Do doświadczenia użyto larw inwazyjne *S. feltiae* pochodzące z biopreparatu Ovinema oraz dziki szczep pozyskany z terenu (Biała Podlaska). Nicienie były poddane działaniu promieniowania gamma (źródło ^{60}Co). Wykazano różnice w działanie poszczególnych dawek promieni gamma oraz odmienne reakcje użytych szczepów nicieni na zastosowane promieniowanie.

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

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Monika RAJKOWSKA[1] and Mikołaj PROTASOWICKI

DISTRIBUTION OF SELECTED METALS IN BOTTOM SEDIMENTS OF LAKES INSKO AND WISOLA (POLAND)

ROZMIESZCZENIE WYBRANYCH METALI W OSADACH DENNYCH JEZIOR IŃSKO I WISOLA (POLSKA)

Abstract: The study was focused on the occurrence and geochemical partitioning of metals (Fe, Mn, Zn, Cu, Pb, Cd and Al) in the upper layer of sediments from mesotrophic Lake Insko and eutrophic Lake Wisola. In both lakes, the lowest concentrations of trace metals were found in the mineral sediments. Average total metal concentrations in sediments decreased in the order Al > Fe > Mn > Zn > Pb > Cu > Cd, and only in Insko mineral sediments contained more Fe than Al. The residual phase was the major phase binding metals, which indicates that recently anthropogenic influence on these sediments was weak. Fe-Mn oxides were the next important metal binding phase. The results indicate, that in the examined lakes no serious metal pollution occurred.

Keywords: sequential extraction, metals, bottom sediments, lakes

Lake ecosystems are particularly susceptible to metal pollution. In water, metals undergo precipitation and sorption on mineral and organic suspensions, which contributes to scavenging the elements in bottom sediments [1]. Metals bound by physical adsorption and chemisorption are in balance with water pores in the sediments, and can easily penetrate into the water column. Metals bound by coprecipitation with hydrated Fe-Mn oxides and with carbonates are more strongly associated with sediments and penetrate to water column only under strongly reductive or significantly acidic conditions. Metals associated with the organic matter, in case of its decomposition, may be released into the water column or transformed to other insoluble forms [2]. Biological and biochemical processes, as well as dissolving and desorption, that occur in the bottom sediments may remobilize metals which have been deposited in the sediments for years, and cause secondary water pollution [2–5]. In the sediments, metal concentrations do not change as frequently as in the water, therefore sediment metal

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levels may be better indicators of potential pollution of water bodies [3,6]. Knowledge of metal behaviour in changeable environmental conditions may enable prediction of the elements uptake by the resident aquatic organisms [7]. Metal speciation analysis provides information on metal mobility and bioavalability, which are related to their binding forms in sediments [8, 9].

We examined metal concentrations and their geochemical partitioning in the upper layer of sediments from two lakes in Western Pomerania, Poland. Insko is an α-mesotrophic lake, with hypolimnetic oxygen saturation exceeding 20 % [10]. Its surface area is 486.6 ha, and maximum depth 41.7 m [11]. Differently, Wisola is a β-mesotrophic lake, with hypolimnetic oxygen saturation below 20 % [10], surface area equal 181.5 ha and maximum depth 15.4 m [11]. Lake Wisola receives treated recycled water from the nearby wastewater treatment plant (mechanical and biological treatment), which is discharged through the Insko Channel. We assessed the sediment quality using Polish regulations on dredged material management and the criteria of geochemical background levels [12, 13].

Materials and methods

The study material had been collected from lakes Insko and Wisola (Fig. 1), once per quarter, since autumn 2003 to summer 2005. The samples were taken from 14 sites, 7 sites in each lake. Sediment samples collected have been divided into two groups: organic – containing above 5 % of organic matter, and mineral – with organic matter content below 5 %. At the same time samples of near-bottom water were collected. Sediment samples were taken with a Van Veen bottom sampler, placed in polyethylene bags, and stored in a cooler box. In the laboratory, pH of sediments and near-bottom

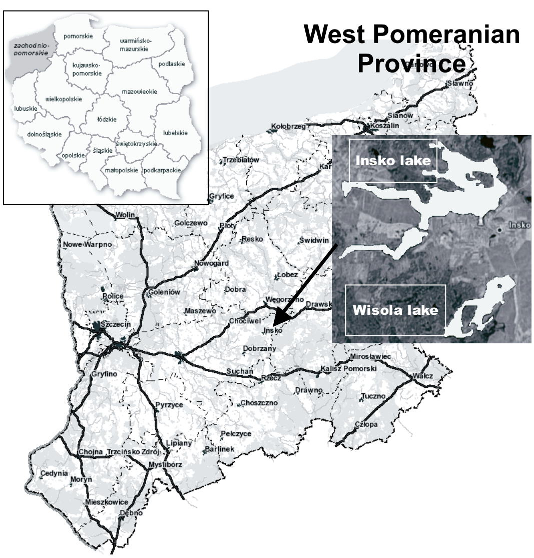

Fig. 1. The study area

water were measured to the nearest 0.1 pH using a pHScan BNC pHmeter. Next, the sediment samples were freeze dried with a Heto LyoLab 3000 apparatus. Dry sediment was ground in an agate mortar, shifted through a 350 μm mesh nylon sieve, and stored in tightly closed polyethylene boxes.

All laboratory procedures involved Merck chemicals of the highest purity grades and deionized water from Barnstead EASY pure UV apparatus. Material was weighed to the nearest 0.001 g in a RADWAG WPS 360/C analytical balance. All samples and reagent blanks were prepared in triplicate. For total metal analysis, the sediment samples were digested with concentrated nitric(V) acid in a MDS 2000 microwave oven, in accordance to the EPA 3015A procedure [14]. Metal partitioning in different geochemical fractions was determined with a six step sequential extraction procedure [15] (Table 1). Concentrations of Al, Fe, Mn, Zn and Cu were determined by *Inductively Coupled Plasma – Atomic Emission Spectrometry* (ICP-AES), while Pb and Cd concentrations – by *Graphite Furnace Atomic Absorption Spectrometry* (GF-AAS). Organic matter content in sediments was estimated by *Loss on Ignition* (LOI) method. The accuracy and precision of the methods were verified using certified reference material MESS-2. Statistical analyses were conducted with StatSoft Statistica 7.1 software. The statistical treatment involved the analysis of variance (ANOVA, Duncan's Test) and determination of linear correlation coefficients, at the significance level $p \leq 0.05$.

Table 1

Six step sequential extraction scheme for sediments [14]

No	Fraction	Forms of metals	Extractant	Phase ratio solid/liquid	Shaking time [h]
I	Exchangeable	metals at exchangeable positions	1 M NH_4OAc; pH 7	1/20	2
II	Carbonate bound	metals bound to carbonates	1 M NaOAc; pH 5	1/20	5
II	Easily reducible	metals bound to Mn oxides and amorphous Fe oxides	1 M $NH_2OH \cdot HCl$; pH 2	1/100	12
IV	Moderately reducible	metals bound to amorphous and poorly crystalline Fe oxides	0.2 M $(NH_4)_2C_2O_4$ + 0.2 M $H_2C_2O_4$; pH 3	1/100	24
V	Organic/sulphidic bound	metals bound to amorphous sulphides and organic mater	30 % $H_2O_2 + HNO_3$; pH 2 NH_4OAc; pH 7	heating 85 ^oC 1/100	12
VI	Residual	Metals built in the crystal structure of minerals (silicates, sulphides, crystalline Fe oxides)	65 % HNO_3	microwave digestion	

Results

In Lake Wisola, sediments (both organic and mineral) contained significantly more organic matter than in Lake Insko. Sediment pH in both lakes was similar and ranged from 6.0 to 7.8. In such conditions aluminium was stably bound to sediments, and was not realised to the water column, which was evidenced by its scanty occurrence in the most mobile geochemical fractions (Table 2).

Table 2

Geochemical partitioning of metals in bottom sediments of lakes Insko and Wisola in period 2003–2005 [mg/kg d.m.]

Lake	Fraction	Al	Fe	Mn	Zn	Cu	Pb	Cd
Insko	I	0.1	0.9	0.5	0.0	21.2	5.7	0.2
	II	0.2	0.5	2.4	0.3	11.5	1.0	8.1
	III	0.8	51.8	8.4	8.2	9.6	2.2	22.5
	IV	10.1	28.1	51.0	15.2	8.5	72.2	23.7
	V	0.1	5.0	2.5	0.1	4.1	1.2	0.9
	VI	88.8	13.8	35.3	76.2	45.3	17.6	44.6
Wisola	I	0.1	0.8	0.8	0.1	29.8	5.4	0.4
	II	0.2	0.4	2.6	0.0	14.8	0.7	4.6
	III	0.3	22.4	9.5	0.5	10.0	1.1	15.7
	IV	15.5	57.4	59.5	8.4	8.9	65.4	50.4
	V	0.3	13.3	3.4	0.9	5.0	2.7	2.0
	VI	83.6	5.7	24.2	90.1	31.6	24.8	27.0

Aluminium levels in the organic sediments were below the background level in clay minerals, and in the mineral sediments – below the background level in sandstones and carbonate rocks [13]. This may indicate, that in the examined sediments aluminium originated mainly from the bedrock geological composition. Iron levels in the sediments were within the range (20000–47000 mg/kg) reported by various authors as geochemical background [4, 13, 16, 17]. In both lakes, the organic sediment iron levels were about 10-fold higher than in the mineral deposits (Table 3). Mean manganese levels were significantly higher in Lake Insko sediments. In both lakes, however, sediment manganese levels were not high (Table 3) and were within the range recognised as natural background [4, 13, 17].

Similar sediment levels of the metal are reported in Lake Piaseczno [18], fish ponds in south-western Poland [19], or the Eastern and Western Oder River [20]. Zinc levels in organic sediments averaged 138.3 mg/kg d.m. in Lake Insko, and 149.6 mg/kg d.m. in Lake Wisola. Bojakowska et al [6] and Moore and Ramamoorthy [21] claim, that unpolluted sediments contain up to several dozen mg/kg. In both examined lakes, sediment zinc levels exceeded the natural background level determined for Polish lakes [6] and background levels reported by other authors [4, 17]. They exceeded also the *Threshold Effect Level* (TEL), above which adverse effects to aquatic organisms may occur, but were below the *Probable Effects Level* (PEL), above which adverse effects are predicted to occur frequently [22]. In the mineral sediments, zinc levels were nearly 10-fold lower than in the organic deposits (Table 3). However, zinc occurred in the sediments of both lakes in much lower concentrations, than reported in Polish water bodies under anthropopression, like Lake Dlugie [9], the Oder River [20], Miedzyodrze within the area of Widuchowa Polder [23], or in polluted Canadian lakes [7]. Copper

Table 3

Mean metal levels in organic and mineral sediments from lakes Insko and Wisola in period 2003–2005 [mg/kg d.m.]

Sediments	Metal	Lake Insko					Lake Wisola				
		n		± SD	min.	max	n		± SD	min.	max
Organic	Al	96	24780	± 6090	10137	36191	48	16896	± 4548	5350	25964
	Fe		20559	± 5087	10837	31166		13774	± 3429	7401	22019
	Mn		254	± 40	171	357		189	± 63	89	283
	Zn		138.3	± 21.2	79	233.1		149.6	± 57.1	21.5	300.3
	Cu		17.5	± 3.4	9.2	24.6		19.5	± 6.7	3.4	32.1
	Pb		54.1	± 16.0	3.8	99.6		63.4	± 36.4	2.1	148
	Cd		1.05	± 0.31	0.04	1.9		1.36	± 0.74	0.32	3.42
Mineral	Al	96	2902	± 889	1092	9452	120	2857	± 1907	899	10254
	Fe		2919	± 857	1110	5393		3177	± 2263	874	10587
	Mn		62	± 14	25	110		52	± 25	12	114
	Zn		12	± 4.6	0.6	27.5		17.8	± 13.4	1.2	48.5
	Cu		2.2	± 1.0	0.2	7.5		2.6	± 2.2	0.3	11
	Pb		3.4	± 1.5	0.1	11.6		4.4	± 3.1	0.5	11.4
	Cd		0.05	± 0.02	0.01	0.14		0.11	± 0.17	< 0.01	0.85

* Sediments from sites: Lake Insko – I1, I2, I3, I5; Lake Wisola – W1 and W5; ** sediments from sites: Lake Insko – I4, I7, I8; Lake Wisola – W2, W3, W4, W6, W7.

levels in the organic sediments averaged 17.5 mg/kg in Lake Insko, and 19.5 mg/kg in Lake Wisola (Table 3). They exceeded the natural background level in Poland [6], but met the background criteria applied in some other countries [13, 16, 17]. Sediments of unpolluted rivers and lakes are reported to contain Cu below 20 mg/kg [6]. In the examined lakes, sediment copper levels were similar to those reported in Lake Kerkini, Greece [24] and lakes situated in unpolluted regions of Sweden [25]. Mean lead levels in the organic sediments ranged from 54.1 mg/kg d.m. in Lake Insko to 63.4 mg/kg d.m. in Lake Wisola, and significantly exceeded the mineral sediment levels (3.4 in Insko and 4.4 mg/kg d.m. in Wisola). Moreover, they exceeded TEL values [22], which means that they could have produced an adverse effect on aquatic life, depending on the proportion of organic sediments in the bottom deposits of the lakes. The mineral sediment lead levels corresponded to the natural background levels in Polish lakes [6]. Organic sediments in Lake Wisola contained significantly more cadmium (1.36 mg/kg) than in lake Insko (1.05 mg/kg). However, in both lakes, the organic sediment cadmium levels were higher than in the mineral deposits (Table 3). In Poland, cadmium background level equals 0.5 mg/kg d.m. [6], while values reported for other countries range from 0.26 to 0.6 mg/kg [13, 17, 26]. In both examined lakes, the organic sediment cadmium levels exceeded TEL values [22].

In the mineral and organic sediments from Lake Wisola and organic sediments from Lake Insko, mean metal levels were arranged in the following decreasing sequence: Al > Fe > Mn > Zn > Pb > Cu > Cd. In the mineral sediments in Lake Insko the sequence was only slightly different: Fe > Al > Mn > Zn > Pb > Cu > Cd. Similar quantitative metal order was also observed in Tuskegee Lake, USA, only Pb exceeded Zn [26]. Depending on the water/sediment accumulation factors, the extent of metal accumulation from water decreased in order: organic sediments: Pb > Fe > Al > Cd > Cu > Mn > Zn; mineral sediments: Pb > Fe > Al > Mn > Cu > Cd > Zn. Overall, high metal levels in sediments may occur even when their concentrations in water are low. Positive linear correlation coefficients indicate, that Fe, Mn and Al played a significant role in binding other metals in examined sediments. Similarly, organic matter content significantly contributed to scavenging metals in the sediments. Especially clear relationships were found in both mineral and organic sediments in Lake Wisola. According to Pruysers et al [27] Fe and Mn sulphides, together with a high concentration of organic matter, are involved in the redox process associated with metal enrichment of sediments. However, the metals may be released back to the water column under reductive or acidic conditions [14]. In the sediments from lakes Insko and Wisola the highest proportion of metals was bound to the residual fraction, which indicates that recently anthropogenic influence on these sediments was weak. Aluminium showed the lowest mobility (up to 95 % bound to the residual fraction), but periodically its proportion in the moderately reducible fraction increased to 20 % (Table 2). In both lakes, copper occurred in the highest proportion (up to 70 %) in the mobile fractions, of which up to 37 % was in the exchangeable fraction (the most labile and bioavailable). Manganese occurred mostly in the moderately reducible and easily reducible fractions. Iron also showed high mobility, its proportion in the fractions bound to Fe-Mn oxides and hydroxides being up to 68 %. In Lake Wisola, iron was bound mostly to the moderately reducible fraction (Table 2).

The results indicate, that in the examined lakes no serious metal pollution occurred.

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ROZMIESZCZENIE WYBRANYCH METALI W OSADACH DENNYCH JEZIOR IŃSKO I WISOLA (POLSKA)

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Abstrakt: W pracy dokonano oceny zdolności osadów dennych pochodzących z jezior Ińsko i Wisola do zatrzymywania oraz uwalniania metali, oraz potencjalnego ryzyka zanieczyszczenia środowiska wodnego. W badaniach oznaczono zawartość całkowitą metali oraz ich udział w poszczególnych frakcjach geochemicznych. W obu jeziorach osady mineralne charakteryzowały się mniejszą zawartością metali śladowych niż osady organiczne. Średnia zawartość metali w osadach obu jezior malała w następującej kolejności: Al > Fe > Mn > Zn > Cu > Pb > Cd, a jedynie w osadach mineralnych jeziora Ińsko stwierdzono większą zawartość Fe w porównaniu z Al. Główną frakcją wiążącą metale była frakcja rezydualna, co wskazuje na niewielki wpływ antropogenny w zanieczyszczeniu metalami badanych osadów. Ważną rolę w wiązaniu metali odgrywały również frakcje związane z tlenkami żelaza i manganu. Badane osady nie wykazywały poważnego zanieczyszczenia metalami.

Słowa kluczowe: ekstrakcja sekwencyjna, metale, osady denne, jeziora

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IMPACT OF MUNICIPAL LANDFILL SITE IN TARNOW ON THE OCCURRENCE OF BENEFICIAL BEETLES

WPŁYW SKŁADOWISKA ODPADÓW KOMUNALNYCH W TARNOWIE NA WYSTĘPOWANIE POŻYTECZNYCH CHRZĄSZCZY

Abstract: The investigations on the occurrence of beneficial epigeal enthomofauna were conducted in 2006 and 2007 on plots situated in the immediate vicinity of the solid waste landfill site in Tarnow. The structure of enthomofauna domination differed considerably depending on the plot location towards the active landfill sector. The greatest diversity of *Col. Carabidae* was observed on the spots located at a longer distance from the landfill site. The vicinity of forest areas positively impacted the presence of predatory beetles.

Keywords: municipal landfill sites, *Col. Carabidae*, *Col. Staphylinidae*

Municipal landfill sites may impact the neighbouring areas among others through the emission of microbial and chemical pollutants. Gaseous, dust and microbial pollutants may pose a serious hazard to human health and life [1–3] and also lead to degradation of the surrounding soils, surface and underground waters and greenery. This may result in disturbing the balance in the environment and in consequence to changes among others in the numbers and species composition of fauna and flora [4]. The factor which additionally affects the fauna of municipal landfill sites is organic waste which is deposited there together with other wastes. A great amount of organic waste favours the presence of some organisms, such as birds or rodents. Also numerous appearances of dipterans from the *Muscidae*, *Calliphoridae* and *Sarcophagidae* genera are observed, which may carry pathogenic microorganisms.

Municipal landfill sites are often located next to agricultural areas and wastelands. An important element of fauna in these areas consists of beneficial epigeal enthomofauna. Beetles from *Col. Carabidae* and *Col. Staphylinidae* families play a particular

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role. Most of beetle species from these families are predatory and hunts other insects, including many species which are crop pests. These beetles are sensitive to environmental pollution and other changes brought about by human activities [5, 6]. Therefore, maintaining the balance in the natural environment and agrocenoses is a crucial challenge.

The present paper aimed to investigate the occurrence of beneficial epigeal fauna in the zone immediately adjoining a municipal landfill site.

Material and methods

The research was conducted in 2006 and 2007 in Tarnow. The solid waste landfill site in the vicinity of which the research was carried out is situated in the city northern quarter called Krzyz. The landfill area is surrounded by ploughlands, wastelands and forest. Observations were conducted on experimental plots located in the immediate vicinity of the landfill site. Plots were established at each side of the landfill in two zones: less than 250 m and between 250 and 500 m from its boundaries. The plot marking was presented in Table 1. Horse bean, Nadwislanski c.v. was cultivated on each plot with an area of 20 m^2. The experiment was set up in four replications and identical cultivation measures were applied on each plot.

Table 1

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

Plot	Location of plots in relation to landfill site		Surroundings
	Direction	Zone [m]	
W I	West	Less than 250	arable land, hay meadow
W II	West	250–500	arable land, hay meadow
N I	North	less than 250	wasteland
N II	North	250–500	wasteland, arable land
E I	East	less than 250	wasteland, forest
E II	East	250–500	wasteland, forest
S I	South	less than 250	forest, wasteland
S II	South	250–500	forest, arable land
Z	Reclaimed sector	0	active sector, hay meadow

The western winds dominate in the area of Tarnow city, average wind speed is 2.2 m/s, pointing to light wind. Detailed data on wind distribution are as follows: north winds – 6 %, northeast winds – 7.1 %, east winds – 16.7 %, southeast winds – 4.8 %, south winds – 14.8 %, southwest winds – 7.4 %, west winds – 22.6 %, northwest winds – 8.8 % and calm air – 11.8 %. Chemical properties of soils in plots are presented in Table 2.

Table 2

Chemical properties of soils occurring in the vicinity of municipal landfill site in Tarnow

Plot	pH_{H_2O}	Heavy metals				
		Cd	Cu	Ni	Pb	Zn
		$mg \cdot kg^{-1}$				
W I	5.1	1.09	5.46	4.87	22.92	57.37
W II	5.1	1.24	4.45	5.32	30.46	60.70
N I	5.6	1.17	9.01	5.24	30.73	67.72
N II	4.8	0.93	5.64	4.33	27.39	58.27
E I	4.8	0.99	5.48	4.62	25.38	58.55
E II	4.9	0.94	7.16	5.26	29.29	71.50
S I	7.5	1.08	10.43	8.61	26.92	97.70
S II	4.7	0.91	5.66	5.15	22.51	62.46
Z	4.7	0.99	7.96	7.19	24.32	60.78

Enthomofauna was captured into Barber's traps (glass jars dug into the ground level with the soil surface). The traps were dug in the middle of each plot. They did not contain the conserving liquid. They were covered with roofs to protect them against rain water. The collected insects were taken to a laboratory for their classification into orders and families using the appropriate keys [7, 8]. Beetles from *Col. Carabidae* family were classified into species or genus on the spot and live specimens were set free. 10 analyses were performed in 2006 and 12 in 2007.

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

Results and discussion

A total of 2461 *Carabidae* beetles were caught and 224 from *Staphylinidae* family. The solid waste landfill site in Tarnow affected the number and species composition of the captured *Carabidae* (Table 3). The greatest species diversity was found on the plots located on the southern side of the landfill site where the area is forested and thus these beetles might have migrated from there to the arable lands. A marked decrease in *Carabidae* numbers was registered on the plots situated in zone I – less than 250 m from the landfill boundaries. In this zone species the diversity of *Carabidae* was smaller than in zone II situated further from the landfill site.

The greatest number of *Carabidae* was captured on the plot located on the southern side of the landfill site in zone II. The smallest number of the specimens was spotted on the plot situated on the northern side of zone I (Table 4). *Staphylinidae* occurred far less numerously than *Carabidae*. Generally, they were more frequently captured on the plots in zone II. More *Staphylinidae* specimens were trapped in the second year of the investigations.

Table 3

Number of specimens and species of *Col. Carabidae* from analysed plots

Carabidae	Year	Plots							
		W		N		E		S	
		I	II	I	II	I	II	I	II
Number of specimens	2006	135	140	97	144	108	141	133	168
	2007	170	198	125	165	130	166	184	257
Number of species	2006	12	17	11	16	13	18	17	25
	2007	14	15	16	16	16	17	24	26

Table 4

Occurrence of beneficial epigeal enthomofauna depending on location of plots towards the landfill site

Taxon	Year	Average number of captured specimens per season [pcs.]								$LSD_{p<0.05}$
		Plots								
		W		N		E		S		
		I	II	I	II	I	II	I	II	
Carabidae	2006	33.8	35.0	24.3	36.0	27.0	35.3	33.3	42.03	
	2007	42.5	49.5	31.5	41.3	32.5	41.5	46.0	64.30	
	Mean	38.2	42.3	27.9	38.7	29.8	38.4	39.7	53.20	*13.75*
Staphylinidae	2006	3.3	3.8	2.8	4.0	2.5	2.3	2.3	4.5	
	2007	4.0	4.8	4.0	3.8	2.5	2.8	3.3	5.0	
	Mean	3.7	4.3	3.4	3.9	2.5	2.6	2.8	4.8	*n.s.*

n.s. – non-significant differences.

The structure of *Carabidae* domination on the analyzed plots was similar (Table 5). Two species: *Pterostichus cupreus* and *Bembidion properans* dominated on all plots. However, it may be noticed that both species had the greatest share on the plots situated on the western, northern and eastern sides. Particularly in zone I the number of eudominants was small. Ground beetles from the *Carabus* species occurred only on the plots situated close to the forest site. Big zoophages prefer forest sites [9]. These big beetles might have migrated from the forest to arable plots.

Beetles from *Carabidae* family play an important role in agrocenose environment because of their predatory lifestyle. Therefore, it is important to maintain high numbers of these beetles on farmlands. As was shown by the research conducted by other authors [10], *Carabidae* are particularly numerous on fields adjoining forested areas or fallows. In the presented investigations also more numerous presence of *Carabidae* was noticed on the fields adjacent to the forest site. A negative effect of pollution on *Carabidae* populations is also well known [11]. *Carabidae* are often used as bioindicators of environmental pollution and the state of its equilibrium. A decline in *Carabidae*

Table 5

Percentage of the most numerous species from *Col. Carabidae* genus and biodiversity indicators

Taxon	Percentage of share – domination structure							
	Plots							
	W		N		E		S	
	I	II	I	II	I	II	I	II
Pterostichus cupreus	29.5 ED	27.8 ED	34.2 ED	24.3 ED	35.3 ED	27.0 ED	22.1 ED	17.2 ED
Pterostichus lepidus	3.6 SD	8.3 D	0.0	7.8 D	4.2 SD	4.6 SD	2.8 SD	3.3 SD
Amara plebeja	7.9 D	9.2 D	8.1 D	10.4 ED	4.2 SD	6.8 D	8.8 D	7.1 D
Amara aenea	1.3 R	0.9 SR	1.8 R	1.6 R	0.0	0.0	1.6 R	5.2 D
Harpalus rufipes	7.2 D	8.9 D	8.1 D	12.3 ED	8.0 D	12.7 ED	11.7 ED	13.6 ED
Bembidion properans	31.5 ED	24.9 ED	30.2 ED	25.6 ED	29.8 ED	24.8 ED	18.6 ED	14.4 ED
Poecilus lepidus	6.6 D	8.9 D	3.6 D	8.1 D	4.6	9.1 D	11.7 ED	13.9 ED
Platynus dorsalis	0.7 SR	0.3 SR	4.1 SD	1.6 R	2.5 SD	2.0 R	5.0 SD	1.2 R
Carabus coriaceus	0.0	0.0	0.0	0.0	0.4 SR	1.6 R	2.5 SD	5.4 D
Carabus granulatus	0.0	0.0	0.0	0.6 SR	1.7 R	2.6 SD	3.5 SD	5.2 D
Other *Carabidae*	11.7	10.8	9.9	7.7	9.3	8.8	11.7	13.5

Eudominants (> 10 %) – ED, Dominants (5–10 %) – D, Subdominants (2–5 %) – SD, Recededents (1–2 %) – R, Subrecedents (< 1 %) – SR.

biodiversity observed in the zone adjoining the landfill site points to a strong negative impact of such facilities upon the natural environment. It is necessary to surround landfill sites with forest areas to minimize their negative environmental effect on the environment. Agrocenoses are especially exposed to such an effect. Therefore, a buffer zone between a landfill site and arable fields is necessary. Semi-natural, non-farmed sites provide convenient conditions for the survival of beneficial organisms [12].

Conclusions

1. Changes in the environment brought about by the active municipal landfill site influenced a decrease in *Carabidae* biodiversity in the agricultural crops.
2. Forest sites have an influence on the diminishing of the negative effect of landfill site on the neighbouring farmlands, owing to maintained biodiversity of predatory beetles.

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WPŁYW SKŁADOWISKA ODPADÓW KOMUNALNYCH W TARNOWIE NA WYSTĘPOWANIE POŻYTECZNYCH CHRZĄSZCZY

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Abstrakt: Badania nad występowaniem pożytecznej entomofauny naziemnej przeprowadzono na poletkach zlokalizowanych w bezpośrednim sąsiedztwie składowiska odpadów komunalnych w Tarnowie w 2006 i 2007 r. Struktura dominacji entomofany różniła się w zależności od lokalizacji poletek względem czynnego sektora składowiska. Największą bioróżnorodność biegaczowatych stwierdzono na poletkach położonych w większej odległości od składowiska. Korzystnie na występowanie drapieżnych chrząszczy wpływała bliska lokalizacja terenów leśnych.

Słowa kluczowe: składowiska odpadów komunalnych, biegaczowate, kusakowate

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Barbara SKWARYŁO-BEDNARZ[1]

INFLUENCE OF THE CONTENTS OF TOTAL FORMS OF LEAD ON THE NUMBER OF SELECTED GROUPS OF MICROORGANISMS IN THE FOREST SOILS OF THE PROTECTED ZONE IN THE ROZTOCZE NATIONAL PARK

WPŁYW ZAWARTOŚCI OŁOWIU OGÓLNEGO NA LICZEBNOŚĆ WYBRANYCH GRUP DROBNOUSTROJÓW W GLEBACH LEŚNYCH OTULINY ROZTOCZAŃSKIEGO PARKU NARODOWEGO

Abstract: The aim of the study was to determine the influence of the contents of total forms of lead on the number of selected microorganisms in the light soils situated in the protected zone in the Roztocze National Park. The experiment was carried out in spring in pine forest that belongs to Senderki Forest Sub-District. The total number of ten soil outcrops was done in the area. The samples were taken from humus levels and mother rock levels. The study reveals that the norms for protected zones concerning the contents of total forms of lead were not exceeded in the forest soils. Nevertheless, a significant enrichment in total lead was observed in surface levels, as compared with mother rock levels. The amount of total lead in humus levels influenced the number of marked colonies of bacteria, *Actinomycete* and fungi, which was also proved by the statistical analysis that was carried out. The amount of total lead in the humus level was significantly negatively correlated with the number of marked colonies of microorganisms. It should be noted, that this study should be treated as preliminary investigation. Further detailed investigation of the content of total lead in the protected soils of south-eastern Poland will allow to determine the influence of this element on the number of soil microflora.

Keywords: total lead, forest soils, soil microorganisms, protected areas

Pollution of soil environment with heavy metals has been studied by many researchers for decades. In spite of decreasing emission of industrial pollution with dust and gas in the whole area of Poland, studies on the contents of heavy metals in soils are still being undertaken. These studies are often treated as monitoring. It is necessary to conduct such studies because heavy metals have enormous abilities to bioaccumulate –

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they systematically accumulate in the environment, especially live environment, which increases the intensity of their negative effects [1].

The protected zone in the Roztocze National Park is one of the areas that are under special supervision as far as the condition of soil and contents of heavy metals are concerned in Lublin Province. The protected zone consists mainly of light soils, which are prone to accumulation of heavy metals. Excessive amounts of heavy metals in soil causes changes in both quantity and quality of organisms living there, which often leads to changes of biological activity of pedosphere [2]. It is often emphasized that biological activity of soils should be used to determine soil quality and estimate changes taking place in soil, that are caused by the use of potentially dangerous factors [3, 4].

The aim of the study was to determine the influence of the contents of total forms of lead on the number of colonies of bacteria, *Actinomycete* and fungi in the light forest soils situated in the protected zone in the Roztocze National Park.

Material and methods

The soils under pine forests in Senderki Forest Sub-District, situated in the protected zone in the Roztocze National Park (which is located in south-eastern part of Poland), were investigated in this study. These soils had granulometrical composition of loose sands and slightly loamy sands.

The samples for the analysis were taken in May from the humus level and the mother rock level from ten soil profiles without preserving their natural structure.

In the fresh soil material that was taken from the humus levels, the following were marked: the number of colonies of bacteria and *Actinomycete* on nutrient agar with soil extract, and the number of fungi on Martina's nutrient agar [5].

In air-dry soil material from the humus levels and the mother rock levels the following were marked: contents of C organic with Tiurin's method in Simakow's modification, pH in H_2O and in 1 mol $\cdot$ dm^{-3} KCl potentiometrically, total absorptive capacity of the soil (T), total contents of Pb in extract of $HClO_4$ and HNO_3 with atomic absorption spectrometry (AAS).

Results and discussion

The investigated genetic levels of forest soils in the protective zone in the Roztocze National Park revealed acidic pH or strongly acidic pH (Table 1). The values of pH_{KCl} in the humus levels ranged from 4.5 to 5.0, and in the mother rock levels – from 3.8 to 4.2. The contents of C organic in the surface levels was 1.6–2.2 % (Table 1). The value of absorptive capacity decreased with the depth of the soil profile. In the humus levels it was within the range 4.7–5.6 cmol(+) $\cdot$ kg^{-1}, and 2.8–3.6 cmol(+) $\cdot$ kg^{-1} in the mother rock levels.

According to Kabata-Pendias and Pendias [6], the natural amount of lead in soil should not exceed 50 mg $\cdot$ kg^{-1}, and the permitted amount of lead in soil should not exceed 100 mg $\cdot$ kg^{-1}. Special norms for permitted amounts of metals in protected areas [7] say that permitted amount of lead in such areas should not exceed 50 mg $\cdot$ kg^{-1}.

Table 1

Basic chemical properties of forest soils

Horizon	pH_{H_2O}	pH_{KCl}	$C_{org.}$ [%]	Absorption capacity of soils (T) [cmol(+) · kg^{-1}]
Ap	5.0	4.6	1.8	4.9
C	4.4	4.0	not investigated	3.2
Ap	5.1	4.9	1.7	5.2
C	4.6	4.2	not investigated	2.9
Ap	4.9	4.5	2.1	4.7
C	4.2	4.0	not investigated	3.3
Ap	4.8	4.5	1.6	5.0
C	4.1	3.9	not investigated	2.9
Ap	5.0	4.8	2.2	5.6
C	4.2	4.0	not investigated	3.6
Ap	5.2	4.8	1.9	4.8
C	4.4	4.1	not investigated	3.1
Ap	5.0	4.7	2.0	4.9
C	4.2	4.0	not investigated	3.3
Ap	5.3	5.0	1.9	5.1
C	4.5	4.0	not investigated	3.1
Ap	5.1	4.8	1.7	4.9
C	4.4	4.0	not investigated	2.8
Ap	5.0	4.7	1.9	5.3
C	4.2	3.8	not investigated	3.1

Table 2 reveals that the permitted amount of lead was not exceeded in the investigated soils. The content of lead in the humus levels ranged from 19.0 to 39.0 mg · kg^{-1} and was noticeably higher than in the mother rock levels (6.8–9.4 mg · kg^{-1}).

Despite the lack of a direct threat that the content of lead (lower than permitted) could have caused in the investigated soils, attention should be paid to the amounts of lead in the humus levels and the mother rock levels. This study revealed enrichment of the humus levels in lead, as compared with mother rock levels that were geo-chemical background. This enrichment was very high and ranged from 228.9 % to 443.2 %. Ciesla and others [8] emphasize the fact that chemical properties of forest soils like acidic pH and high contents of C organic enhance accumulation of lead in these soils.

Heavy metals, including lead, can be present in soil for hundreds or even thousands of years [9]. They can have a toxic effect on soil microorganisms. This effect depends on the concentration of a metal, correlation with other elements and ecological condition [10].

Many authors investigated the influence of heavy metals on the susceptibility of soil microorganisms in pot experiments [11, 12]. Such investigation allows to analyse in detail the influence of increasing doses of selected heavy metals on soil microflora. In the soils that occur naturally in the area, relations between the concentration of heavy metals and the number of soil microflora are more complicated and more difficult to estimate [2]. In spite of that, the literature presents field experiments that try to estimate the influence of heavy metals on the number of selected groups of microorganisms in soils located in the vicinity of the emitters of these elements [2].

Table 2

Content of heavy metals in forest soils and number of colonies of investigated microorganism

Horizon	Pb [mg · kg^{-1}]	Enrichment of Ap level as compared with C [%]	Bacteria and *Actinomycete* [10^9 cfu kg^{-1} d.m. soil]	Fungi [10^6 · cfu kg^{-1} d.m. soil]
Ap	21.3	300	21.0	105
C	7.1	100	not investigated	
Ap	21.8	320.6	12.5	90
C	6.8	100	not investigated	
Ap	29.5	364.2	5.2	92
C	8.1	100	not investigated	
Ap	27.1	343.0	12.1	80
C	7.9	100	not investigated	
Ap	21.7	319.1	14.8	100
C	6.8	100	not investigated	
Ap	38.0	427.0	7.6	85
C	8.9	100	not investigated	
Ap	31.1	330.9	11.0	104
C	9.4	100	not investigated	
Ap	39.0	443.2	7.2	80
C	8.8	100	not investigated	
Ap	19	228.9	14.3	100
C	8.3	100	not investigated	
Ap	25.0	316.5	13.0	90
C	7.9	100	not investigated	

Preliminary studies carried out in this work aim at negative changes in the monitoring potential that take place in the closest vicinity of the Roztocze National Park. The area of the investigation was purposefully located in the forest soils in the protected zone of the park, because in protected areas contents of heavy metals should not exceed the norms – even the contents slightly below the norms are not satisfactory.

The humus levels of forest soils had different numbers of colonies of bacteria, *Actinomycete* and fungi. The number of colonies of bacteria and *Actinomycete* ranged from 5.2 to 21.0 · 10^9 cfu · kg^{-1} d.m. of soil, and in the case of fungi it ranged from 80 to 105 · 10^9 cfu · kg^{-1} d.m. of soil (Table 2).

The statistical analysis that was carried out revealed that the contents of total lead in the humus levels influenced the amount of colonies of bacteria, *Actinomycete* and fungi. Significant negative correlations were obtained between the contents of total lead and the number of colonies of bacteria, *Actinomycete* (r = –0.754), and fungi (r = –0.580).

Moreover, it was observed that the content of total lead was significantly, positively correlated with pH, content of C organic and absorption capacity (Table 3).

Table 3

Correlation coefficients between Pb and basic chemical properties of forest soils at p = 0.01

	pH_{H_2O}	pH_{KCl}	C_{org}	T	Bacteria and *Actinomycete*	Fungi
Pb	0.897	0.887	0.913	0.859	–0.754	–0.580

The preliminary results of the study suggest that it is necessary to continue further studies in this matter, which will allow to monitor in detail the changes in soil environment in protected areas.

Conclusions

1. Total contents of lead in the investigated soils did not exceed the permitted norms for protected areas. However, significant enrichment of the surface levels in lead was observed, as compared with the mother rock levels (geo-chemical background).

2. The statistical analysis that was carried out revealed that the amount of Pb in the humus level significantly, negatively correlated with the number of colonies of bacteria, *Actinomycete* and fungi that were present in the investigated soil environment.

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WPŁYW ZAWARTOŚCI OŁOWIU OGÓLNEGO NA LICZEBNOŚĆ WYBRANYCH GRUP DROBNOUSTROJÓW W GLEBACH LEŚNYCH OTULINY ROZTOCZAŃSKIEGO PARKU NARODOWEGO

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Abstrakt: Celem badań była próba określenia wpływu zawartości ogólnej ołowiu na liczebność wybranych grup drobnoustrojów w glebach lekkich położonych w otulinie Roztoczańskiego Parku Narodowego. Badania przeprowadzono w okresie wiosennym na terenie lasu sosnowego Leśnictwa Senderki. Ogółem w terenie wykonano 10 odkrywek glebowych, z których pobierano próbki z poziomów próchnicznych i skały macierzystej. Z przeprowadzonych badań wynika, iż w glebach leśnych nie zostały przekroczone dopuszczalne normy zawartości ogólnych form ołowiu opracowane dla trenów chronionych. Stwierdzono jednak znaczne wzbogacenie w ołów całkowity poziomów wierzchnich w stosunku do skał macierzystych. Ilość ołowiu ogólnego w poziomach próchnicznych miała wpływ na ilość oznaczonych kolonii bakterii i promieniowców oraz grzybów. Prawidłowość tą potwierdza przeprowadzona analiza statystyczna. Ilość ołowiu ogólnego w poziomie próchnicznym istotnie ujemnie korelowała z liczebnością kolonii oznaczonych drobnoustrojów. Należy podkreślić, iż badania przeprowadzone w niniejszej pracy mają charakter badań wstępnych. Ich kontynuowanie pozwoli nie tylko na szczegółowe śledzenie zawartości ołowiu ogólnego w glebach chronionych południowo-wschodniej Polski, ale także na ocenę wpływu tego pierwiastka na liczebność mikroflory glebowej.

Słowa kluczowe: ołów ogólny, gleby leśne, drobnoustroje glebowe, tereny chronione

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Maciej WALCZAK[1] and Aleksandra BURKOWSKA[1]

UV RADIATION IMPACT ON ENZYMATIC AND RESPIRATORY ACTIVITY OF NEUSTONIC AND PLANKTONIC BACTERIA

WPŁYW PROMIENIOWANIA UV NA AKTYWNOŚĆ ENZYMATYCZNĄ I ODDECHOWĄ BAKTERII NEUSTONOWYCH I PLANKTONOWYCH

Abstract: The surveys were carried out in the pelagic zone of Brzezno lake (the Tuchola Forest). Water samples were collected from the surface microlayer and the subsurface water. The surveys covered respiratory activity of bacteria, using a measurement system OxiTop Control. Also, general activity of hydrolytic enzymes was estimated measuring rates of fluorescein release from fluorescein diacetate. The research was conducted in two experimental layouts: with and without humic substances. Conducted surveys proved substantially lower respiratory and hydrolytic activity of bacteria influenced by UVB radiation compared with bacteria without UVB radiation. The experiments did not prove unambiguous protective operation of HS – only for selected strains with bacteria subjected to UVB radiation in presence of HS demonstrated lower decrease of respiratory and hydrolytic activity.

Keywords: UV radiation, surface microlayer, bacterioneuston, enzymatic activity, respiratory activity

In the vertical plane, the external layer of water body is so-called surface microlayer. This layer constitutes a particular chemical and physical environment, which differs substantially from the subsurface water. The surface microlayer is formed by adhesion forces, which are a result of intermolecular attraction and surface tension on the interface of two media: air and water. This leads to the accumulation of organic and inorganic compounds within the layer. The surface microlayer often contains an elevated number of bacteria, called bacterioneuston. Due to the fact that bacterioneuston inhabits the surface microlayer, its members are exposed to stressful ecological factors to a greater degree than organisms inhabiting the water column.

The insolation is one of the primary factors affecting the number and activity of the bacterioneuston. The highest levels of solar radiation, including UV, that reach a water body are concentrated in surface layers. The most insolated water layer in aquatic

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systems is certainly the surface microlayer. The amount of radiation penetrating the surface microlayer affects organisms living within that water layer as well as decomposition and matter circulation processes.

Considering the entire range of solar radiation reaching the air-water interface, medium wave UV radiation, ie UVBλ = 290-320 nm and UVA λ = 320-400 nm, is of the highest biological importance due to its harmful effects. Radiation in this wavelength range causes DNA damage (lethal effect) or inhibits the growth of organisms by inhibiting enzyme synthesis, reducing active transport and inducing mutations, all of which are sublethal effects [1].

There are numerous studies that demonstrate that solar radiation, in particular UVB radiation, is detrimental to the production of bacterial biomass and exoenzyme activity [2–4]. It is also noteworthy that photooxidation of *dissolved organic matter* (DOM) and *particulate organic matter* (POM), which results in the release of considerable quantities of easily assimilable organic matter to the environment and may increase bacterioplankton activity [5, 6], occurs under the influence of UV.

Experimental

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

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

Surface microlayer water samples were collected by a Garrett [7] technique using a Plexiglas plate, which collects a 150 μm water layer. Subsurface water was sampled from a depth of 25 cm using an automatic pump. Taken water samples were poured into sterile glass containers.

Isolation of bacterial strains. In order to isolate bacterial strains there was a surface screening carried out on the *tryptone soy agar* (TSA) (Difco) medium surface. After 6 days of incubation at 20 ^{o}C a representative strains collection was detached and transferred onto TSA medium bevels.

Preparation of test bacterial strains suspension. Isolated bacteria strains were generated for 3 days at 20 ^{o}C in 50 cm^3 of liquid *tryptone soy broth* (TSB) medium. Afterward, from each culture taken was 30 cm^3 and spun for 5 min at 10 000 rpm, temperature of 10 ^{o}C. The supernants was used as crude enzyme solution, bacterial deposit was suspended in 30 cm^3 of sterile Ringer's solution. Optic density of each strain bacterial suspension was driven to equal value of 0.5 applying sterile Ringer's solution as a diluting agent.

Exposure to UVB radiation. Prepared bacterial suspension and crude enzyme solution of given strain was divided into 3 parts, 10 cm^3 each, and transferred into three parallel sterile Petri dishes. First one was a controlling agent and was not subjected to

UVB radiation. Two other dishes containing bacterial suspension were exposed to UVB radiation (lamp Philips; 15 min., 50 μW/cm^2), while before exposure to radiation there was sterile solution of humic substances (Fluca) added to one of them (final concentration HS 100 mg/dm^3).

Activity of hydrolytic enzymes – by measuring the rate of *fluorescein released* from fluorescein diacetate (FDA) [8]. The quantity of released fluorescein was measured using a Hitachi f-2500 spectrofluorometer at an excitation wave of λ = 480 nm and emission wavelength of λ = 505 nm.

The respiration activity of bacteria was determined with the measurement system OxiTop Control 12. The measurement of *biochemical oxygen demand* (BOD) with OxiTop®-Control was carried out according to the operating instruction provided by the supplier [9]. The incubation was carried out for 12 hours at 22 ^{o}C. The respiration activity was expressed in mg O_2/dm^3 of bacterial suspension.

Results and discussion

Exposure of hydrolytic enzymes solution to UVB radiation brought about reduction of their hydrolytic activity to 88–76 % of not exposed samples value. Stronger inhibition of enzymatic activity UVB radiation caused for enzymes produced by planktonic strains (Fig. 1). In case of 9 for 20 investigated strains found was minor protective effect of humic substances (Table 1). Enzymes produced by these strains demonstrated higher activity exposed to radiation at HS presence.

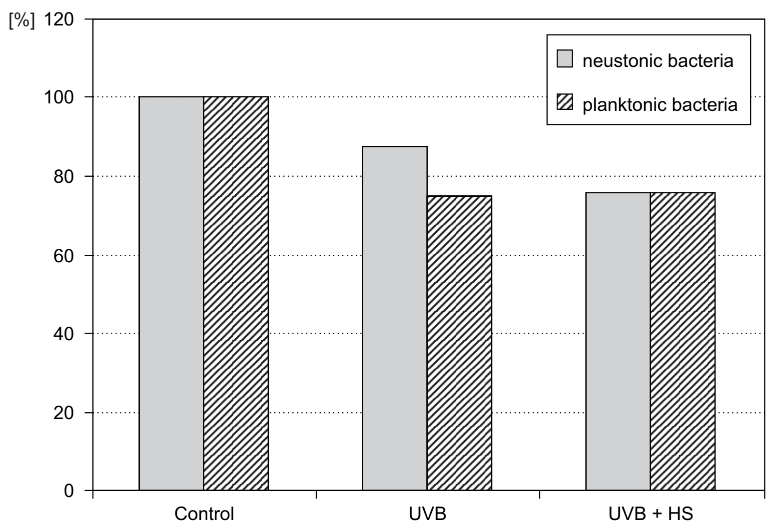

Fig. 1. The activity of hydrolytic enzymes after exposure to UVB radiation (average)

Prior to this study Walczak [10] noticed also directly in the lake water significant differences of hydrolases activity depending on solar radiation. Similar results obtained

Boavida and Wetzel [11] who surveyed phosphatases activity as well as Jorgensen [4] who investigated urease and glucosidase.

Table 1

UVB radiation impact on enzymatic activity of bacteria

Number of strain	Control	UVB	UVB in presence of HS
Neustonic bacteria			
1N	0.365*	0.243	**0.271**
2N	0.714	0.672	0.432
3N	0.183	0.152	0.151
4N	0.347	0.261	**0.300**
5N	0.262	0.210	0.207
6N	0.628	0.467	**0.511**
7N	0.095	0.100	0
8N	0.073	0.072	**0.079**
9N	0.493	0.548	0.427
10N	0.381	0.354	**0.385**
Planktonic bacteria			
1P	0.025	0.026	0.023
2P	0.530	0.372	0.231
3P	0.248	0.116	**0.173**
4P	0.341	0.235	0.229
5P	0.552	0.577	0.511
6P	0.116	0.030	**0.076**
7P	0.396	0.384	0.347
8P	0.134	0.114	**0.116**
9P	0.488	0.328	**0.401**
10P	0.415	0.334	0.320

Expanations: * – concentration of fluorescein (μg/cm^3); HS – humic substances; ■ – protective operation of HS.

All of these enzymes (urease, phosphatase, and glucosidase) are categorized as extracellular hydrolases. Therefore, they are secreted to the external environment, where there is no protection against the harmful effect of UV. Furthermore, it was also observed that extracellular enzymes unbound to organic matter are inhibited to a much higher degree (50–60 %). In contrast, inhibition of enzymes bound with organic matter equaled only to 30 % [3].

Experiments on respiratory activity of bacteria with application of measurement system OxiTop Control proved significant reduction of oxygen use in samples exposed to UVB radiation compared with not expose bacteria (Fig. 2). Oxygen use for exposed samples made up 50–66 % of bacteria not subjected to UVB radiation values on average

(Fig. 3). Inhibition of respiration rate was found for both, planktonic and neustonic bacteria. No statistically significant difference was observed in respiratory activity whether or not HS accompanied bacteria exposed to UVB radiation (Fig. 2).

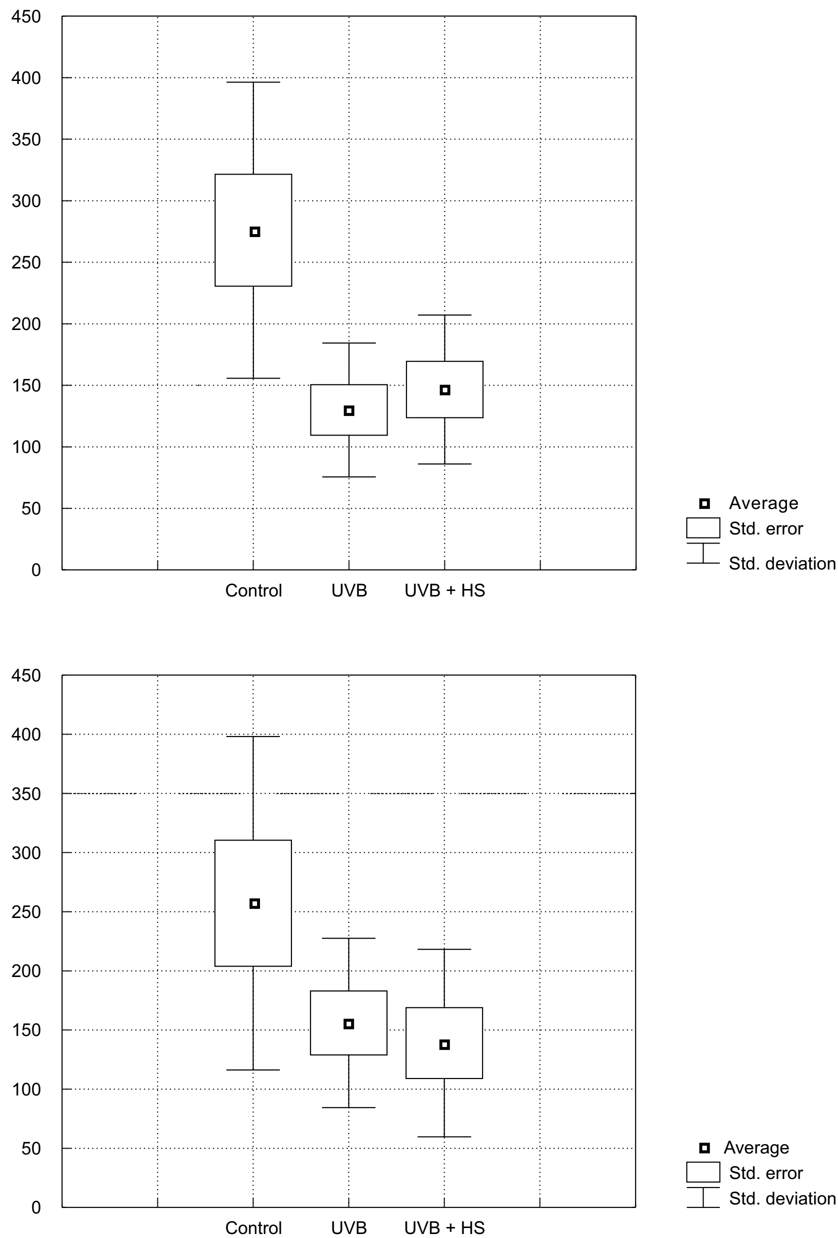

Fig. 2. UVB radiation impact on respiratory activity of bacteria (the *average respiration activity* was expressed in [mg O_2/dm^3 of bacterial suspension])

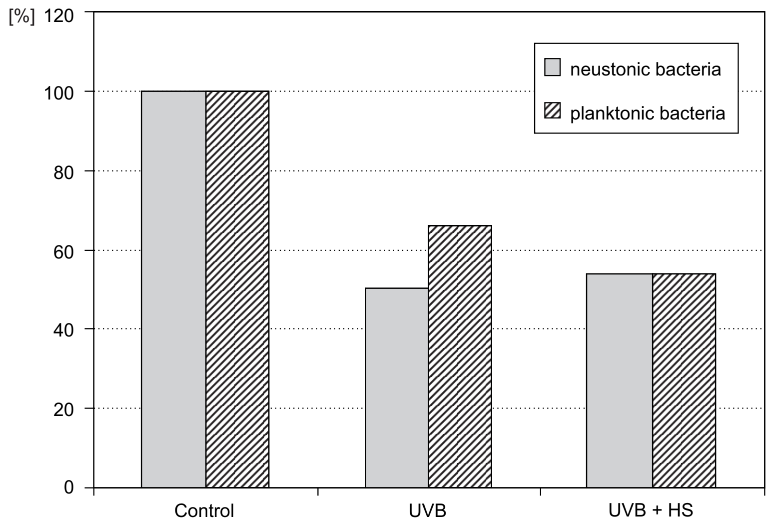

Fig. 3. The respiratory activity of bacteria after exposure to UVB radiation (average)

Unfortunately, no prior results of studies analyzing the effect of solar radiation on respiratory activity of neustonic bacteria have been found in the available literature, which prevents broader comparison of the presented results. Former Walczak study [10] regarding cellular dehydrogenases activity participating in electrons' transport within respiratory chain did not prove significant impact of solar radiation on these enzymes activity. Beside dehydrogenases, in cellular respiration take part also many other enzymes. Hindering their activity by UVB radiation can result in reduction of bacteria respiratory activity.

The experiments did not prove unambiguous protective operation of HS. Only for selected bacteria strains exposure to UVB radiation at HS presence resulted in lower reduction of respiratory and hydrolytic activity. On one hand, HS absorb some part of radiation. On the other hand, photochemical reactions produce many compounds that are harmful for bacteria. According to Scully et al [12], reactive forms of oxygen also play an important role in the decomposition of organic matter under the influence of UV. These compounds are produced in water in the presence of UV and organic matter and may indirectly inhibit the activity of extracellular enzymes.

Summing up, the conducted research confirmed and expanded earlier reports regarding the importance of solar radiation and UV on the activity of bacterial enzymes in aquatic environments. However, it should be noted that the effect of solar radiation, including UV, is not limited to simple and direct impacts on bacterial cells. Radiation affects the environment through a wide range of indirect means, eg organic matter photooxidation or the impact on phyto- and zooplankton, which also has a considerable impact on the activity of aquatic bacteria.

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WPŁYW PROMIENIOWANIA UV NA AKTYWNOŚĆ ENZYMATYCZNĄ I ODDECHOWĄ BAKTERII NEUSTONOWYCH I PLANKTONOWYCH

Zakład Mikrobiologii Środowiskowej i Biotechnologii
Uniwersytet Mikołaja Kopernika w Toruniu

Abstrakt: Badania prowadzono w strefie pelagialu jeziora Brzeźno (Bory Tucholskie). Próbki wody pobierano z mikrowarstwy powierzchniowej (MP) i wody podpowierzchniowej (WPP). W trakcie badań oznaczono aktywność oddechową bakterii z zastosowaniem systemu pomiarowego OxiTop Control oraz ogólną aktywność enzymów hydrolitycznych, mierząc tempo uwalniania fluoresceiny z dioctanu fluoresceiny. Badania prowadzono w dwóch układach doświadczalnych: bez *substancji humusowych* (SH) i w obecności tych substancji. W wyniku przeprowadzonych badań stwierdzono zdecydowanie niższą aktywność oddechową i hydrolityczną bakterii po naświetleniu UVB, w porównaniu z bakteriami nie naświetlonymi. Nie stwierdzono jednoznacznie ochronnego działania SH, jedynie w przypadku niektórych szczepów naświetlanie bakterii UVB w obecności SH powodowało mniejszy spadek aktywności oddechowej i hydrolitycznej.

Słowa kluczowe: promieniowanie UV, mikrowarstwa powierzchniowa, bakterioneuston, aktywność enzymatyczna, aktywność oddechowa

Varia

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