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Elżbieta BOLIGŁOWA[1], Katarzyna GLEŃ[1]
and Dariusz ROPEK[1]

PRELIMINARY RESEARCH ON AN ASSESSMENT OF THE EFFECT OF MINT AND EUCALYPTUS OIL ON SELECTED PLANT PATHOGENIC FUNGI

WSTĘPNE BADANIA NAD OCENĄ ODDZIAŁYWANIA OLEJKU MIĘTOWEGO I EUKALIPTUSOWEGO NA WYBRANE GRZYBY CHOROBOTWÓRCZE ROŚLIN

Abstract: The research compared the effect of peppermint oil (*Mentha x piperita* L. var. *Officinalis*) and Tasmanian blue gum (*Eucalyptus globulus* Labill.) oil and their doses on linear growth, biomass and sporulation of *Fusarium culmorum* (W.G.Sm.) Sacc., *F. solani var. coeruleum* (Sacc.) Booth and *Sclerotinia sclerotiorum* (Lib.) de Bary Schlecht. The research has shown that the fungistatic activity of ethereal oils under conditions *in vitro* depends on the kind of plant oil, its dose and fungus species. Among the tested pathogenic microorganisms, *Sclerotinia sclerotiorum* fungus is more sensitive to the presence of mint and eucalyptus oil. On the other hand, higher oil doses (0.8 and 1.0 mm/cm^3) reveal a better fungistatic effect on *Fusarium* fungi.

Keywords: plant oils, phytopathogenic fungi, laboratory tests

The harmful impact of synthetic pesticides on the environment has evoked an interest in seeking plant protection materials of natural origin based on microorganisms and compounds isolated from plants [1–3]. Application of preparations based on natural compounds allows for elimination or reduction of the use of plant protection chemicals, improvement of the quality of plant materials used for organic food production, protection of the environment owing to their weaker effect and easier biodegradation [4]. Currently, several plant preparations have been registered and attempts are made to extend the assortment of these substances. Various plant species are potential sources of natural compounds revealing fungistatic properties [5]. Compounds with an antibacterial and antifungal character comprise glycosides, alkaloids, phenols, saponines and essential oils [5, 6]. According to Łakota et al [5] the number and quality of these

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compounds in plants depend on many factors: plant species, its development stage, weather and soil conditions.

Some authors [7–9] think that extracts from oil-bearing plants show bactericidal and fungicidal properties. These plants include among others: peppermint (*Mentha* x *piperita* L. var. *officinalis*) and Tasmanian blue gum (*Eucalyptus globulus* Labill.). These plants produce ethereal oils which are used by pharmaceutical industry [9, 10]. According to Ożarowski and Jaroniowski [11] an essential oil, in which menthol dominates, is the basic component of peppermint. Besides, mint raw material contains also tannins, flavonoids, phenol acids and mineral salts. The chemical composition of Tasmanian blue gum aromatic oil is similar to oils from myrtle family plants [10]. According to Strzelecka and Kowalski [12] eucalyptus oil contains mainly cineole (ca 80–90 %) and also cymene, pinene, limonene, camphene and geraniol.

There are few publications [8, 13, 14] which point to potential use of antibacterial properties of ethereal oils obtained from aromatic plants (oregano, thyme, rosemary, coriander, dill or others) for plant pathogenic bacteria and fungi control.

The research aimed at comparing, in laboratory conditions, the effect of peppermint oil (*Mentha* x *piperita* L. var. *officinalis*) and Tasmanian blue gum oil (*Eucalyptus globulus* Labill.), as well as their doses on linear growth, biomass and sporulation of *Fusarium culmorum* (W.G.Sm.) Sacc., *F. solani var. coeruleum* (Sacc.) Booth and *Sclerotinia sclerotiorum* Schlecht.

Material and methods

Preliminary research on the effect of selected oils on phytopathogenic fungi under conditions *in vitro* were undertaken at our Department of Agricultural Environment Protection. The experiment used essential oils of Dr Beta series, made by Pollena Aroma. Mint oil was obtained from peppermint (*Mentha* x *piperita* L. var. *officinalis*) and the eucalyptus oil from Tasmanian blue gum (*Eucalyptus globulus* Labill.).The method used was described in a paper by Daferer et al [8]. The oils were added into PDA medium in doses of 0.1; 0.25; 0.5; 0.8 and 1.0 mm^3. Petri dishes with pure PDA medium were the control. The prepared media were inoculated with a 5 mm agar ring, overgrown with a two-week old mycelium of *Fusarium culmorum* (W.G.Sm.) Sacc., *F. solani var. coeruleum* (Sacc.) Booth and *Sclerotinia sclerotiorum* (Lib.) Schlecht. Pathogenic microorganisms tested in the experiment originated from the own collection of the Department of Agricultural Environment Protection. The fungi were cultured in a thermostat at 23 $^{o}C \pm 1$ ^{o}C) in five replications.

Linear growth of the analyzed microorganisms was presented as a difference between fungus growth on the control dishes and on dishes containing media with added plant oils. After three weeks since the experiment outset conidial sporulation of the test fungi was assessed by means of counting the number of macroconidia in Bürker haemocytometer.

The tested fungi biomass was grown in 300 dm^3 Erlenmayer flasks on 100 cm^3 of modified medium (without agar) with the use of ethereal oils, the same as in the experiment on fungi linear growth. In the inoculation chamber the fungi inoculum was added to the medium prepared in flasks. The fungi were cultured for 21 days at room temperature of ca 22 ^{o}C and afterwards the culturing liquid with mycelium was filtered through filter paper. Subsequently the mycelium was dried in a sterile glass at 80 ^{o}C to constant weight.

Results and discussion

The present research revealed stronger activity of peppermint oil in inhibiting linear growth of plant pathogenic fungi than of eucalyptus oil (Fig. 1, 2). A different response of phytopathogenic organisms was observed not only to the kind of plant oil applied but also to its dose in the PDA medium. Authors of other publications are of similar opinion

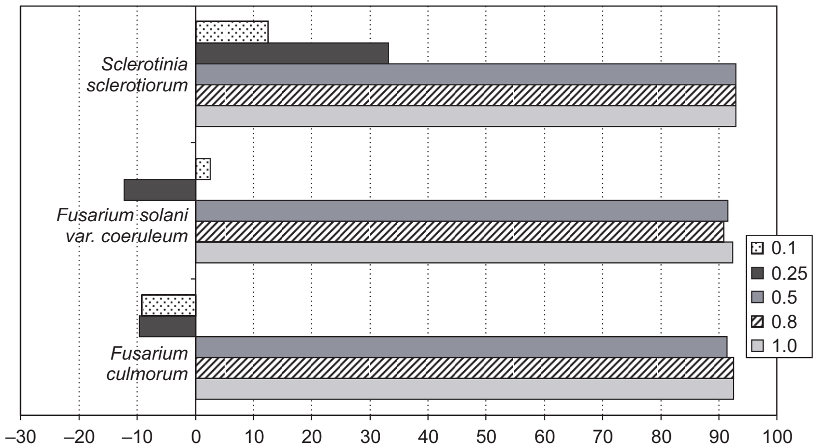

Fig. 1. Effect of mint oil on tested fungi linear growth inhibition [%]

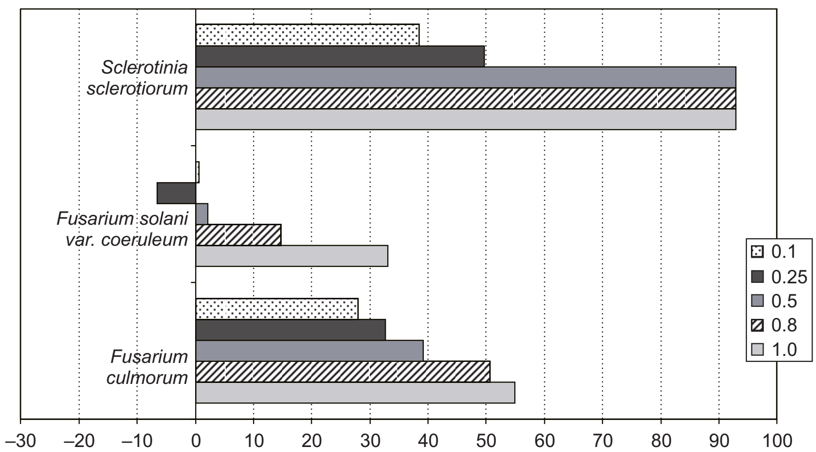

Fig. 2. Effect of eucalyptus oil on tested fungi linear growth inhibition [%]

[8, 14]. Among the tested organisms, *Sclerotinia sclerotiorum* showed greater sensitivity. A supplement of 0.25 cm^3 of mint oil caused a 33 % inhibition of this fungus linear growth. The same dose of eucalyptus oil inhibited the growth of *S. sclerotiorum* in 49 %. The presence of a higher dose (0.5 mm^3/cm^3 PDA) of both analysed oils inhibited the fungus linear growth even in 94 %. Daferera et al [8], who tested essential oils from aromatic plants (oregano, thyme, rosemary and lavender), observed growth inhibition of: *Botritis cinerea* and *Fusarium solani* var. *coeruleum*. On the other hand peppermint oil used in a dose of 0.5 mm^3 revealed stronger fungistatic properties towards *Fusarium* fungi. Introduction of this amount of oil to the medium inhibited *Fusarium culmorum* and *F. solani* var. *coeruleum* growth in 92 %. On the other hand, the fungistatic effect of the eucalyptus oil on *Fusarium* fungi, particularly on *F. solani* var. *coeruleum*, was weak. Diversified results of laboratory experiments obtained are the consequence of the chemical composition of the analysed oils. Other authors [8, 13, 14] are of the same opinion demonstrating that the activity of the isolated oils with respect to micro-organisms is not unanimous.

Biomass and sporulation also depended on the fungus species, plant oil and its dose (Tables 1, 2). Generally, the tested ethereal oils and their doses reduced biomass increments and sporulation of the tested fungi. Among the tested microorganisms the *S. sclerotinium* fungus was more sensitive to the oil presence. Already 0.10 mm^3 of mint and eucalyptus oil supplied to the medium caused inhibition of biomass increment and sporulation of the *S. sclerotiorum* fungus. *Fusarium* fungi and especially *F. solani* var. *coeruleum* revealed smaller sensitivity to the presence of oil in the medium. Reduction of biomass increment and sporulation were observed under the influence of higher

Table 1

Effect of tested ethereal oils on fungi biomass [g]

Oil dose [mm^3/cm^3]	*Fusarium culmorum*	*Fusarium solani*	*Sclerotinia sclerotiorum*
Control	0.266	0.300	0.566
Mint oil			
0.10	0.201	0.456	0.077
0.25	0.204	0.381	0.095
0.50	0.231	0.327	0.112
0.80	0.180	0.300	0.087
1.00	0.186	0.171	0.134
Mean	0.211	0.322	0.178
Eucalyptus oil			
0.10	0.212	0.171	0.074
0.25	0.240	0.313	0.065
0.50	0.183	0.269	0.046
0.80	0.151	0.193	0.084
1.00	0.192	0.158	0.359
Mean	0.207	0.234	0.199

Table 2

Conidial sporulation of fungi under the influence of ethereal oils

Oil dose [mm^3/cm^3]	*Fusarium culmorum*	*Fusarium solani*	*Sclerotinia sclerotiorum*
Control	57.5×10^6	41.2×10^7	39.7×10^7
Mint oil			
0.10	72.5×10^6	27.2×10^7	70×10^6
0.25	32.5×10^6	35.5×10^7	90×10^6
0.50	X	17.5×10^6	45×10^6
0.80	X	X	65×10^6
1.00	X	5×10^6	30×10^6
Eucalyptus oil			
0.10	45×10^6	15.7×10^7	29×10^7
0.25	15×10^6	29.2×10^7	18×10^7
0.50	X	29.7×10^7	14×10^7
0.80	10×10^6	45×10^6	65×10^6
1.00	20×10^6	34.5×10^6	65×10^6

X – no spores.

doses (0.8 and 1.0 mm/cm^3 PDA). A phenomenon of diversified effect of peppermint and Tasmanian blue gum oil on plant pathogenic microorganisms is connected with the specific character of chemical compounds in the composition of these oils, their complex effect and sensitivity of the phytopathogen itself, as reported by Daferera [8] and Lo Cantore et al [14].

Conclusion

The fungistatic activity of essential oils *in vitro* depends on the kind of plant oil, its dose and fungus species. Among the tested pathogenic microorganisms, *Sclerotinia sclerotiorum* fungus proved more sensitive to the presence of mint and eucalyptus oil. On the other hand, higher doses of the oils (0.8 and 1.0 mm/cm^3 PDA) reveal fungistatic properties towards *Fusarium* fungi.

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WSTĘPNE BADANIA NAD OCENĄ ODDZIAŁYWANIA OLEJKU MIĘTOWEGO I EUKALIPTUSOWEGO NA WYBRANE GRZYBY CHOROBOTWÓRCZE ROŚLIN

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Abstrakt: Badania laboratoryjne dotyczyły porównania wpływu olejku mięty pieprzowej (*Mentha* x *piperita* L. var. *Officinalis)* i olejku eukaliptusa gałkowego (*Eucalyptus globulus* Labill.) oraz ich dawek na wzrost liniowy, biomasę i zarodnikowanie *Fusarium culmorum* (W.G.Sm.) Sacc., *F. solani var. coeruleum* (Sacc.) Booth*, Sclerotinia sclerotiorum* (Lib.) de Bary. Z badań wynika, że aktywność fungistatyczna olejków eterycznych w warunkach *in vitro* zależy od rodzaju olejku roślinnego, jego dawki i gatunku grzyba. Z testowanych mikroorganizmów chorobotwórczych roślin, grzyb *Sclerotinia sclerotiorum* jest bardziej wrażliwy na obecność olejku miętowego i eukaliptusowego. Natomiast właściwości fungistatyczne w odniesieniu do grzybów z rodzaju *Fusarium* wykazują większe dawki olejków (0,8 i 1,0 mm/cm^3).

Słowa kluczowe: olejki roślinne, grzyby fitopatogenne, testy laboratoryjne

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HEAVY METALS IN THE SOIL AND IN THE ORGANISMS OF THE INVERTEBRATES INHABITING THE SOIL

METALE CIĘŻKIE W GLEBIE I ORGANIZMACH BEZKRĘGOWCÓW GLEBOWYCH

Abstract: The heavy metals that get into the organisms influence their vital processes. In order to evaluate the toxicity of metals to mesofauna of the soil the diversity, the number and the content of the Cd, Pb, Ni and Zn in the soil and in the body of the fauna of grass habitats were analysed. The chosen areas were situated near the roads with different rates of traffic flow and, to compare, in the city park. The soils were characterized by similar pH reaction and the low humidity. They differ in the content of the heavy metals. The soil in the city park showed the lowest concentration of Pb and low for the other heavy metals and, at the same time, the highest density of mesofauna.

Keywords: soil mesofauna, abundance, diversity, heavy metals

The heavy metals derived from antropogenic pollution of the air, water and soil disperse in these habitats and contaminate directly or indirectly through the plants or organic matter the organisms living there [1]. Their harmful influences consist of the possibility of cumulating in the living organisms and of their chronic toxicity [2].

The high density of heavy metals influence negatively the biological processes of the soil by reducing its fertility, enzymatic activity and by changes in its acidification. The high content of the metals can be toxic to microflora, plants and soil animals as well as for the man, because they become in less or higher degree the part of the food chain [3, 4]. The mechanisms of the harmful influences of the heavy metals on the living organisms are diverse and can lead to physiological changes causing the death of cells

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and tissues, and can be responsible for mutation- and cancer-causing changes in the organisms [5].

In order to evaluate the toxicity of the metals to mesofauna of the soil the diversity, number and the content of Cd, Pb, Ni and Zn were examined in the soil as well as in the fauna dwelling there.

Materials and methods

The material analysed was the soil from four lawn situated near roads with different rates of traffic flow and, for comparison, the soil from city park 200 m distant from the road.

Locality I situated around 1 m from the street;
Locality II situated around 5 m from the street;
Locality III situated around 4 m from the street;
Locality IV situated around 0.5 m from the street;
Locality V situated around 200 m from the street.

With the use of the soil frame of the size of 25 × 25 cm the set of samples was taken on the selected localities in spring 2008. The soil frame was thrust into the soil on the depth of 10 cm. Each series consisted of 16 tests on the surface of around 1 m^2.

Mesofauna was scampered away by employing the dynamic method in the modified Tullgren apparatus. After marking the select mesofauna its density and diversity were analysed.

Soil moisture and its pH, its temperature as well as the content of Cd, Pb, Ni and Zn were determined by using AAS method in the soil and in the mesofauna scampered away.

Dry samples of the soil and of the mesofauna were mineralized. For this purpose dry samples of the soil and the mesofauna was poured over 3 cm^3 of 65 % HNO_3, heated to the temperature of 120 ^oC and left for 4 hours. The filtered liquid was poured into measuring flasks and filled with distilled water to the volume of 25 cm^3. In solutions of the soil prepared in this way the content of heavy metals was determined by atomic absorption spectrometer (AAS – Cole-Parmer, BUCK 200A).

Results

The soil analysed were characterized by similar, slightly alkaline reaction (pH 7.33–7.74) and similar humidity within the range 16.1 to 20.4 % (Table 1).

Table 1

Comparison of selected parameters of the soils in the studied localities in Krakow

Selected parameters	Locality I	Locality II	Locality III	Locality IV	Locality V
Soil moisture [%]	16.5	16.1	20.4	18.9	18.85
Soil pH [-]	7.33	7.38	7.72	7.74	7.53
Area temperature [^oC]	11.5	9.8	12.6	12.9	8.5
Soil temperature [^oC]	9.3	11.1	11.4	11.9	8.8

These small differences in the humidity have no influences on the density of the mesofauna. The noticeable differences in the diversity on the analysed areas were not detected. The important differences in the research were connected with the content of the heavy metals in the soil as well as in the mesofauna dwelling there (Table 2).

Table 2

Comparison of mesofauna in the soils of the selected localities in Krakow

Selected parameters	Locality I	Locality II	Locality III	Locality IV	Locality V
Abundance of pedofauna [sp. no. per m^2]	440	566	396	592	1484
Diversity [number of taxonomic groups]	9	9	10	9	11

However, in the body of the mesofauna the highest concentration of Cd was detected in the I area, Ni in the V area and Zn in the I area (Fig. 1), where the soil was characterized by the lowest concentration of the above-mentioned elements. Whereas as far as Pb is concerned, its highest concentration was detected in the body of the mesofauna on the II area, where, at the same time, the highest content of this element in the soil was noted (Fig. 1).

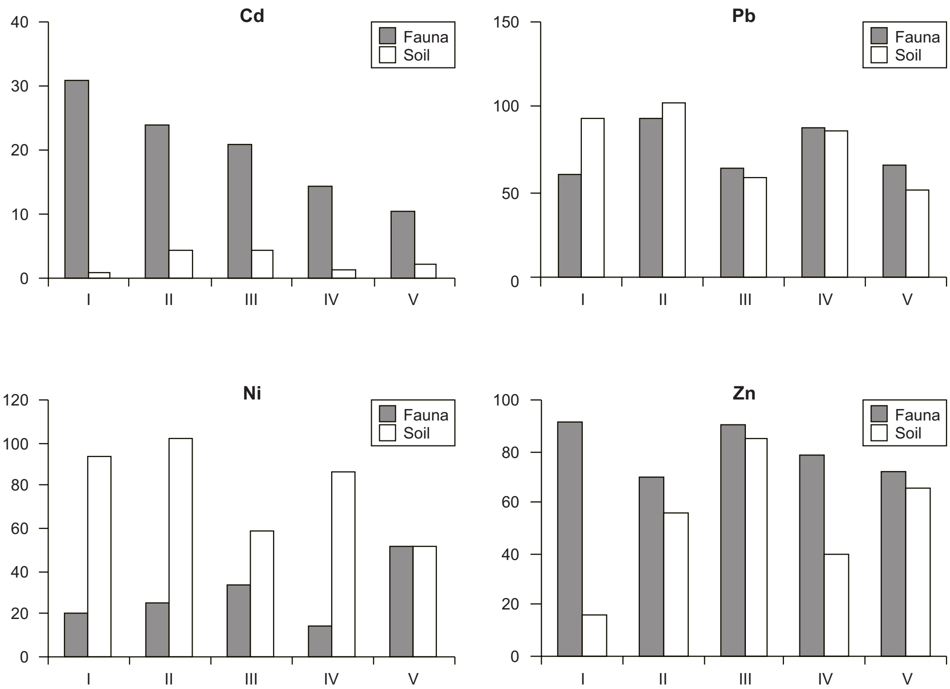

Fig. 1. Heavy metals content in the selected localities

The soil in the city park had the lowest concentration of Pb and relatively low of Cd and Ni. At the same time the highest density of mesofauna was detected in this area. In this area 1484 species in m^2 were noted, whereas in the remaining areas the density of the mesofauna was from more than two to more than three times lower (Table 2). The mesofauna in the city park contained the lowest concentration of Cd, relatively low of Pb and Zn and the highest concentration of Ni in spite of its low content in the soil (Fig. 1). It can be the prove of the fact that direct connection between the content of Ni in the soil and the fauna dwelling there does not exist.

In the areas with the high rates of traffic flow (locality II, III) the highest density of Cd, Ni and Zn and simultaneously the law density of the mesofauna and high content of Cd in their bodies were detected (Table 2, Fig. 1).

Conclusions

1. The small differences in the pH of the soil analysed have no influences on the density of the mesofauna.
2. The high contents of Cd, Pb, Ni and Zn in the soil limits the number of the mesofauna dwelling there.
3. The high contents of Cd and Zn in the soil as well as in the body of the mesofauna is the limiting factor of its density.
4. As far as Cd, Ni and Zn are concerned direct connection between their number in the soil and its number of these metals in the body of the mesofauna dwelling there was not detected.
5. A considerable amount of trees and bushes as well as remoteness from roads protect the soil against Pb.

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METALE CIĘŻKIE W GLEBIE I ORGANIZMACH BEZKRĘGOWCÓW GLEBOWYCH

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Abstrakt: W celu oceny toksyczności metali dla mezofauny glebowej zbadano zróżnicowanie, liczebność oraz zawartość Pb, Cd, Ni, Zn zarówno w glebie, jak i w ciele zasiedlającej ją fauny wybranych siedlisk trawiastych. Badane stanowiska były położone blisko traktów komunikacyjnych o różnym stopniu nasilenia ruchu pojazdów mechanicznych i dla porównania w parku miejskim. Gleby charakteryzował podobny odczyn pH oraz mała wilgotność. Różniły się one natomiast zawartością metali ciężkich. Gleba w parku miejskim wykazała najmniejszą koncentrację Pb i małą pozostałych metali ciężkich, a jednocześnie największe zagęszczenie mezofauny. Natomiast w organizmach badanych zwierząt glebowych stwierdzono duże stężenia metali ciężkich na stanowiskach o dużej koncentracji tych metali w glebie.

Słowa kluczowe: mezofauna, zagęszczenie, metale ciężkie

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SANITARY ANALYSES OF SURFACE WATER IN THE INFLUENCE AREA OF MUNICIPAL WASTE DUMP BARYCZ IN KRAKOW

BADANIA SANITARNE WÓD POWIERZCHNIOWYCH W STREFIE ODDZIAŁYWANIA SKŁADOWISKA ODPADÓW KOMUNALNYCH BARYCZ W KRAKOWIE

Abstract: Microbiological analyses of surface water and soaking water were carried out in the area of the municipal waste dump Barycz in Krakow. The surface water samples were taken from June 2004 to May 2005 in monthly intervals from 5 measuring points, located following the direction of Malinowka stream water flow, nearby the waste dump, and the sample of soaking water from the drainage trench. While comparing the results from the period in which the II section was exploited, and then the III section, it may be ascertained, that the waste and mostly soaking water are the main sources of different microorganisms' penetration into the surface water. Amount of the tested indicating bacteria in the samples was mainly dependent on the distance of the measuring points from the borders of waste dump sections. On the basis of the regulations from the Ordinance of Ministry of Environment (DzU Nr 32, poz. 284) it was ascertained that in four measuring points – from above the III section, which is the inflow to the waste dump, until the point about 2 000 m further – the water in Malinowka stream is in class IV. Only in further part of the stream, even 2 700 m further, the water was classified into class III.

Keywords: microbiological indexes, surface water, municipal waste dump, soaking water

Waste dumps may be the source of different contamination, not only for soil, but also for groundwater and surface water. Threat to the quality of water nearby the waste dumps depends mainly on amount and composition of waste, technology of deposition, and location of the object. The highest danger comes from the waste dumps with wrong ground impregnation, where the contamination may infiltrate even to the significant distances and depths [1, 2].

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Influence of municipal waste dumps on the groundwater and surface water may be various: from the local contamination – e. g. soaking water, surface flows which carry the contamination from the near waste dumps, drained by the watercourses and collectors of water-bearing beds as well as washing away of waste during surface waters overflow. These factors may occur individually or in complex [3, 4].

Until now, microbiological analyses of surface water in the areas of waste dumps have been minor. The reason for this is lack of legal norms (in the Ordinance of the Minister of Environment from January 2003 about the range, time, way and conditions of monitoring of waste dumps, the biological factor was totally disregarded) and universal, standardized methods of evaluation of waste dumps influence on the environment and human health. Appreciating the role of such analyses, the main scientific aim of this work was the research of sanitary state of surface water nearby the big municipal waste dump.

This paper aimed to determine the effect of an active municipal waste landfill on microorganisms participating in nitrogen transformation in the soil environment.

Material and methods

Microbiological analyses of surface water and soaking water were carried out in the area of the municipal waste dump Barycz in Krakow. It is the biggest and the longest exploited object of such type in the area of Malopolska province and one of the biggest in Poland (its total area is 37 ha). The area of waste dump was divided into three parts: one was exploited from the end of 1974 to 1992, second to 2005, and currently the III section is being exploited – 11 ha syncline. At present, the municipal waste dump Barycz in Krakow may be considered as one of the best organized in Poland.

Samples of water were taken from June 2004 to May 2005 in monthly intervals from 5 measuring points, located following the direction of water flow of Malinowka stream, nearby the waste dump, and one sample of soaking water was taken from the drainage trench.

The chosen measuring points were marked on the map (Fig. 1) and described as following:

A – "background" – Malinowka stream before the III section, by the fence closing the waste dump (570 m from the gateway to the waste dump in the South-West direction),

B – Malinowka stream near the middle part of the III section, 260 m from the gateway to the waste dump in the South-East direction,

C – Malinowka stream, by the approaching road – nearby the unused collector for the soaking water, 720 m from the gateway in the North-East direction,

D – Malinowka stream near the P-8 piezometer, 660 m to the North from the borders of the I section,

E – Malinowka stream near the G piezometer, 1230 m to the North from the borders of the II section,

O – soaking water from the drainage trench by the II section of the waste dump, 450 m from the gateway to the waste dump in the North-East direction.

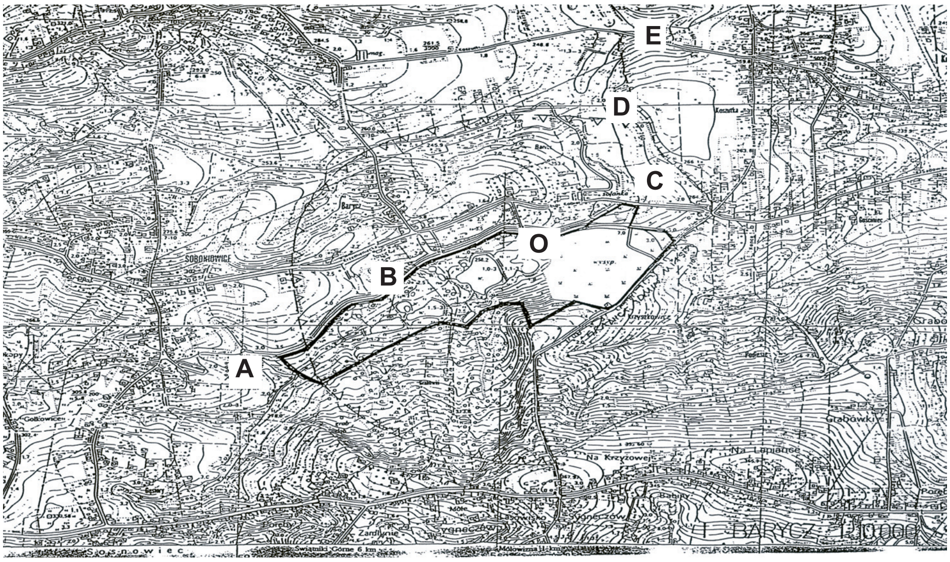

Fig. 1. Location of measuring points

It has to be mentioned, that the D and E measuring points were placed beyond the 500 m of protective zone measured from the borders of the waste dump.

Samples of water and soaking water were transported to the lab of Department of Microbiology at the University of Agriculture in Krakow, where the microbiological analyses were carried out, which consisted of:

1. evaluation of general number of mesophilic bacteria on nutritious agar,
2. evaluation of number of general coliforms and thermotolernat coliforms on Kessler–Swenarton agar, and in order to confirm the presence of coliform group bacteria – on Endo agar and on brilliant green bile agar,
3. evaluation of count of fecal *Streptococci* on APB medium and on AFE medium in order to confirm the presence of fecal *Streptococci*.

Number of microorganisms was defined using inoculation of diluted and non-diluted water and soaking water on the media as well as using membrane filters. The results were counted over 1 cm^3 or 100 cm^3 or were presented as a count.

Results and discussion

Waste dumps and inconveniences related to them become more and more visible issue in environment protection during last years. Waste cultivation, especially municipal and industrial waste, has to be included into very important but also very difficult tasks. In Poland, it was not possible to solve all of these problems for a large scale [5].

While searching for the solution of these problems, from June 2004 to May 2005 microbiological analyses were performed on surface water of Malinowka stream, which flows through the area and surroundings of the municipal waste dump Barycz in Krakow. The gained results are presented in the Table 1 and in the Figures 2, 3 and 4.

On the basis of gained analytical data (Table 1, Fig. 2) a visible tendency of mesophilic bacteria increase was observed, as well as other microbiological indexes from March 2005, which is from the beginning of active usage of the II section of the waste dump. This fact evidences the influence of the waste dump on the microbiological state of the tested water. It is worth to mention, that the maximal amount of mesophilic bacteria was observed in March 2005 (point A) in the month in which the III section of the waste dump became active. The quatitative analyses also show that the highest number of mesophilic bacteria was after the water of Malinowka stream flew along all three sections of the waste dump and the old collector for the soaking water (from 2900 to 4895250 cfu/cm^3, mean value 800410 cfu/cm^3) – point C. The further the stream flew, the lower number of mesophilic bacteria was found, until the D measuring point (about 650 m from the C point) where the amount of mesophiles was from 6000 to 630000 cfu/cm^3. However mean number of mesophilic bacteria (77810 cfu/cm^3) was over ten times lower than in the C point. Very slow, gradual self-purification process was observed with the flow of water to the North. The first symptoms of this phenomenon occurred in the water taken 950 m, and 1230 m to the North from the borders of the waste dump.

According to Smylla [6] the amount of bacterio-plankton in 1 cm^3 of the surface water ranges from 102 to 109 in water non-contaminated with the sewage. The results of analyses performed in 1992 in the border of the protective zone (500 m) showed that the water in this part has not been self-purified [7]. Such phenomenon was also presently observed, but the fact of decreasing of the microbiological contamination in the further part of the stream is encouraging.

The current sanitary evaluation of water is based on the indirect conclusions about the presence of pathogens and indicators which normally live as saprophytes in the gastrointestinal tract of humans and higher animals. The sanitary indicators play the warning role from the infections, because there is a direct dependence between the amount of indicating bacteria in water and the amount of pathogens [6, 8, 9]. Water is not the natural environment for the pathogens, it is only their transport, and it is just in the period in which these microorganisms may stay alive [10, 11].

The basic indicator of bad sanitary state are coliforms, which amount decides, according to the Ordinance of the Ministry of Environment (DzU Nr 32, poz. 284) [12], of the division of surface water into five classes. Taking these criteria into account, it should be concluded that water of Malinowka stream in the part from the A to D measuring points include in the IV class, which means it is of bad quality. However, water from E point (located 2740 m from A point and 800 m from D point) was classified into class III (water of satisfactory quality regarding the microbiological state) But it should be pointed out that in August 2004, the amount of fecal coliforms reached the top border of this class. It is also worth to mention that these bacteria were not found in 8 among 12 analyses in 100 cm^3 of water taken from E point. Water quality evaluation is based on the border amounts of indicators, which for the IV class count: coliforms – from 5000 to 50000, fecal coliforms from 2000 to 20000 in 100 cm^3 of water.

Table 1

Amount of microorganisms in surface water in Malinowka stream in the area of municipal waste dump Barycz in Krakow

Measuring point	Dates of sampling											
	30 VI 2004	29 VII 2004	31 VIII 2004	26 IX 2004	27 X 2004	25 XI 2004	28 XII 2004	27 I 2005	25 II 2005	29 III 2005	28 IV 2005	25 V 2005
	Mesophilic bacteria [cfu in 1 cm^3 of water]											
A	553330	930250	549330	17910	250	200	190	680	347890	950000	721890	400000
B	319000	580670	1206350	1659750	200	150	850	6300	18470	78000	138950	152000
C	4895250	1138000	70500	3396700	17000	10000	13560	27900	14780	2900	8300	10000
D	41000	6000	61000	630000	54500	21100	35800	14540	19800	7300	18650	24000
E	35300	46180	58100	46180	21300	16210	8100	7800	6900	12340	21520	23100
O	3000330	920670	4267500	20890000	17000	16000	43320	893500	654200	1090000	3870000	28000000
	Fecal streptococci [count]											
A	>1	>1	0.0001	0.1	0.1	>1	>1	>1	0.01	0.0001	0.001	0.0001
B	0.1	0.01	0.01	0.1	0.01	>1	>1	>1	0.1	0.0001	0.001	0.0001
C	0.001	0.01	0.001	>1	0.1	>1	>1	>1	0.1	0.0001	0.01	0.01
D	0.1	0.001	0.001	>1	>1	>1	0.1	>1	0.1	0.001	0.001	0.0001
E	>1	0.1	0.1	0.1	>1	>1	>1	>1	>1	0.1	0.01	>1
O	0.00001	0.001	0.001	0.001	0.01	0.01	0.01	0.001	0.0001	0.00001	0.001	0.00001

A (above the III section) and B (on the height of the middle part of the III section) points had in 100 cm^3 of water as following: from 500 to 18200 cfu of coliforms and from 300 to 7600 cfu of fecal coliforms (thermotolerant), from 3200 to 18900 cfu of coliforms and from 500 to 10600 cfu of fecal coliform. However the mean amount was similar to the first group – 7708 and 7467 cfu, 4200 and 4125 cfu for the second group of the tested bacteria. During 12 months of the research, samples of water from A point

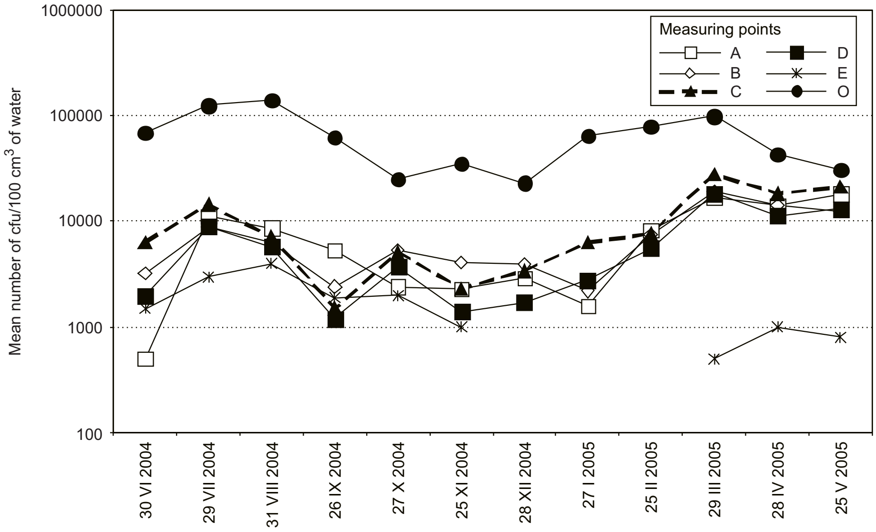

Fig. 2. Amount of coliforms (log scale) in surface water and in soaking water depending on the date of sampling in the area of the municipal waste dump Barycz in Krakow

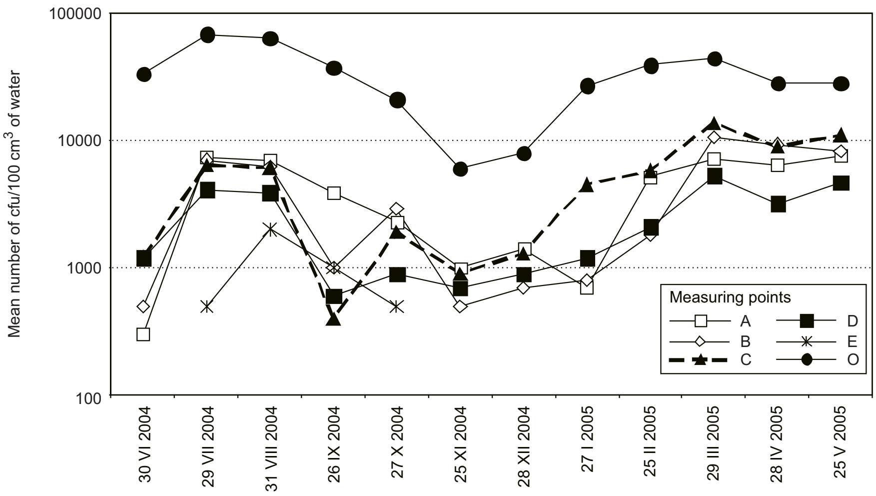

Fig. 3. Amount of thermotolerant coliforms (log scale) in surface water and in soaking water depending on the date of sampling in the area of the municipal waste dump Barycz in Krakow

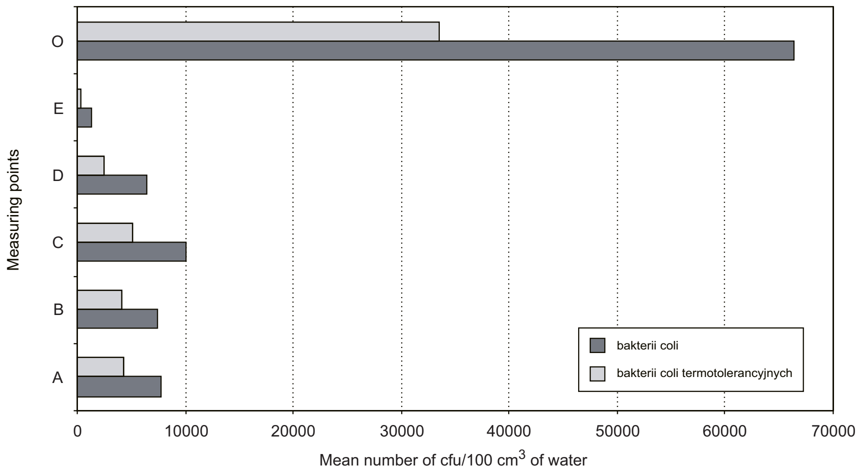

Fig. 4. Average amount of coliforms and thermotolerant coliforms (linear scale) in surface water and soaking water in the area on the municipal watse dump Barycz in Krakow

8 times, and from B point 6 times did not transgress the border values of fecal coliforms for the IV class. According to the general coliform index, 7 out of 12 analyses classified the water from A and B point into the IV class.

Many more bacteria were isolated from water from C point, taken from Malinowka stream nearby the old collector for the soaking water. In 100 cm^3 of this water, coliforms occurred in the amount from 400 to 13600 cfu. The border value of coliform index for the II class was transgressed 9 times, whereas for fecal coliforms – 7 out of 12 analyses, which caused classification of this water into the IV class. In the further parts of Malinowka stream the amount of both groups of bacteria decreased, but as far as 1 450 m from the C point it could be classified into the III class.

In 1 cm^3 of water taken from A, B, C and D points – periodically no fecal *Streptococci* were found, or they were found even in 10–4 dilution. The gained results of bacteriological analyses were given as a count, and its range was defined from > 1 to 0.0001. Only in water from E point, the range of fecal *Streptococci* was from > 1 to 0.01, and the minimal value was found only once – 28th April 2005. In other 7 months these bacteria were not found, and in 4 dates of sample taking their presence was found only in water ten times diluted, taken from the point located the furthest from the waste dump (Table 1).

Soaking water has the greatest influence on the contamination of the surface water. Decrease of their occurrence is synonymous with the decrease of the degrading influence of the municipal waste dump on the environment. However, it should be mentioned that municipal waste collecting leads to the occurrence of soaking water. The amount of soaking water depends on the type of waste, way of its collection, period of the waste dump exploitation and on the amount of precipitation. The composition of the soaking water depends on the stage of decay and the waste substance [13].

Basing on the chemical analysis in 2004, it was found that the soaking water in the area of the municipal waste dump Barycz in Krakow is mainly contaminated with organic compounds and is highly salted. The border pH values were 6.9 and 9.5 but most often pH was on the level of about 7–8 [14].

The analyses of the soaking water taken from the drainage trench nearby the II section of the waste dump Barycz in Krakow showed very profuse occurrence of different microorganisms groups during the whole year of tests. On the basis of the analyses, occurrence in 1 cm^3 of the soaking water from 16000 to 28000000 cfu of mesophilic bacteria was found from 30th June 2004 to 25th May 2005. The sanitary indexes were on the high level, because the count of fecal streptococci in the soaking water was from 0.01 to even 0.00001, and coliforms were from 23000 to 141000 cfu/100 cm^3 whereas fecal coliforms were from 6000 to 68000 cfu/100 cm^3. Comparing these results with surface water classification, a high transgression of the bottom border values for the last V class of water may be found. These values are: coliforms > 50000 and fecal coliforms > 20000 in 100 cm^3. Mean monthly amount of these bacteria in the tested soaking water was as follows: 33583 and 66417 cfu in 100 cm^3. In summer (July and August) maximal value of coliforms index was found: over 100000 for the general group and over 60000 for the fecal – thermotolerant group. It should be mentioned that the soaking water temperature was on the level 2.9–20.7 ^{o}C during the research year. The high temeration in summer was promoting the growth of most microorganisms.

Comparing the results gained in the period of II section exploitation, and then the III section of the municipal waste dump, in the samples of surface water, the soaking water may be considered to have the influence on spreading of microorganisms in the area and in the surroundings of this waste dump.

According to PIOS [15] the soaking water is many times overloaded than the city sewage, because it shows large quantitative differentiation of bacteria. Improvement of the soaking water quality was usually found after protection of the waste heap, by eg reinforcing the slopes, tightening the soil and surrounding it by the screen as deep as the impermeable level [2].

Conclusions

1. Microbiological analyses of the surface water and the soaking water performed from June 2004 to May 2005 nearby the municipal waste dump Barycz in Krakow showed the differentiation of indicating bacteria occurrence.

2. The collected waste and the soaking water are the basic source of different microorganisms' penetration into the surface water. Amount of the tested indicating bacteria in the samples depended on the distance of the measuring points from the borders of the waste dump sections.

3. Analyzing the results, it may be concluded that on the whole part of the Malinowka stream – 2740 m, occurrence of the fecal bacteria was found. According to this, there is a danger of the presence of other microorganisms, which may negatively influence people and animals health.

4. On the basis of the microbiological research and on the Ordinance of the Minister of Environment (DzU Nr 32, poz. 284) in four measuring points – above the III section (the place of inflow to the waste dump) to the point about 2 000 m further – water in

Malinowka stream may be of the IV class. Only in the further part of the stream distant over 2 700 m the water was classified into the III class.

5. The exact knowledge of the influence of the municipal waste dumps on the environment and human health is very important issue, which needs the complex, interdisciplinary, long-term research. This is why it is necessary to perform microbiological analyses of surface water in the area of waste dump influence, not only the physicochemical ones, and it should be legally defined.

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BADANIA SANITARNE WÓD POWIERZCHNIOWYCH W STREFIE ODDZIAŁYWANIA SKŁADOWISKA ODPADÓW KOMUNALNYCH BARYCZ W KRAKOWIE

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Abstrakt: Badania mikrobiologiczne wód powierzchniowych oraz odcieku prowadzone były w rejonie składowiska odpadów komunalnych Barycz w Krakowie. Próbki wód powierzchniowych pobierano w okresie od czerwca 2004 r. do maja 2005 r. w odstępach miesięcznych z 5 stanowisk pomiarowych ułożonych kolejno w kierunku spływu wody potoku Malinówka w sąsiedztwie składowiska oraz próbkę odcieku z rowu opaskowego. Porównując uzyskane wyniki w okresie, kiedy był eksploatowany sektor II, a następnie sektor III składowiska odpadów komunalnych Barycz w Krakowie można stwierdzić, że składowane odpady, a przede wszystkim odcieki są zasadniczym źródłem przenikania różnych grup mikroorganizmów do wód powierzchniowych. Liczebność badanych bakterii wskaźnikowych w pobranych próbkach wody uzależniona była przede wszystkim od odległości wyznaczonych stanowisk pomiarowych od obrzeży sektorów składowiska. Na podstawie zaleceń zawartych w Rozporządzeniu Ministra Środowiska (DzU Nr 32, poz. 284) stwierdzono, że w czterech punktach pomiarowych – od powyżej III sektora składowiska, czyli miejsca wpływu na teren składowiska aż do punktu oddalonego około 2000 m – woda w potoku Malinówka odpowiada klasie IV. Dopiero w dalszym biegu potoku oddalonym ponad 2700 m zaliczono badaną wodę do III klasy.

Słowa klucze: wskaźniki mikrobiologiczne, wody powierzchniowe, składowisko odpadów komunalnych, odciek

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CHANGES IN NUMBERS OF MICROORGANISMS PARTICIPATING IN NITROGEN METABOLISM IN SOIL UNDER HORSE BEAN CULTIVATION AROUND A MUNICIPAL WASTE LANDFILL SITE IN TARNOW

ZMIANY LICZEBNOŚCI DROBNOUSTROJÓW BIORĄCYCH UDZIAŁ W METABOLIZMIE AZOTOWYM W GLEBIE POD UPRAWĄ BOBIKU WOKÓŁ SKŁADOWISKA ODPADÓW KOMUNALNYCH W TARNOWIE

Abstract: Field research connected with the subject of the paper was conducted from March 2006 to September 2007. For the sake of the experiment 8 experimental points were established on each side of the municipal waste landfill in Tarnow in two zones: 50–200 m and 250–500 m from its boundaries and samples were collected from the soil in which horse bean, Nadwiślański c.v. was cultivated. An additional ninth point was located in the landfill area, in the inactive, reclaimed landfill sector. The results of microbiological tests show obvious differences in the quantitative composition of microflora participating in nitrogen metabolism depending on the distance from the active landfill sector. Comparison of all results revealed an apparent increase in the number of microorganisms participating in nitrogen metabolism during the horse bean vegetation period in comparison with their occurrence before and after the completed vegetation season.

Keywords: soil, microflora, municipal waste landfill site

Soil, as the natural living environment of various microorganism taxonomic groups, for many of them is a suitable ecological niche where numerous abiotic and biotic factors affect one another. Soil quality depends not only on its physical and chemical properties but is also strictly connected with its biological properties, and in the first place with microbiological processes [1, 2].

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Soil microflora is the component of environmental biocenosis which is the fastest growing and responding to changes in environmental parameters. It is conditioned by diversity of biochemical functions, specific for microorganisms and their very high physiological activity. There is also a distinct interrelation among soil environment parameters, microorganisms settling soil and plants [3, 4].

Therefore, soil microorganisms are the factor which together with the vegetal cover determines both the direction and character of biochemical processes and all basic biological transformations connected with biological activity and physicochemical properties of arable soils. The results of the activity comprise not only mineralization and humification of various organic compounds (including humus synthesis) but also mobilization of many mineral compounds which are of primary importance for the life of plants and soil animals [2, 3]. Therefore, it is most important to identify precisely and analyze biocenotic relationships formed in the soil environment, which allow for observation of the scale of changes in soil microorganism biodiversity [5]. The present paper aimed to determine the effect of an active municipal waste landfill on microorganisms participating in nitrogen transformation in the soil environment.

Material and methods

Field research connected with the subject matter was conducted from March 2006 until September 2007 as a model field experiment set up in the vicinity of a municipal waste landfill site in Tarnow. Eight experimental points for soil sampling were established on each side of the investigated object in two zones: below 250 m and 250–500 m from its boundaries and experimental plots were designated in them. Horse bean, Nadwiślański c.v. was cultivated on the plots. An additional ninth point was localized in the landfill area, on an inactive sector, which was formerly reclaimed. Labelling of the points has been presented in Table 1.

Table 1

Plots situated in the vicinity of the municipal waste landfill site in Tarnow 2006–2007

No.	Point	Location of plots with respect to landfill site	
		Direction	Zone [m]
1	W I	West	50–200
2	W II – control	West	250–500
3	N I	North	50–200
4	N II	North	250–500
5	E I	East	50–200
6	E II	East	250–500
7	S I	South	50–200
8	S II	South	250–500
9	Z	Landfill site – reclaimed sector	

Soil samples for microbiological analyses were collected four times in 2006 and 2007 (from March till September) from the root zone at various periods of horse bean vegetation. Collected soil samples were brought to the microbiological laboratory of the Agricultural University in Krakow, where soil moisture and pH were measured and microbiological analyses were conducted. These comprised determination of the numbers of ammonificators, proteolytic microorganisms and aerobic atmospheric nitrogen assimilators of the genus *Azotobacter*. Moreover the course of nitrification and denitrification processes was determined. The number of microbial colony forming units (CFU) was assessed using the method of dilution inoculation and converting the assessment result into 1 gram of soil dry matter or the count was assayed in diluted soil starting from 10–1, ie in 0.1 g.

An important agent contributing to pollutant spread from a landfill are winds. Characteristics of wind directions in the region of the municipal landfill site in Tarnow was presented in Table 2.

Table 2

Wind directions in Tarnow

Wind direction	Proportion
North wind	6.0
North-eastern	7.1
Eastern wind	16.7
South-eastern wind	4.8
South wind	14.8
South-western wind	7.4
Western wind	22.6
North-western wind	8.8
Silences	11.8

Results and discussion

The numbers of soil microorganisms is one of the parameters allowing for an assessment of processes occurring in the polluted soils and their potential ability for pollutant biodegradation. Mineralization of organic compounds containing nitrogen is the basic microbiological process supplying nitrogen for plants and microorganisms in an easily available mineral form. Proteins and other nitrogen containing substances occurring in the soil environment undergo gradual microbiological decomposition by proteolytic, ammonification and denitrifying bacteria [1, 6, 7].

Results of microbiological analyses show apparent differences in the quantitative composition of the microflora participating in nitrogen metabolism depending on the distance from the active landfill. Analytical data presented in Figure 1 show that between 3000 and 172000 CFU proteolytic bacteria occur in 1 g of soil dry matter in the municipal waste landfill in Tarnow and in the surrounding area, in soil under horse bean, Nadwiślański c.v. cultivation. The maximum value for this group of bacteria was assessed in September 2006 on an experimental plot situated in the 50–250 m zone south from the landfill area (Fig. 1, plot S I). The minimum numbers of proteolytic bacteria were registered in September 2007 on the control plot – W II, which was located in 250–500 m zone in front of the landfill entrance gate (to the west). A slightly higher number of these bacteria was also observed on S II point (Fig. 1) in September 2006. Quantitative analyses of proteolytic bacteria at 9 experimental points shows their different numbers, which were larger in soils on the plots situated to the north and south, at the distance below 250 m from the landfill (plots S I and N I). Attention should be paid to an apparent decrease in proteolytic bacteria numbers in the initial and final period of the experiment, ie in March 2006 and September 2007 (Fig. 1).

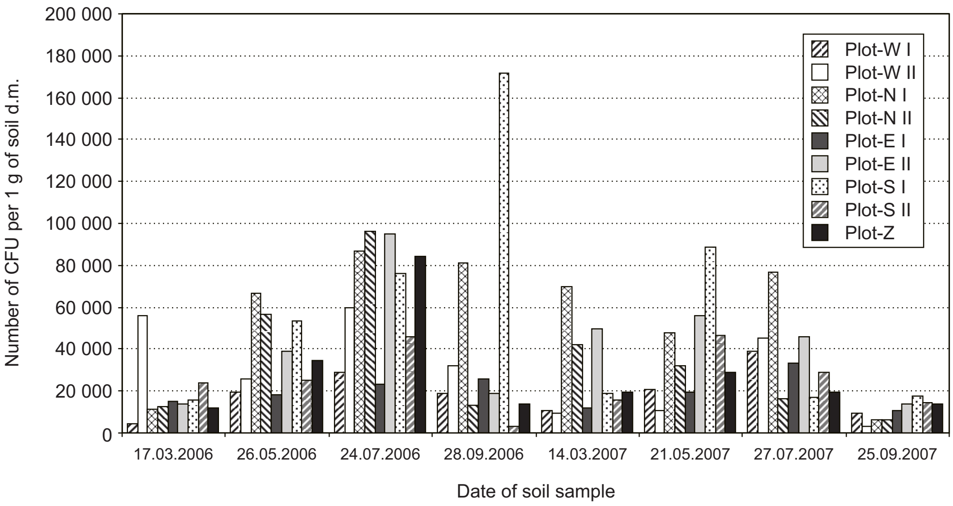

Fig. 1. Changes in proteolytic bacteria numbers in soil under horse bean, Nadwiślański c.v. cultivation in the vicinity of the municipal waste landfill site in Tarnow (in colony forming units (CFU) per 1 g of soil d.m.)

The analysis of the changes in ammonification bacteria numbers according to the date and place revealed considerable changes in their numbers, fluctuating depending on the research point from 113500 (March 2007) on E I plot to 7881066 CFU I 1 g soil d.m. (March 2006) at the research point located 250 m north of the landfill (N I plot) (Fig. 2). Definitely the lowest values for ammonification bacteria were registered on the experimental plot E II located in the 250–500 m zone east of the landfill. A comparison of these bacteria numbers in the experiment on all plots shows an apparent increase in their numbers during horse bean growing season from May till July 2006–2007, which was most probably caused by intensive mineralization of the soil organic matter due to the thermal and moisture condition in this period prevailingly advantageous for the microflora development [8].

The obtained results evidence that the course of ammonification process depends on various environmental factors, among others the soil type, total carbon and organic nitrogen concentrations, mineral and organic fertilization [9].

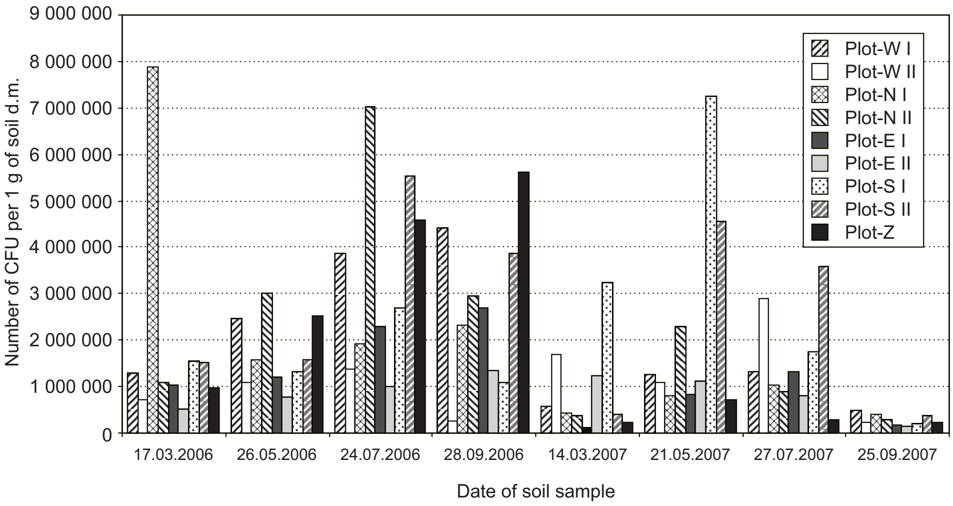

Fig. 2. Changes in ammonification bacteria numbers in soil under horse bean, Nadwiślański c.v. cultivation in the vicinity of the municipal waste landfill site in Tarnow (in colony forming units (CFU) per 1 g of soil d.m.)

Among bacteria participating in the atmospheric nitrogen assimilation are *Azotobacter* genus. The numbers of these free living soil assimilators under plots where horse bean was cultivated ranged between 0 at all experimental points to 460 colony forming units (CFU) per 1 g of soil d.m. at W II point (Fig. 3). Comparison of changes in *Azotobacter* numbers suggests the fact that in the presented experiment they were the

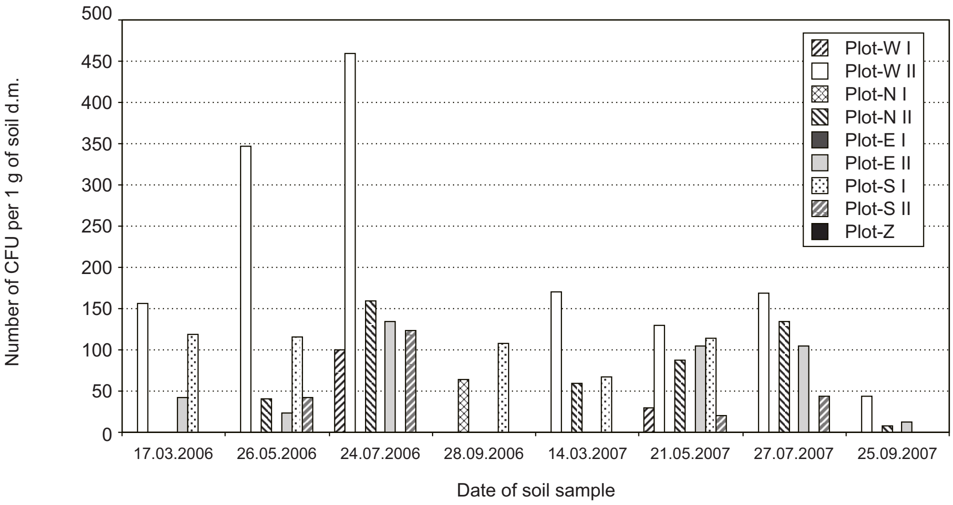

Fig. 3. Changes in *Azotobacter* bacteria numbers in soil under horse bean, Nadwiślański c.v. cultivation in the vicinity of the municipal waste landfill site in Tarnow (in colony forming units (CFU) per 1 g of soil d.m.)

most frequent and in greatest numbers present on the field located in front of the entrance to the landfill (to the west) at point W II and on the plots considerably distant from the landfill, in the 250–500 m zone – experimental points N II, E II and S II. No presence of *Azotobacter* was detected over the whole period of the experiment at the experimental point situated in the landfill area in the reclaimed sector (point Z) and in the point located east of the landfill in the 50–500 m zone.

It confirms that the numbers of *Azotobacter* depended on the soil pollution, which has a toxic effect visible either as a considerable decline in its numbers or leading to a total elimination of this microorganism group in the soil in the area of the municipal waste landfill or close to it [9].

Considering the dates of analyses, the increase in *Azotobacter* populations may be noticed in the soil under horse bean cultivation in the presented experiment in the summer period (from May till June 2006 and 2007) in comparison with the spring-autumn season (March, September 2006 and 2007).

The research testified that the nitrifying and denitrifying activity of the soil depended both on the location of the experimental plot and on the period of the experiment. As results from Table 2, the nitrification count at the experimental points was determined on the level from 0.01 to 0.000001. An apparent predominance of nitrification process is visible on the plots localized to the north and south of the landfill site (experimental points N I and S II) in comparison with the plot situated in the landfill area in the reclaimed sector. General seasonal changes of the nitrification process were regular and distinctly evidencing that the highest activity of the nitrification process occurred during horse bean vegetation in the summer and early autumn period, ie in July and September 2006 and in July 2007.

The obtained research results prove that nitrification process may be disturbed by chemical compounds present in soil. The course of this process may be disturbed also when other conditions, unfavourable for nitrifiers occur in soil since the nitrification process is determined by pH, organic matter content and heavy metal concentrations in soil [10].

Different denitrification counts were registered in soil samples collected from the experimental plots situated in the landfill and its neighbourhood, ranging from 0.001 at the points situated west of the landfill in the below 500 m zone from the landfill entrance – points W I and W II and at the point located to the north at the distance of 250 m – point N I (Table 3). An apparent prevalence of the denitrification process was visible in the soil on plots localized in the zone between 250 and 500 m from the landfill and in the area of the reclaimed landfill sector in comparison with the plots situated in its vicinity.

It should be emphasized that soil provides an excellent substratum for the growth and development of miocroorganisms because it is usually well supplied in organic and mineral nutrients, has suitable moisture, pH and favourable aerobic conditions. Owing to these physicochemical properties, it is the natural habitat for large numbers of various forms of microflora and microfauna [11].

Table 3

The course of the nitrification and denitrification process in soil under horse bean, Nadwiślański c.v. cultivation in the vicinity of the municipal waste landfill site in Tarnow

Count	Sampling dates							
	17 March 2006	26 May 2006	24 July 2006	28 Sept. 2006	14 March 2007	21 May 2007	27 July 2007	25 Sept. 2007
	Plot W I – Control							
Nitrification	0.0001	0.001	0.000001	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
	Plot – W II							
Nitrification	0.00001	0.00001	0.0001	0.000001	0.00001	0.00001	0.0001	0.0001
Denitrification	0.0001	0.0001	0.0001	0.001	0.0001	0.000001	0.00001	0.0001
	Plot – N I							
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
	Poletko – N II							
Nitrification	0.0001	0.0001	0.000001	0.01	0.00001	0.00001	0.000001	0.001
Denitrification	0.00001	0.00001	0.000001	0.0001	0.0001	0.000001	0.000001	0.00001
	Plot – E I							
Nitrification	0.00001	0.00001	0.001	0.001	0.0001	0.00001	0.001	0.01
Denitrification	0.001	0.001	0.0001	0.0001	0.001	0.001	0.00001	0.0001
	Plot – E II							
Nitrification	0.00001	0.0001	0.000001	0.00001	0.001	0.00001	0.001	0.001
Denitrification	0.0001	0.00001	0.0001	0.0001	0.0001	0.000001	0.00001	0.0001
	Plot – S I							
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
	Poletko – S II							
Nitrification	0.00001	0.0001	0.000001	0.000001	0.0001	0.000001	0.000001	0.0001
Denitrification	0.00001	0.00001	0.000001	0.000001	0.0001	0.000001	0.000001	0.00001
	Plot – Z							
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

On the basis of the investigated soil texture analysis they were classified to sandy, weakly loamy, light and strong loamy deposits (plots; W II, E I, E II, S I and S II), to light loams (plots W I, N I, N II, Z). Deposits with heavier (loamy) granulation occurred only on the plots established on the northern side of the landfill and on the plot Z situated on the already reclaimed landfill sector. The conducted measurements reveal considerable fluctuations of the soil pH from 4.8 to 7.1 (Fig. 4) in the investigated soil

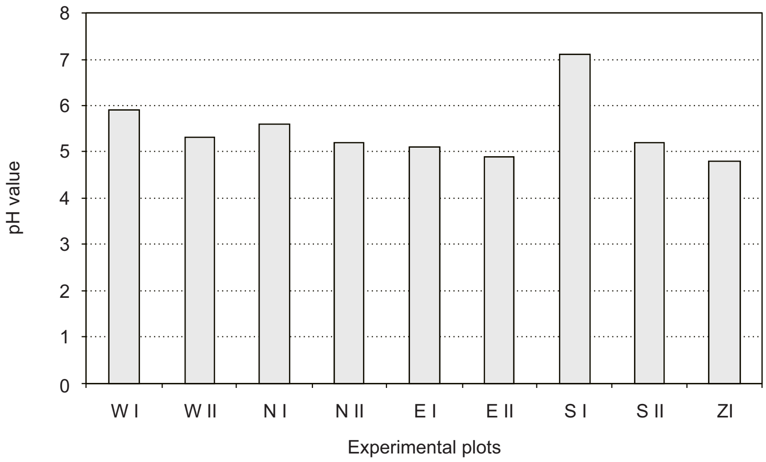

Fig. 4. Values of soil pH at experimental points localized around the municipal waste landfill site in Tarnow

environments. The reaction of all analyzed soils was acid at all experimental points except the soil taken from S I plot, which revealed alkaline pH. These conditions caused changes in the quantitative occurrence of microorganisms active in nitrogen transformation processes in the soil environment of the presented experiment.

On the basis of the obtained results it may be stated that a clear increase in the numbers of microorganisms active in nitrogen metabolism has been visible during the horse bean growing season. It confirms the fact that also various crop species affect the occurrence of various microorganisms in the soil environment, first during their growth in the vegetation season, through root system development and finally through the residue which remains after their die-back. In this way they also influence biological, chemical and physicochemical soil properties [12–14].

Conclusions

1. Numerous changes in the occurrence of microorganisms participating in nitrogen metabolism were found in the soil under horse bean cultivation in the vicinity of the active municipal waste landfill in Tarnow.

2. Microorganism numbers depended in the first place on the experimental plot localization and the period of the experiment.

3. A comparison of the obtained results allows for a conclusion that during the horse bean growing period an apparent increase in microorganism number participating in the nitrogen metabolism was noted in comparison with their presence after the season completion.

4. Analyses of the microbiocenotic composition of soil surrounding the landfill allow to improve knowledge about the dynamics but also on the range of pollutant spread in its vicinity and to decide upon the environment protection strategy.

Acknowledgement

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ZMIANY LICZEBNOŚCI DROBNOUSTROJÓW BIORĄCYCH UDZIAŁ W METABOLIZMIE AZOTOWYM W GLEBIE POD UPRAWĄ BOBIKU WOKÓŁ SKŁADOWISKA ODPADÓW KOMUNALNYCH W TARNOWIE

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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 od składowiska odpadów komunalnych w Tarnowie w dwóch strefach 50–200 i 250–500 m od jego granic wyznaczono w sumie 8 stanowisk badawczych pobierania próbek gleby, na których uprawiano bobik odmiany Nadwiślański. Dodatkowy punkt dziewiąty zlokalizowano na terenie składowiska odpadów na nieczynnym sektorze, który został wcześniej zrekultywowany. Wyniki przeprowadzonych badań mikrobiologicznych wskazują na wyraźne różnice w ilościowym składzie mikroflory biorącej udział w metabolizmie azotowym w zależności od odległości od czynnego składowiska. Porównując wszystkie wyniki, można wysunąć wniosek, że w okresie wegetacji bobiku widoczny jest wyraźny wzrost liczebności drobnoustrojów biorących udział w metabolizmie azotowym w porównaniu do ich występowania zarówno przed, jak i po zakończonym sezonie wegetacyjnym.

Słowa kluczowe: gleba, mikroflora, składowisko odpadów komunalnych

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COMPARISON OF THE EFFECT OF LIMING AND MAGNESIUM TREATMENT ON *Sitona* sp. HARMFULNESS ON BROAD BEAN (*Vicia faba* L., ssp. *maior*) IN CONDITIONS OF SOIL HEAVY METAL POLLUTION

PORÓWNANIE ODDZIAŁYWANIA WAPNOWANIA I NAWOŻENIA MAGNEZOWEGO NA SZKODLIWOŚĆ OPRZĘDZIKÓW (*Sitona* sp.) NA BOBIE (*Vicia faba* L. ssp. *maior*) W WARUNKACH SKAŻENIA GLEBY METALAMI CIĘŻKIMI

Abstract: The present study aimed at determining the effect of liming and magnesium treatment on harmfulness of *Sitona* sp. for broad bean plants growing in conditions of soil contaminated with single heavy metals on the III level of pollution in the IUNG, Pulawy, PL classification. The observations were conducted on broad bean (*Vicia faba* L. ssp. *maior*), White Windsor c.v. cultivated in three series: 1. in limed soil; 2. in soil receiving magnesium treatment; 3. in non-limed soil and in soil without magnesium. In each series the plants were cultivated in the following objects: unpolluted soil with natural heavy metal content (Control); unpolluted soil with natural heavy metal content receiving mineral fertilization (NPK); soil polluted with cadmium dosed 4 mg $\cdot$ kg^{-1} d.m.; soil polluted with 530 mg $\cdot$ kg^{-1} d.m. of lead; soil contaminated with copper dosed 85 mg $\cdot$ kg^{-1} d.m.; soil polluted with zinc dosed 1000 mg $\cdot$ kg^{-1} d.m. and soil polluted with nickel dosed 110 mg $\cdot$ kg^{-1} d.m. Liming was conducted on the basis of hydrolytic acidity analysis of soils from individual objects. The following calcium doses were applied: Control – 619 mg CaO $\cdot$ kg^{-1} d.m.; Control + NPK – 672 mg CaO $\cdot$ kg^{-1} d.m.; cadmium contaminated soil – 630 mg CaO $\cdot$ kg^{-1} d.m.; lead contaminated soil – 596 mg CaO $\cdot$ kg^{-1} d.m.; copper contaminated soil – 798 mg CaO $\cdot$ kg^{-1} d.m.; zinc contaminated soil – 1142 mg CaO $\cdot$ kg^{-1} d.m.; nickel contaminated soil – 818 mg CaO $\cdot$ kg^{-1} d.m. Equal magnesium fertilization with 20.4 mg Mg $\cdot$ kg^{-1} d.m. was used in all objects.

In conditions of soil contamination with heavy metals the effect of the liming and magnesium treatment on the harmfulness of *Sitona* beetles on broad bean may be modified by the atmospheric conditions in a given season. The liming and magnesium treatment of soil polluted with copper, lead, nickel and zinc on the III level of pollution according to the IUNG classification does not lead to any greater degree of broad bean leaf injuries due to *Sitona* beetles. Magnesium fertilization of soil polluted with cadmium on the III level according to the IUNG classification may contribute to enhanced attractiveness of broad bean plants to *Sitona*.

Keywords: heavy metals, magnesium fertilization, liming, *Sitona* sp.

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Liming and magnesium treatment are recommended as a method of reducing the unfavourable effect of heavy metals on plant. It may be connected with smaller heavy metal uptake by plants [1] and changes in macroelement concentrations [2]. Liming of soil contaminated with single heavy metals (copper, cadmium, nickel, lead and zinc) on the III level of pollution in the IUNG, Pulawy, PL classification caused a significant (between 2 and 4 fold) limiting of the contents of all above-mentioned heavy metals in broad bean roots and considerable (between 4 and 10 fold) limiting of cadmium, nickel and zinc accumulation in broad bean shoots. The same measure also reduced, though to a lesser degree, the content of lead and copper in the shoots [1, 3]. Magnesium fertilization caused between a 2 and 5-fold decrease in the content of analyzed heavy metals in broad bean roots, but it did not affect the content of copper or cadmium in broad bean shoots. The measure might also have affected a decline of zinc concentrations. On the other hand, in soil contaminated with lead and nickel, magnesium treatment may lead to an increase in the content of these elements in broad bean aboveground parts [1]. Measures such as liming or magnesium application may also change the host plant usefulness for prospective herbivorous [4].

The research was undertaken to compare the effect of liming and magnesium treatment on *Sitona* sp. beetles on broad bean in conditions of soil contaminated with single heavy metals on the level of medium pollution according to the IUNG classification.

Material and methods

The experiment was conducted in 2005 on degraded chernozem developed from loess, with acid reaction and 1.13 % content of organic carbon. Observations were conducted on broad bean (*Vicia faba* L., ssp. *maior*), White Windsor c.v. cultivated in three series: 1. in limed soil; 2. in soil receiving magnesium treatment; 3. in non-limed soil and in soil without magnesium. The doses of heavy metals, lime and magnesium were given in Table 1. The analyzed level of soil contamination with heavy metals corresponded to the III class of pollution in the IUNG classification [5]. The method of heavy metal application, the amount of basic fertilization used and methods of performed chemical analyses were presented in other papers [6, 7]. Liming was conducted on the basis of an analysis of soil hydrolytic acidity from individual objects. The dose was applied according to 1 Hh. The magnesium fertilizer dose was established on the basis of soil analysis performed by the Agro-Chemical Station in Krakow. The content of biovailable magnesium in the initial soil was 7.2 mg $\cdot$ 100 g^{-1} od soil dry mass.

The intensity of *Sitona* sp. beetles' feeding was assessed by measuring leaf area losses, leaf area consumed and by counting injured and uninjured leaves. The analysis of injuries was conducted twice: at the early stage of plant development (several leave stages – 21.05.05) and two weeks later (2.06.05). The significance of differences between the means were examined using one way ANOVA. The means were differentiated using the Duncan test at the significance level p = 0.05.

Table 1

Experimental design

Object	Metal dose per mg/kg soil d.m.	CaO dose per mg/kg soil d.m.	Mg dose per mg/kg soil d.m.
Unfertilized control	—	619	
Control with mineral treatment	—	672	
Cadmium contaminated soil	4	630	
Copper contaminated soil	85	798	20.4
Lead contaminated soil	530	596	
Nickel contaminated soil	110	818	
Zinc contaminated soil	1000	1142	

Results and discussion

Liming caused an increase in soil pH by ca 0.6–1 unit, whereas magnesium treatment did not lead to any major changes in the soil reaction [1].

At the first analyzed date *Sitona* sp. beetles injured up to 18 % of leaves, depending on the analyzed object (Fig. 1A). The beetles injured the largest number of leaves on plants growing in the soil polluted with lead and limed. Also the mean leaf area consumed per plant was the largest here. However, the differences were statistically significant mainly with respect to the objects where beetles did not start to feed at that time, ie in conditions of zinc contaminated soil, in the soil polluted with zinc and receiving magnesium treatment, in the soil polluted with nickel and limed, and on the control where magnesium fertilization was applied. At the same time, no significant differences were detected between the analyzed objects in the leaf blade loss due to *Sitona* beetles' feeding (Fig. 2A). In the later period (2.06.05) *Sitona* sp. beetles injured between 25 and 60 % of leaves (Fig. 1B). The loss of leaf area as a result of their feeding ranged between 1.35 % and 4.42 % (Fig. 2B). Liming of control soil without mineral fertilization contributed to a decrease in the percentage of both the leaves injured by *Sitona* beetles and the consumed leaf area. In this case magnesium treatment even slightly intensified this effect. However, the differences in the percentage of loss of leaf blade area between the objects mentioned above were not significant (Fig. 2B). In the Author's earlier studies liming, irrespective of the dose, did not cause notable differences in the degree of injuries to the control plants either the unfertilized ones or the ones receiving mineral treatment when observations were conducted at the early stage of development. On the other hand, as in the presented experiment, a decline in the degree of leaf blade damage was observed in a later period as a result of beetle feeding under the influence of liming [8]. A similar result was obtained also in former studies [4, 9].

Under conditions of cadmium contaminated soil, liming did not affect significantly beetle feeding on broad bean leaves at the second of the analyzed dates, whereas magnesium treatment contributed to an increase in injured area and to the percentage of loss of leaf blade (Fig. 2B). A slight weakening of plant growth as an effect of

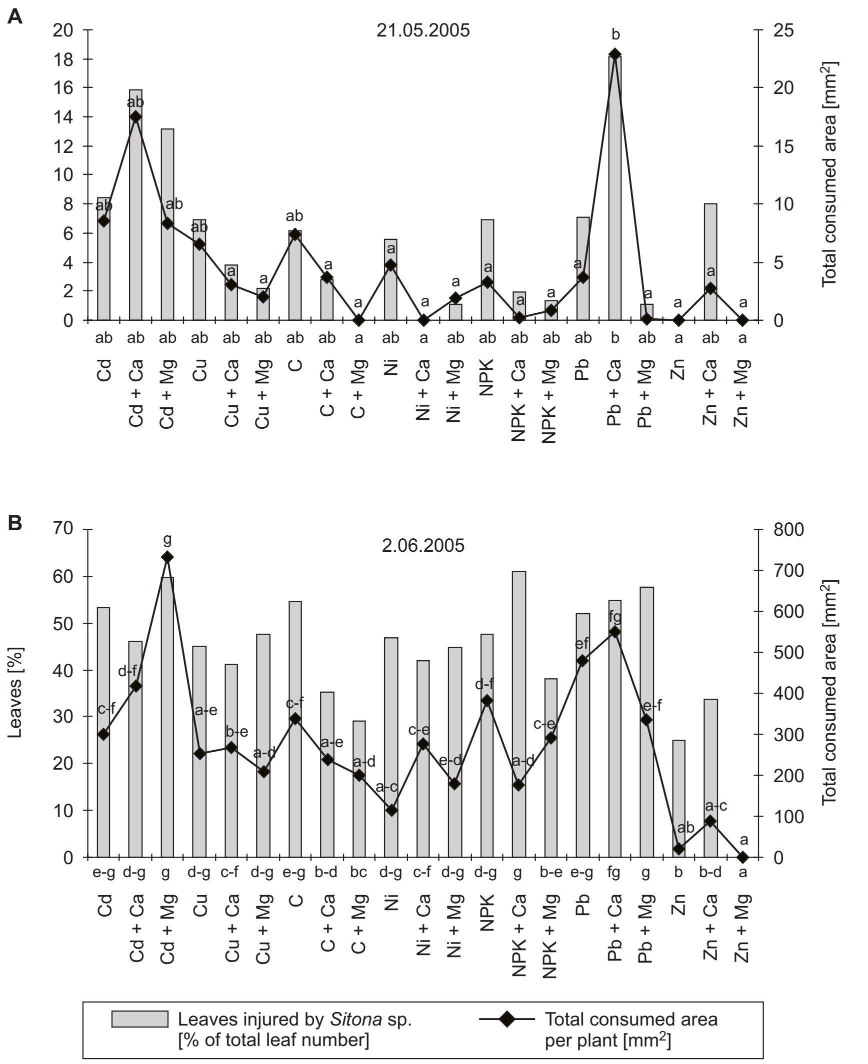

Fig. 1. Injuries caused by *Sitona* beetles on broad bean growing in natural soil (C – Control, NPK), in soil polluted with individual heavy metals and after application of liming or magnesium treatment. Means for each feature marked with the same letter do not differ at $p = 0.05$

magnesium treatment might have been the cause of increased value of the latter indicator. In the Author's former studies liming of soil contaminated with cadmium variously affected the degree of leaf injuries caused by *Sitona*. In one year it kept in check beetle feeding, whereas in another, particularly when the analysis was conducted in a later period (as in the present research), it might be observed that liming with a dose established acc. to 1 Hh contributed to slightly bigger plant injuries by *Sitona* beetles than in the cadmium polluted object, but without liming. Liming with a larger dose

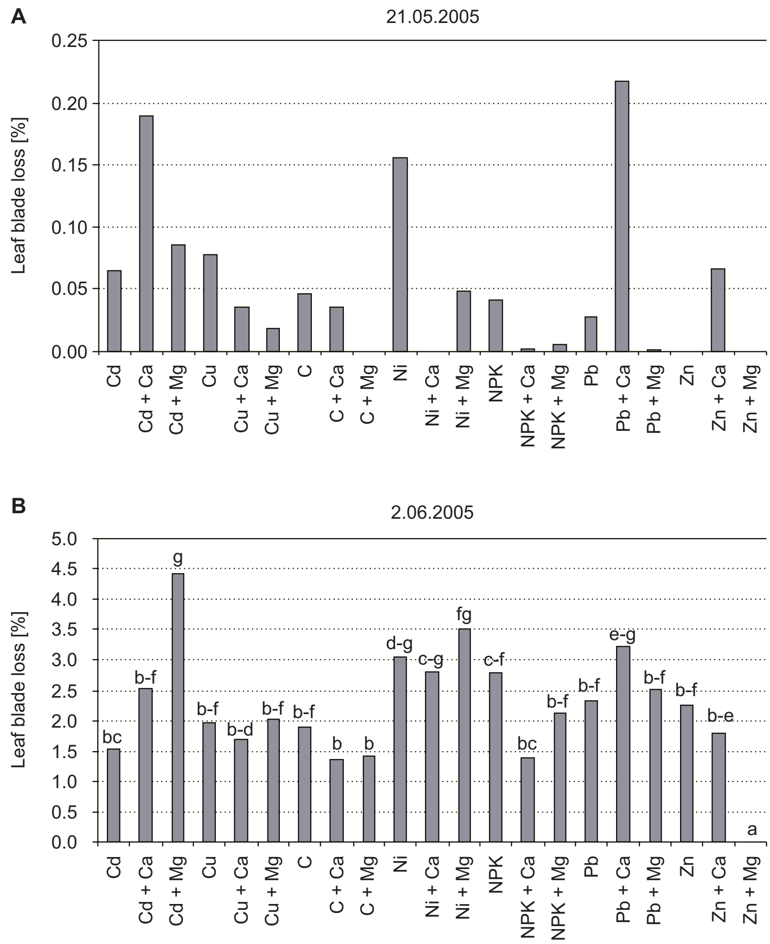

Fig. 2. Leaf blade loss due to *Sitona* beetle feeding [% of total Leaf area] in broad bean growing in natural soil (Control – C, NPK), in soil polluted with individual heavy metals and after application of liming or magnesium fertilization. Means marked with the same letter do not differ at p = 0.05. Assessments were presented only when statistical differentiation between means existed

partly leveled these differences [8, 9]. Liming and magnesium treatment of soil polluted with a combined dose of heavy metals on the I level of pollution did not lead to an increase in the degree of broad bean leaf injuries due to *Sitona* beetles [10].

In conditions of copper polluted soil neither liming nor magnesium treatment significantly affected the degree of broad bean leaf injuries caused by *Sitona* beetles (Fig. 1, 2). A similar situation was observed in the Author's former research [8, 9].

In lead-contaminated soil at the second date of observations no significant differences were detected either between the objects limed and receiving magnesium treatment or the object where no such measures were applied (Fig. 1B, 2B). A beneficial effect of liming which reduced *Sitona* sp. harmfulness at the early development stage, ie when their feeding most affects plant development, was observed in earlier studies [9, 10]. No notable differences between the limed object and one without liming were found either in the percentage of injured leaves or consumed leaf area when the analysis was performed at a later date.

Liming of nickel contaminated soil at a later date of observations did not affect significantly broad bean plant attractiveness for *Sitona* beetles (Fig. 1B, 2B). On the other hand, during earlier observation in the object with nickel contaminated soil and limed, no leaf injuries were detected (Fig. 1A, 2A). Leaf blade loss due to *Sitona* beetles feeding when the soil was fertilized with magnesium was slightly higher than in conditions of nickel contaminated soil without liming and without magnesium treatment (Fig. 2A). Also in former studies an apparent decline in the percentage of loss of leaf blade was observed under conditions of nickel polluted but limed soil [8].

In conditions of zinc polluted soil both at an earlier and later date of observations slightly more injuries caused by *Sitona* beetles were noted on plants growing in limed soil than in non-limed (the differences ranged within an experimental error) (Fig. 1, 2). Similar relationships were observed in former research [8, 9]. It may be caused by a considerable improvement of the growth of plants contaminated with this element after soil liming, owing to which these plants provide more attractive food for pests. Plants growing in conditions of zinc polluted soil under magnesium treatment were characterized by very handicapped growth and were not injured by *Sitona* sp. beetles.

Conclusions

1. The effect of liming and magnesium treatment applied under conditions of soil contaminated with heavy metals on harmfulness of *Sitona* sp. beetles for broad bean may be modified by atmospheric conditions in a given season.
2. Liming and magnesium treatment of soil contaminated with copper, lead, nickel and zinc on the III level of pollution according to the IUNG classification do not lead to an increase in the degree of leaf injuries caused by *Sitona* beetles.
3. Magnesium treatment of soil polluted with cadmium on the III level of pollution according to the IUNG classification may contribute to better attractiveness of broad bean plants for *Sitona* sp.

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PORÓWNANIE ODDZIAŁYWANIA WAPNOWANIA I NAWOŻENIA MAGNEZOWEGO NA SZKODLIWOŚĆ OPRZĘDZIKÓW (*Sitona* sp.) NA BOBIE (*Vicia faba* L. ssp. *maior*) W WARUNKACH SKAŻENIA GLEBY METALAMI CIĘŻKIMI

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Abstrakt: Celem podjętych badań było określenie wpływu wapnowania i nawożenia magnezowego na szkodliwość oprzędzików *Sitona* sp. dla roślin bobu rosnących w warunkach gleby zanieczyszczonej pojedynczymi metalami ciężkimi na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG. Obserwacje prowadzono na bobie (*Vicia faba* ssp. *maior*) odm. Windsor Biały uprawianym w trzech seriach: 1. na glebie wapnowanej; 2. glebie poddanej nawożeniu magnezowemu; 3. niewapnowanej oraz nienawożonej magnezem. W każdej serii rośliny uprawiano w następujących obiektach: gleba niezanieczyszczona – o naturalnej zawartości metali ciężkich (Kontrola); gleba niezanieczyszczona – o naturalnej zawartości metali ciężkich nawożona mineralnie (NPK); gleba zanieczyszczona kadmem w dawce: 4 mg · kg^{-1} s.m.; gleba zanieczyszczona ołowiem w dawce: 530 mg · kg^{-1} s.m.; gleba zanieczyszczona miedzią w dawce: 85 mg · kg^{-1} s.m.; gleba zanieczyszczona cynkiem w dawce: 1000 mg · kg^{-1} s.m.; gleba zanieczyszczona niklem w dawce: 110 mg · kg^{-1} s.m. Wapnowanie przeprowadzono na podstawie analizy kwasowości hydrolitycznej gleby z poszczególnych obiektów. Zastosowano następujące dawki wapna: Kontrola – 619 mg CaO · kg^{-1} s.m.; Kontrola + NPK – 672 mg CaO · kg^{-1} s.m.; gleba zanieczyszczona kadmem – 630 mg CaO · kg^{-1} s.m.; gleba zanieczyszczona ołowiem – 596 mg CaO · kg^{-1} s.m.; gleba zanieczyszczona miedzią – 798 mg CaO · kg^{-1} s.m.; gleba zanieczyszczona cynkiem 1142 mg CaO · kg^{-1} s.m.; gleba zanieczyszczona niklem 818 mg CaO · kg^{-1} s.m. Nawożenie magnezowe zastosowano jednakowe dla wszystkich obiektów: 20,4 mg Mg · kg^{-1} s.m.

Wpływ zabiegów wapnowania i nawożenia magnezowego w warunkach skażenia gleby metalami ciężkimi na szkodliwość chrząszczy oprzędzików dla bobu może być modyfikowany przez warunki atmosferyczne panujące w danym sezonie. Wapnowanie i nawożenie magnezowe gleby zanieczyszczonej miedzią, ołowiem, niklem i cynkiem na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG nie powoduje wzrostu stopnia uszkodzenia liści bobu przez chrząszcze oprzędzików. Nawożenie magnezowe gleby zanieczyszczonej kadmem na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG może przyczyniać się do wzrostu atrakcyjności roślin bobu dla oprzędzików.

Słowa kluczowe: metale ciężkie, nawożenie magnezowe, wapnowanie, *Sitona* sp.

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STUDY ON THE AEROBIC BIODEGRADATION OF CHELATING AGENTS UNDER THE STATIC CONDITIONS

BADANIE TLENOWEJ BIODEGRADACJI ZWIĄZKÓW CHELATUJĄCYCH MIKROELEMENTY NAWOZOWE W WARUNKACH TESTU STATYCZNEGO

Abstract: In this paper results of biodegradation of DTPA, HEEDTA and a new, perspective chelating agents – MGDA and GLDA are shown. The assessment of susceptibility of these compounds to biodegradation was carried out in accordance with the Polish Standard PN-88/C-05561 – Study on the aerobic biodegradation of organic compounds in water under the static conditions. Analysis showed, that GLDA and MGDA are characterized by considerably quicker and more effective biodegradation than DTPA or HEEDTA and in this connection they can replace it with success in many branches of industry.

Keywords: Biodegradation, chelating agents, static test

Nowadays exists a possibility of providing for the agriculture the new generation of fertilizers of greater effectiveness and controlled activity, having the maximally limited negative influence on the quality of the water sources, soil and atmosphere. Liquid micronutrient fertilizers are the fertilizers meeting such requirements. Micronutrients contained in them are found in a form of chelate – the complex with the organic compound. These connections guarantee fast and safe micronutrients uptake by plants creating fertilization as the effective method of supplementing nutrients. However the stability and the period of the availability of micronutrients are dependent on the properties of the ligand. Thus studying the specificity – first of all the biodegradability and the potential time of affecting in the soil – of chelating agents applied in micronutrient fertilizers is essential [1–6].

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For complexation of micronutrients different chelating agents are being used. The role of the ligands perform both natural occurring organic substances and synthetic compounds. Natural organic complexones are for example lignosulphonic acids, sulphonic tannins or humic acids. However for the production of liquid micronutrient fertilizers it is beneficial to apply first of all synthetic organic compounds, which by the formation a few ionic or ligand bonds, are able to enclose element permanently. Among them most commonly used compounds are the following: ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), hydroxy-2-ethylenediamine-triacetic acid (HEEDTA), nitrilotriacetic acid (NTA) and their salts [1, 2, 5].

However research indicate, that so far applied chelates are characterized by insufficient biodegradability and do not meet the primary rules of environmental protection. Their negative influence on the environment and related danger for people lead to start searching for new compounds, of the same or even higher effectiveness, but first of all being characterized by a fast and effective decomposition through microorganisms. The example of such biodegradable substitute can be methylglycine-diacetic acid (MGDA) and glutamic acid *N,N*-diacetic acid (GLDA) [1, 2, 4, 5].

Diethylenetriaminepentaacetic acid – DTPA

Diethylenetriaminepentaacetic acid (DTPA) is universally used chelating agent which complex metal cations. It creates complexes via five carboxylic and three amino groups with free pairs of electrons. DTPA has broad applications in different branches of industry. It is used in production of household detergents, herbicides, in the paper and fertilizer industry. Moreover its ability to bind as well as to remove radioactive substances from the organism such as plutonium, americium and the curium finds application in medicine [6–11].

Several information are known about the influence of this compound on the natural environment. However some sources inform that high concentrations of DTPA inhibit growth of microorganisms. It can suggest the toxicity of this substance. Furthermore above-mentioned chelate has small degree of biodegradability or it is even non-bio-degradable [11, 12].

Hydroxy-2-ethylenediaminetriacetic acid – HEEDTA

HEEDTA belongs to hydroxycarboxylic acids and differs from aminopolycarboxylic acids with it, that in its structure one carboxylic group is replaced with the hydroxyl group. Such structure of HEEDTA contributes to increasing its solubility in the water [8, 13].

This chelate, similarly to DTPA, is a compound with strong complexing properties. It has also similar applications, however its world scale consumption is much lower. It usually appears in the trisodium salt form – Na_3HEEDTA [8, 13].

The biochemical decomposition process of this compound is difficult and is carried out slowly. It is assumed that the degree of its biodegradation does not exceed the 20 % [8].

Methylglycinediacetic acid – MGDA

Methylglycinediacetic acid is a new, synthetic chelating agent which forms complexes of the high stability in a wide range of the pH and the temperature values. Nowadays it has been applied already in detergents, household detergents, cosmetics, in processes of galvanization and in the textile industry [14, 15].

Many standard OECD tests has proved that MGDA is a readily biodegradable chelating agent. Moreover, unlike other complexing substances, MGDA does not require adapted bacteria for decomposition, but instead is degraded under the standard conditions defined by the OECD. Furthermore after carrying out many toxicological and environmental tests it has been proved that MGDA is completely safe for the human health and it is not triggering off negative effects in the natural environment [5, 13, 15].

Glutamic acid *N,N*-diacetic acid – GLDA

GLDA is, similarly to MGDA, new, strongly complexing synthetic compound. In a wide range a pH range is characterized by a good solubility as well as the unprecedented stability in high temperatures. Moreover it has been not observed that applying this compound triggered off any ecological or toxicological effects [16, 17].

GLDA molecule is built from two carboxymethyl groups linked to the atom of nitrogen of glutamate. This synthetic chelate has been released on the market relatively recently and it is produced under the commercial name Dissolvine® GL. However this product includes only L-GLDA, isomer D-GLDA is not undergoing the biodegradation [16–18].

L-GLDA is considered to be biodegradable. Degree of its biodegradation in the sequence of 28 days in the test of the closed bottle exceed the 60 %. Moreover it was possible to isolate microorganisms that were able to carry out the effective decomposition of this isomer. Microorganisms that carried out the L-GLDA biodegradation were Gram negative bacteria from the *BG-1* strain. They were characterized by the 100 % homology with *Rhizobium radiobacter* and therefore they were categorized to *α-Proteobacteria* [16–18].

The optimal balance between the degree of the biodegradation and the ability of strong and permanent complexes formation by GLDA causes that this compound is an excellent alternative for the conventional synthetic chelating agents and can be applied without reservations in different branches of industry [16].

Materials and methods

Assessment of the biodegradation of fertilizer micronutrients complexing agents was carried out according to Polish Standard PN-88/C-05561 – Examination of the aerobic biodegradation of organic compounds in the aqueous environment under the conditions of the static test [19].

The progress of the process of the biochemical disintegration of these compounds was determined on the basis of the decay of the substance in the sample and with the usage of changes in the COD degree reduction.

The degree of the organic compound decay and the chemical oxygen demand – COD reduction, during the biodegradation test under the static test conditions, inform us about the susceptibility to the biochemical decomposition. It is assumed that compounds are readily biodegradable when the degree of elimination reach level of 70 % during 5 days or the 45 % after twenty-four hours. The substance is being regarded biodegradable when its elimination reaches 50–70 % in sequence 20 days or 70 % in the sequence of 6–20 days. If the decay reaches level below the 50 % in the sequence of 20 days such compound can be classified as a hardly biodegradable. The compound is considered to be resistant to the biodegradation, when during the time of the test (20 days) is not undergoing the biodegradation [19].

Study on the aerobic biodegradation of organic compounds in the aqueous environment under the static test conditions – PN-88/C-05561

The method is based on determining the degree of degradation of organic compound placed in mineral medium inoculated with standard activated sludge. The process of the biodegradation lasted maximal for 20 days and it was carried out under aerobic conditions, in the room temperature, without the access of the light.

Mineral medium and standard activated sludge were placed in three flasks. Solution of the tested organic compound was inserted into first two flasks. The third flask served as a control test. Moreover in the process it has been applied, for comparison, the glucose as the factor supporting the biochemical decomposition.

Flasks prepared in this way were secured with corks and supplied with wires delivering compressed air. Then they were put on electromagnetic mixers, in the overshadowed place, at room temperature.

Immediately after preparing samples and connecting apparatus, after 1, 3, 6, 24 hours, and every next day through maximal 20 days from each of three flasks a determined amount of the sample has been collected. After draining them off they were used for indicating the concentration of tested compound and the COD value.

COD was examined with the usage of two methods: potassium dichromate and potassium permanganate. The concentration of tested compounds was determined using complexometric titration [20, 21].

Diethylenetriaminepentaacetic acid (DTPA), hydroxy-2-ethylenediaminetriacetic acid (HEEDTA), 40 % trisodium salt of methylglycinediacetic acid (Trilon®M, BASF, Germany) and 38 % tetrasodium salt of glutamic acid-*N,N*-diacetic acid (Dissolvine®GL, Akzo Nobel, The Netherlands) were subjects to the research on the biodegradation.

Results and discussion

In this paper the determination of biodegradation degree of chelating agents, applied in micronutrient fertilizers manufacturing: the DTPA and HEEDTA as well as MGDA and GLDA – compounds that may displace from fertilizer market the ones so far applied, were the aim of presented researches. The progress of the process of the biochemical degradation of tested substances was determined on the basis of the concentrations reduction and with the usage of the nonspecific indicator of mass depletion, which is the COD reduction.

Reduction of the compound concentration (degree of compound reduction, degree of compound biodegradation) in the time (t) has been calculated according to the formula:

$$X = \frac{C_0 - C_t}{C_0} \cdot 100 \ [\%]$$

in which: C_0 – concentration of compound in the time t = 0, [g/dm^3],
C_t – concentration of compound after the t time, [g/dm^3].

Results of the degree of reduction of all tested compounds are shown in Fig. 1. It represents the reduction of the DTPA, HEEDTA, MGDA and GLDA concentration in samples with and without the addition of glucose.

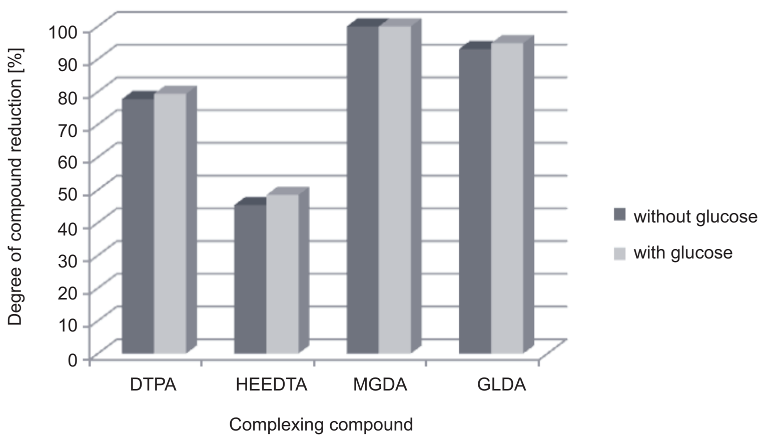

Fig. 1. Degree of reduction of complexing compounds: DTPA, HEEDTA, MGDA and GLDA

The highest degree of biodegradation for all tested compounds, both in the presence as well as at the lack of glucose, was characterized by the MGDA. It is totally biodegradable and the addition of glucose accelerated its decomposition. In the case of the second alternative chelating agent – GLDA, the reduction of the concentration reached the level of 94 % in the sequence of 20 days in both kinds of samples. Thus it is possible to classify this substance among biodegradable substances. Moreover the addition of glucose did not influenced on the rate of its decay.

The most difficult biodegradable chelating agent turned out to be the compound, commonly used so far, HEEDTA. The degree of its degradation reached level of 48.5 % in the case of the addition of glucose and 45.4 % when the biodecay occurred without its presence. Glucose did not also influenced on the rate of the DTPA elimination in the greater degree and the degree of its biodegradation reached level about 78 %.

COD was examined with the two methods: potassium permanganate and potassium dichromate appropriately according to standards: PN-ISO 6060:2006 and PN-EN ISO 8467:2001. The degree of COD reduction for both methods was calculated according to following formula:

$$Y = \frac{a - b - c}{a} \cdot 100 \ [\%]$$

in which: a – COD value at t = 0 time, mg O_2/dm^3,
b – COD value after the t time, mg O_2/dm^3,
c – COD value in the control test at t = 0 time, mg O_2/dm^3.

The degree of COD reduction of organic compounds in examined samples with and without the addition of the glucose is presented in Fig. 2.

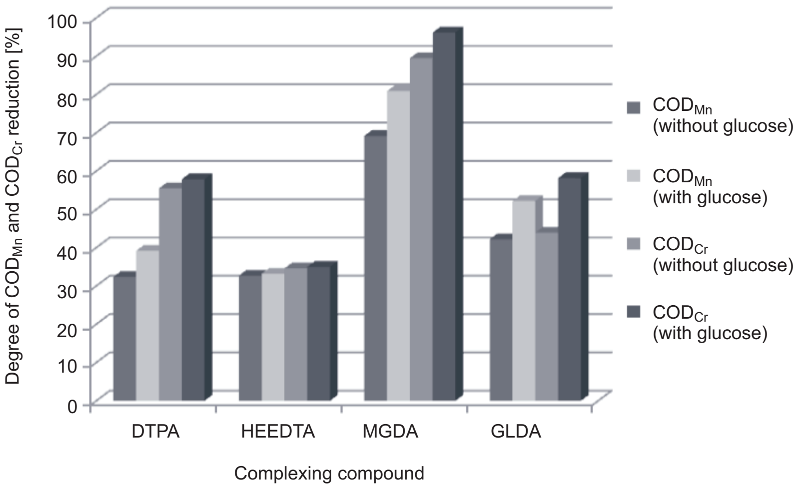

Fig. 2. Degree of COD_{Mn} i COD_{Cr} reduction for DTPA, HEEDTA, MGDA and GLDA

The highest degree of COD reduction, for all tested compounds, both with potassium permanganate as well as potassium dichromate was characterized by MGDA. This prospective chelating agent, compared with different chelates, was characterized by about 70–80 % degree of COD_{Mn} reduction and about 93 % degree of COD_{Cr} reduction. For GLDA the degree of the COD_{Mn} reduction reached level about 43 % and 54 % with the potassium dichromate method. Glucose had the influence on the degree of COD reduction of this compound, because in flasks containing its addition in the case of the potassium permanganate method the degree of the COD reduction increased about 10 %, and in the case of the potassium dichromate method about 14 %.

The lowest COD reduction was found for HEEDTA. Value of the degree of the COD reduction in this case in both methods and irrespective of the glucose presence oscillated from 32 to 35 %. Also degree of DTPA COD_{Mn} reduction reached level over 30 %, however the degree of COD_{Cr} reduction was much higher and reached level about 56 %.

The list of results of the biodegradation for all tested chelating agents are shown in Table 1 and Table 2.

Table 1

Biodegradation of complexing substances under static conditions without glucose

Complexing compound	Degree of compound reduction [%]	Degree of COD reduction [%]	
		COD_{Mn}	COD_{Cr}
DTPA	77.8	32.4	55.5
HEEDTA	45.4	32.7	34.6
MGDA	100 (216 h)	69.2	89.5
GLDA	93.1	42.2	43.9

Table 2

Biodegradation of complexing substances under static conditions with glucose

Complexing compound	Degree of compound reduction [%]	Degree of COD reduction [%]	
		COD_{Mn}	COD_{Cr}
DTPA	79.4	39.2	57.9
HEEDTA	48.5	33.2	35.0
MGDA	100 (192 h)	80.9	96.2
GLDA	94.9	52.2	58.2

The biodegradation tests carried out under the static test conditions has shown, that the most difficult degradable by microorganisms was HEEDTA. The degree of the concentration reduction of this compound reached level 48.5 % in the presence of glucose and 45.4 % with lack of it. The presence of the glucose influenced on increase in the degree of the HEEDTA biodegradation only insignificantly. COD for HEEDTA, examined both with potassium permanganate as well as potassium dichromate method, reached level of degree of COD reduction on average 33 %. The 10 % difference between the degree of the concentration and COD reduction can suggest that during the process of the decomposition of this compound intermediates products of decomposition could be formed.

The degree of the DTPA biodegradation reached level of the 78 %, furthermore glucose only insignificantly influenced the reduction degree of this substance. According to COD results obtained with potassium dichromate it came out that DTPA is undergoing the biochemical disintegration in about 55 %, and in the case of the potassium permanganate method only in about 37 %. The disproportionate reduction in estimated COD to obtained value of the DTPA concentration reduction indicates indirect products of the degradation of this compound appearing in the sample.

The degree of MGDA biodegradation reached level of the 100 %. Total degradation of this chelating agent occurred at 216 hour of the process. The results of the degree of COD_{Mn} and COD_{Cr} reduction reached appropriately level about 70–80 %, without presence of additional glucose, and 89–96 %, when glucose was being added.

The reduction of GLDA concentration reached the level of 94 % and glucose only slightly influenced its rate. However COD reduction examined with both methods reached level 55 % in the presence of the factor supporting the biodegradation and about 43 % at its lack. In this case it is also possible to suspect that during the process of the biodegradation indirect products of the GLDA disintegration could be formed.

Conclusions

On the basis of the carried out researches on the biodegradation under the static test conditions it can be found, that:

1. Complexing compounds such as DTPA and HEEDTA, that are the ones so far applied in the fertilizer industry, were underwent biodegradation in the range of 45 to 80 % in the 20 days of process.

2. The most hardly biodegradable chelating agent was HEEDTA and its biochemical decomposition reached level about 47 %.

3. In the case of conventional chelating agents glucose did not have considerable influence on the rate of their biochemical decay.

4. Researches on the biodegradation of relatively new chelating agent, which is MGDA, showed that it was characterized by a fast and effective degradation by microorganisms. The biodegradation reached level of 100 % in time less than 11 days.

5. The presence of glucose as a readily available source of carbon and energy had influence on MGDA biodegradation rate.

6. The degree of the GLDA biodegradation, as a new prospective chelating agent, reached level about 95 %, however much lower degree of the COD reduction of this compound showed that during its decomposition the intermediates could be formed.

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BADANIE TLENOWEJ BIODEGRADACJI ZWIĄZKÓW CHELATUJĄCYCH MIKROELEMENTY NAWOZOWE W WARUNKACH TESTU STATYCZNEGO

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Abstrakt: Przedstawiono wyniki biodegradacji DTPA i HEEDTA oraz nowych, alternatywnych związków kompleksujących – MGDA i GLDA. Ocena podatności tych związków na biorozkład przeprowadzona została zgodnie z Polską Normą PN-88/C-05561 – Badanie tlenowej biodegradacji związków organicznych w środowisku wodnym w warunkach testu statycznego. Badania wykazały, iż GLDA i MGDA odznaczają się znacznie szybszą i skuteczniejszą biodegradacją niż DTPA czy HEEDTA i w związku z tym mogą zastąpić je z powodzeniem w wielu gałęziach przemysłu.

Słowa kluczowe: biodegradacja, związki chelatujące, test statyczny

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NITROGEN, PHOSPHORUS, AND POTASSIUM BALANCE IN THE SOIL OF MOUNTAIN MEADOW AFTER ITS THREE YEAR UTILIZATION

BILANS AZOTU, FOSFORU I POTASU PO TRZECH LATACH UŻYTKOWANIA ŁĄKI GÓRSKIEJ

Abstract: The research was accomplished on a mountain meadow (650 m above sea level), its type being red fescue (*Festuca rubra* L.) and common bent grass (*Agrostis capillaris* L.), during a period from 2004 to 2006. The experiment was conducted on an acid brown soil containing a medium amount of potassium and a very small amount of phosphorus. The experiment included the total number of 4 objects: 3 objects with three fertilizing variants applied, and one control object. A lysimeter was mounted on each object to collect seepage water. The highest amount of nitrogen was determined in the meadow sward gathered which had been fertilized by applying mineral fertilizers, and the highest amount of potassium – in the meadow sward harvested that had received only barnyard manure. The highest content of phosphorus was determined in the meadow sward that had been fertilized with barnyard manure and mineral fertilizers. The effluent water leached the highest amount of nitrogen of the object fertilized by applying mineral fertilizers. The amount of mineral compounds leached out of the control object was the lowest.

Keywords: meadow, types of fertilizers, and macroelements

The cycle of natural elements in nature should be based on well-balanced principles of sustainable development; and this is possible when natural fertilizers are rationally applied and supplemented with mineral fertilizers, and when self-regulating mechanisms functioning in ecosystems are intentionally implemented und knowingly utilized. Rational application of fertilizers does not include only appropriate amounts of fertilizers to be applied, but also depends on correct proportions of individual fertilizer components, suitable application seasons and times, and types of fertilizers to be applied. In particular, in the mountain regions the soil nutrient balance should be properly determined and stable since the negative consequences of intensification of mineral fertilization for grassland in those regions are more serious than for meadows in lowlands [1]. The majority of fodder production in a mountain region should be located

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on grassland that is predominantly supplemented with natural manures. Such grassland is considered an ecosystem exerting an exceptionally favourable impact on the natural environment [2]. Thus, it is very important for both the economic and the ecological spheres that any grassland is reasonably and effectively managed. The objective of the research initiated and conducted in the region as indicated above was to compare the amounts of components supplied with fertilizers to the meadow soil with the amount of soil nutrients contained in the fodder crops harvested and with the amount of components leached out of the soil by effluent waters.

Conditions and methodology of research

The research project was conducted on a mountain meadow (650 m above sea level, 20 55′34″ E, 49 24′35″ N), its type being red fescue (*Festuca rubra* L.) and common bent grass (*Agrostis capillaris* L.), over a period from 2004 to 2006. The hillside slope where the experimental field was situated was 4^{o} towards NE. The experiment was carried out on a field with an acid brown soil made up of magurski sandstone showing a granulometric composition of medium loam. The chemical profile of this soil was as follows: pH_{KCl} – 3.8; total nitrogen (N_{total}) – 0.29 %; organic matter – 5.0 %; P, K and Mg in the assimilable ions: 9.5, 64.8, and 94.1 mg kg^{-1}, respectively. The research included 4 different objects: 1 control object; 1 object fertilized by applying full mineral fertilizers, their dose being $P_{18}K_{50}N_{100}$; 1 object manured with sheep manure, its dose being 10 Mg ha^{-1}; and 1 object fertilized with barnyard manure plus supplementary mineral fertilizers, ie phosphorus and nitrogen, their amount being the same as when full mineral fertilization was applied. The following forms of fertilizers were applied over the periods indicated below: phosphorus in the form of tri-superphosphate was applied once in spring; potassium in the form of 56 % potash salt was divided into two equal portions and applied for the first and the second regrowth; nitrogen in the form of ammonium nitrate at a rate of 100 kg ha^{-1} was applied in 2 portions, 60 % and 40 % of the total dose, for the first and the second regrowth. The supplementary dose of nitrogen applied to the object with manure was applied in one dose for the first regrowth. The sheep manure was applied each year, in the early spring. The contents of chemical components in the manure utilized in the experiment were as follows: dry mass content – 25.4 %; total nitrogen content (N_{total}) – 0.69 %; P – 0.14 %; K – 0.60 %; Ca – 0.25 %; Mg – 0.08 %; Na – 0.06 %. The manure dose of 10 Mg supplied 69 kg of N, 14 kg of P, and 60 kg of K to the soil.

Lysimeters collecting seepage water were mounted in the early spring of the year 2003; there were 3 lysimeters mounted on the experimental objects. The soil thickness in this region was poor, thus, the lysimeters were situated at a depth of 20 cm, and the collection surface of the individual lysimeter was circle-shaped with a 50 cm diameter. The quantity and quality of water collected by lysimeters was determined several times during the summer period. The results obtained were statistically analyzed. Average values were compared using the Duncan’s test. It was assumed that the significance of the results be 0.05.

The meadow sward was mowed twice during each year of the experiment period. The first regrowth was harvested at the turn of the second and third decade of June, and the second regrowth was in the third decade of August. After the meadow was mown, samples of plant material were collected and macroelements were determined.

Research results

The 3-year average amount of nitrogen determined in the harvested sward fertilized using mineral fertilizers was almost twice as high as the amount of nitrogen found in the sward gathered on the control object (Table 1).

Table 1

Quantities of mineral components determined in the crop yield and taken away by effluent waters (kg ha^{-1})

Variant	Years	Content levels of components determined in the dry mass yield			Quantity of components taken away by effluent waters		
		kg ha^{-1}					
		N	P	K	N	P	K
Control object	2004	67.2	4.8	37.7	2.6	0.1	0.7
	2005	98.3	6.9	109.6	4.1	0.2	1.0
	2006	60.3	5.0	58.6	1.2	2.4	2.6
	I X	75.3 a	5.5 a	68.6 a	2.6 a	0.9 c	1.4 a
PKN	2004	141.3	12.4	106.4	10.4	0.1	1.1
	2005	163.0	12.6	178.6	11.1	0.1	1.0
	2006	132.5	10.6	126.8	4.9	0.5	4.2
	I X	145.6 c	11.9 b	137.3 b	8.8 c	0.3 b	2.1 b
FYM 10 Mg	2004	117.5	11.9	159.3	5.5	0.2	1.9
	2005	149.0	13.4	254.3	7.0	0.2	2.3
	2006	92.7	13.0	151.2	2.4	0.3	2.1
	I X	119.7 b	12.8 b	188.3 c	5.0 b	0.3 b	2.1 b
FYM 10 Mg + PKN	2004	116.8	10.4	93.2	4.2	0.1	2.1
	2005	157.6	16.4	279.3	6.6	0.1	3.0
	2006	114.8	15.1	134.5	2.0	0.1	0.6
	I X	129.8 bc	14.0 c	169.0 c	4.3 b	0.1 a	1.9 b

The amount of nitrogen determined in the dry mass yield harvested on the objects fertilized by applying barnyard manure plus mineral fertilizers was lower by 11 %, and on the object fertilized solely with barnyard manure – by 18 % lower if compared with the amount of nitrogen contained in the object fertilized exclusively with mineral fertilizers. The quantity of nitrogen taken away by effluent waters from the object fertilized by applying only mineral fertilizers was 8.8 kg ha^{-1} and this figure was three times as high as the quantity of water taken away from the control object, and twice as high as from two other objects. The highest amount of nitrogen was determined in the yield of the meadow sward that was fertilized with barnyard manure plus supplementary mineral fertilization (14 kg ha^{-1}), and the loss of nitrogen due to its being leached out of the soil by effluent waters was the lowest in this object. An amount of approximately 12 kg of phosphorus was found in the meadow sward harvested on the remaining objects under fertilization, and the load of phosphorus taken away by the effluent waters was twice as high as the lowest load of phosphorus. The lowest amount of phosphorus, ie 5.5 kg ha^{-1}, was determined in the control meadow sward harvested, and the phosphorus losses in this object were the highest, ie almost 1 kg ha^{-1}.

The gathered meadow sward fertilized by using only barnyard manure contained the highest amount of potassium, and the gathered meadow sward fertilized by a mixture of barnyard manure plus mineral fertilizers contained less potassium, and its amount was lower by 20 kg. With regard to the potassium content in the sward, the harvested meadow sward fertilized by applying mineral fertilizers was in the third position. The control meadow sward delivered the lowest amount of potassium, ie only 69 kg. The amounts of potassium leached out of the objects under fertilization were similar and amounted to nearly 2 kg, and of the control object: 1.4 kg ha^{-1}.

Analysis of experimental data

The dry mass yield decided on the amount of nitrogen content (yield); according to Kasperczyk [3], the nitrogen dose of 100–120 kg N ha^{-1} is mainly utilized in the process of plant mass accretion and does not impact the increase in the nitrogen content in plants. On the other hand, the contents of phosphorus and potassium were impacted by both the dry mass yield and the content of those elements in plants. Another important fact deserving attention is that effluent water leached out the highest amount of nitrogen of the objects fertilized by applying mineral fertilizers of 100 kg ha^{-1}; this result corresponds to the results obtained by other researches [4]. Insignificant losses of soil nutrients caused by effluent waters that leached them out of the objects receiving barnyard manure should be attributed to the strong buffer function and to the long-lasting impact of this fertilizer. Additionally, when mineral nitrogen was added to the object fertilized with barnyard manure plus supplementary fertilizers, the plant development was accelerated in spring. Due to this phenomenon, only limited amounts of water could penetrate through the soil profile and, thus, the amounts of soil nutrients leached out of the soil were lower [5].

Conclusions

The fertilization by applying barnyard manure and mineral fertilizers appears to be the most favourable type of fertilization as regards the amounts of components contained in the dry mass yield. Regarding the impact of this fertilization type on water environment, this fertilization type limits the eutrophication of rivers and lakes.

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BILANS AZOTU, FOSFORU I POTASU PO TRZECH LATACH UŻYTKOWANIA ŁĄKI GÓRSKIEJ

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Abstrakt: Badania przeprowadzono w latach 2004–2006 na łące górskiej (650 m n.p.m.) typu kostrzewy czerwonej (*Festuca rubra* L.) i mietlicy pospolitej (*Agrostis capillaris* L.). Doświadczenie założono na glebie brunatnej kwaśnej średnio zasobnej w potas, a bardzo ubogiej w fosfor. Uwzględniono 3 warianty nawozowe i kontrolę. Na każdym wariancie zainstalowano lizymetry, z których zbierano wodę przesiąkową. Najwięcej azotu zebrano z runią otrzymującą nawożenie mineralne, a potasu z runią otrzymującą sam obornik. Fosforu najwięcej zebrano z runią nawożoną obornikiem wraz z nawozami mineralnymi. Woda odciekowa z obiektu otrzymującego nawożenie mineralne wypłukiwała najwięcej azotu. Najmniej składników mineralnych było wypłukiwanych z obiektu kontrolnego.

Słowa kluczowe: łąka, rodzaj nawozów, makroskładniki

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EVALUATION OF HEAVY METAL LOAD IN SELECTED NATURE RESERVES OF SOUTHERN POLAND

OCENA STOPNIA ZAGROŻENIA METALAMI CIĘŻKIMI WYBRANYCH REZERWATÓW POŁUDNIOWEJ POLSKI

Abstract: In this study we investigated heavy metals (Cd, Pb, Zn, Cu and Fe) accumulation in upper layer of soils (from three levels: 0–10 cm, 10–20 cm, 20–30 cm) and in the leaves of *Pinus sylvestris* L., *Picea abies* [L.] Karst., *Maianthemum bifolium* [L.] F.W. Schmidt and *Hedera helix* L. collected from nature reserves: Bukowa Kepa, Ostreznik, Zielona Gora, and Slotwina situated south of Poland. The investigations were carried out in 2004–2007. In soil samples from reserves Ostreznik and Slotwina higher concentrations of Cd was noted than "normal" level for protected area (1 mg/kg). Elevated Pb content in upper layer of soil were estimated in reserves of Bukowa Kepa (84.9 mg/kg) and Ostreznik (68.9 mg/kg). The level of Pb and Zn in plants leaves was below the values considered as toxic. However the concentration of Cd in leaves of *Hedera helix* L. collected in nature reserves Bukowa Kepa, Ostreznik and Zielona Gora was higher than the level considered as toxic (5–10 mg/kg d.m.). Relatively low Cu concentration in the leaves of investigated plants indicated poor plant nutrition.

Keywords: heavy metals, nature reserves of southern Poland, *Pinus sylvestris* L., *Picea abies* [L.] Karst., *Maianthemum bifolium* [L.] F. W. Schmidt and *Hedera helix* L.

Nature reserves are one of area forms of nature protection enabling a protection of ecosystems valuable from a natural point of view [1]. Protection of nature in a form of nature reserves enables maintenance of those reserves durability, their biodiversity as well as ecological processes proceeding there [2].

Forest ecosystems, including protected reserves, are a subject to different dangers which scale increases all the time. Results of those hazards can be seen, among other things, in increase of number of forest fires, damages of forest stands as a result of weather anomalies, shortening of periods between gradations of pests and increasing acreage of their mass occurrence, dying out of trees and forest stands as a result of emission as well as without any found reason [3]. Rare species of plants disappear, other appear, synanthropic, brought by a man. Plant associations are a subject of

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adverse changes and degradation. Very adverse and dangerous for reserves are changes in site conditions resulting from anthropogenic impact [4]. The most important ingredient contaminating and degrading natural environment are chemical elements emitted in accordance with different processes of human activity. Introduced into ecosystems, they cause a disturbances in homeostasis [5–7]. The most durable and onerous for environment of those elements are heavy metals whose concentration, despite a falling tendency, are still hazardous for environment and living organisms [5, 8, 9]. Plants, particularly chemical content of their assimilation apparatus, are susceptible indicator of biophysical and chemical changes of environment [5, 10–12].

In threatened areas, that is where environment contamination by toxic metals and gases repeatedly exceeds standards for protected areas, there occur 3/4 of Polish national parks and 2/3 reserves [13].

The objective of this piece of research was to determine a load factor of chosen heavy metals (Zn, Cu, Pb, Fe and Cd) in the Slotwina Reserve, Bukowa Kepa Reserve, Ostreznik Reserve and Zielona Gora Reserve on the basis of a chemical analysis of soil as well as assimilation apparatus of chosen plant species.

Material and methods

The investigation was carried out in the soil and leaves and needles of *Picea abies* [L.] H. Karst., *Pinus sylvestris* L., *Maianthemum bifolium* [L.] F.W. Schmidt, *Hedera helix* L. Karst in the Slotwina Reserve, Bukowa Kepa Reserve, Ostreznik Reserve and Zielona Gora Reserve. Samples of soil (from the level 0–10 cm) and leaves and needles *Picea abies* [L.] H. Karst., *Pinus sylvestris* L., *Maianthemum bifolium* [L.] F.W. Schmidt, *Hedera helix* L. Karst abies were collected in the vegetation season of 2003–2004 (Slotwina Reserve) and 2006–2007 (the rest of reserve). In order to determine the heavy metals concentration, plants material dried in 105 ^{o}C to a constant weight, ground to a powder, then mineralized and dissolved in 10 % HNO_3. After filtration Cd, Pb, Zn, Cu and Fe content were measured using 10 % HNO_3. The measurements were carried out using the conventional Atomic Absorption Spectrometry (AAS) [14]. The quality of analytical procedures was controlled by using the reference material (Certified Reference Material CTA – OTL – 1 Oriental Tabacco Leavs).

Results and discussion

Contents of heavy metals in examined samples were presented in Tables 1 and 2.

Natural content of cadmium in soil significantly depends on an occurrence of this element in country rocks. Amount of imission has a significant influence on a noted current concentration of this element.

Average content of cadmium in soils of Poland amounts to 0.2 μg/g [15].

Concentrations of this element in a top layer of a soil, in case of the Bukowa Kepa Reserve and the Zielona Gora Reserve were beyond a limit of determination so they were lower than 0.1 μg/g. In the Ostreznik Reserve and the Slotwina Reserve, content of cadmium varied from 0.98 μg/g to 3.42 μg/g and exceeded permissible concentrations of that element in soil in protected area. In both cases, a decrease of cadmium content was observed together with a depth of collected samples.

Table 1

Content of zinc, copper, lead, iron and cadmium [μg/g d.m.] in leaves and needles of chosen plant species

Nature reserve	Species	Zn	Cu	Pb	Fe	Cd
Slotwina	*Picea bies* L.	18.84	2.04	1.64	17.5	nd
	Pinus sylvestris L.	42.95	3.59	2.88	22.27	nd
	Maianthemum bifolium L.	44.54	3.69	1.66	53.87	nd
	Hedera helix L.	66.82	4.99	2.14	48.88	nd
Bukowa Kepa	*Picea abies* L.	28.3	0.94	1.32	33.3	0.37
	Pinus sylvestris L.	25.4	0.87	2.03	39.1	0.49
	Maianthemum bifolium L.	28.0	2.42	8.61	72.3	3.07
	Hedera helix L.	247.1	2.56	6.70	53.5	5.40
Ostreznik	*Picea abies* L.	21.2	0.38	1.57	24.6	0.11
	Pinus sylvestris L.	23.2	0.73	2.32	21.8	0.5
	Maianthemum bifolium L.	23.9	1.86	4.42	78.1	2.57
	Hedera helix L.	213.0	2.07	4.42	65.1	4.75
Zielona Gora	*Picea abies* L.	nd	nd	nd	nd	nd
	Pinus sylvestris L.	27.3	0.94	1.24	56.4	0.53
	Maianthemum bifolium L.	28.5	2.14	4.42	69.4	1.81
	Hedera helix L.	223.0	3.51	9.75	72.3	4.18

Table 2

Content of zinc, copper, lead, iron and cadmium [μg/g] in the upper layer of the soil

Elements	Layer [cm]	Nature reserve			
		Slotwina	Bukowa Kepa	Ostreznik	Zielona Gora
Cd	0–10	3.42	nd	2.04	nd
	10–20	2.03	nd	1.77	nd
	20–30	1.3	nd	0.98	nd
Pb	0–10	4.97	84.9	68.9	41.7
	10–20	2.46	23.5	22.3	15.0
	20–30	2.46	9.3	12.3	7.9
Zn	0–10	22.42	33.1	42.6	18.4
	10–20	20.68	11.8	32.1	4.6
	20–30	13.44	7.3	23.1	3.0
Cu	0–10	3.61	1.98	1.98	1.67
	10–20	3.21	1.14	1.46	1.03
	20–30	1.22	0.82	1.03	0.72
Fe	0–10	344.0	1301.0	613.0	643.0
	10–20	258.5	1066.0	408.0	419.0
	20–30	125.0	818.0	289.0	326.0

Soil analysis results in this elaboration, regarding content of cadmium, are comparable with results received for other protected areas. Ciepal et al [16] determined an average concentration of Cd in a top layer of soil within a range 0.5–0.9 μg/g on Mt Babia Gora, and on Mt Pilsko 1.9–3.1 μg/g of air-dry soil. Kimsa et al [17] determined 2.7–3.4 μg/g of Cd in a top layer of soil of the Swietokrzyski National Park. Ciepal et al [18] noted 2.0 μg/g of Cd in a top layer of soil in the Bukowica Reserve, whereas in the Lipowiec Reserve it was 4.0 μg/g of Cd. Ciepal and Lipka [19] give out a cadmium content in a top layers of soil in the Smolen Reserve at the level of 17.0 μg/g and for Gora Chelm 16 μg/g. Lukasik [20] noticed in soil of the Parkowe Reserve 1.5 μg/g of Cd.

In case of plants, cadmium is an element not necessary for their growth but still easily absorbed both by root system and leaves, usually proportionally to a concentration in environment [15].

Sawicka-Kapusta [21] gives out a range of cadmium occurrence in plants from not contaminated areas: 0.12–0.5 μg/g d.m., whereas Kabata-Pendias and Pendias [15] 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 as phytotoxic [22].

Szarek et al [23] discovered average amount of cadmium 2.6 μg/g d.m. in plants of ground cover in beech forest in the area of the Ojcowski National Park. Ciepal et al [18] found a cadmium content of 2.4–6.0 μg/g d.m. in the Bukowica Reserve and 4.5–7.0 μg/g in the Lipowiec Reserve, depending on species. Ciepal and Lipka [19] gives out a content of cadmium in plants of ground cover at the level of 0.5–16.0 μg/g d.m. in the Smolen Reserve and 0.7–18 μg/g d.m. in the Gora Chelm Reserve and in both cases the highest concentration regarded *Hedera helix* L. Ciepal [24] gives out a cadmium content in needles of *Pinus sylvestris* L. originating from the Bukowica Reserve and it amounts to 0.5–6.0 μg/g d.m., from the Smolen Reserve 4.0–10.0 μg/g d.m. and from the Gora Chelm Reserve 7.0–14.0 μg/g d.m. Ciepal and Rycman [25] in an analogical material originating from Roztoczanski National Park noted a cadmium content within a range from 0.6 to 1.05 μg/g d.m. For spruce needles, Ciepal [24] gives out following contents of cadmium: 2.0–7.0 μg/g d.m. in the Bukowica Reserve, 12.0–21.0 μg/g d.m. in the Smolen Reserve and 14.0–21.0 μg/g d.m. in the Gora Chelm Reserve. Received results indicate relatively high content of cadmium in plants of a ground cover of examined reserves. Whereas content of cadmium in needles of *Pinus sylvestris* [L.] Karst and *Picea abies* L. does not exceed a level characterizing plants from non-polluted areas. In comparison with discussed protected areas, results received for coniferous trees in examined reserves present that these areas are clean, not threatened by cadmium contamination.

Similarly to cadmium case, content of lead in soil is closely connected with a mineralogical and granulometric content as well as origin of soil bed-rocks but simultaneously, occurrence of this element in a top layer of soil is mostly connected with all anthropogenic factors.

Kabata-Pendias and Pendias [15] give out an average content of lead for soils of Poland not exceeding 20 μg/g. Permissible concentration of Pb in soils in areas protected on the basis of law regulations regarding protection of nature amounts to 50 μg/g.

Kimsa et al [17] determined in the surface layer of soil in the Swietokrzyski National Park a lead concentration amounting to 18.0–19.0 μg/g. Ciepal et al [18] determined in the surface layer of soil in the Bukowica Reserve a lead concentration amounting to 60.0 μg/g and in the Lipowiec Reserve 80.0 μg/g. In the area of the Jurajskie Landscape Parks – a part located in the former Czestochowskie province, Slezanski [26] gives out lead concentrations within a range from 19.90 to 323.0 μg/g.

Received results concerning lead content in a surface layer of soils in examined reserves are between 4.97–84.9 μg/g. Only in two reserves (the Slotwina Reserve and Zielona Gora Reserve) of four taken into consideration the permissible lead contents were not exceeded.

Regular (physiological) content of lead in plants varies from 5 to 14 μg/g d.m., and amount of 30 μg Pb/g d.m. is considered as toxic [15].

Ciepal [24] found contents of lead, depending on a research season, amounting to 0.5–4.0 μg/g d.m. for *Hedera helix* L. in the Bukowica Reserve and 24.0–26.0 μg/g d.m. for *Maianthemum bifolium* [L.] F.W. Schmidt in the Gora Chelm Reserve.

Czarnowska and Stasiak [27] give out a content of lead in needles of *Pinus sylvestris* L. from non-polluted areas and it amounts to 0.5–14 μg/g d.m. In mountain pine communities on Mt Babia Gora and Mt Pilsko the determined lead content was correspondingly 5.9 μg/g d.m. and 8.5 μg/g d.m. Needles of *Picea abies* [L.] Karst contained amounts of this metal within a range 15–21.5 μg/g d.m. regarding Mt Babia Góra and 21–35 μg/g d.m. regarding Mt Pilsko [16].

Received results concerning lead content in assimilation apparatus of chosen plants from examined reserves indicate insignificant threat by this metal. The highest contents found in *Hedera helix* L. or *Maianthemum bifolium* [L.] F.W. Schmidt are far from values considered as toxic.

Average zinc concentration in soils of different countries is within limits of 30–120 μg/g. Average zinc content for non-polluted soils in Poland is 40 μg/g [15]. Permissible content of zinc in soils of protected areas amounts to 100 mg/kg.

Ciepal and Lipka [19] found 350 ěg Zn/g in a surface layer of soil in the Gora Chelm Reserve and in the Smolen Reserve it was 200 μg Zn/g. Lukasik [20] at the upper level of soil in the Parkowe Reserve found that an average concentration of zinc equals 85 μg/g. On Mt Babia Gora a content of zinc was from 105.0 to 215 μg/g and on Mt Pilsko 185.0–325.0 μg/g [16].

In this elaboration, received results were within a range of 18.4–42.6 μg Zn/g. In each examined protected area the permissible concentrations of this metal were not exceeded.

In case of plants, zinc is a necessary for proper growth. To cover physiological requirements of plants, a concentration in leaves at the level of 15–30 μg/g d.m. is sufficient and in aboveground parts of a plant, staying away from pollution influences, is around 10–70 μg/g [15].

Zinc concentrations in examined samples presents predispositions of *Hedera helix* L. to an accumulation of this element. In case of this species, concentration of zinc exceeded a level considered as physiological. Contents found in leaves of other plants of a ground cover and in needles of *Picea abies* [L.] Karst and *Pinus sylvestris* L. did not exceed permissible standards.

Content of copper and iron in examined soils in any case did not exceeded permissible values. Ranges of concentrations were within 1.67–3.61 μg Cu/g and 344–1301 μg Fe/g.

Copper and iron, similarly to zinc, belong to the biogenic group of elements, used by plants in many metabolic traces [15].

Content of iron in plants changes significantly during vegetation period, in a different degree for particular plant, the most often within limits 10–400 μg/g d.m. [15].

Content of iron in plants changes significantly during vegetation period, in a different degree for particular plant, the most often within limits 10–400 μg/g d.m. [15].

Considering copper, Kabata-Pendias and Pendias [15] give out a physiological content of copper in leaves of different species at the level of 5–30 μg/g d.m.

In examined plant material, concentrations of this element varies from 0.38 to 4.99 μg/g d.m. and do not exceed permissible values and present even a deficiency of copper in an assimilation apparatus of chosen plant species.

Conclusions

Conducted research results present that there is no excessive heavy metals load in the Slotwina Reserve, Bukowa Kepa Reserve, Ostreznik Reserve and Zielona Gora Reserve. Content of heavy metals in soil, leaves and needles of chosen plant species growing in the areas of the Slotwina Reserve, Bukowa Kepa Reserve, Ostreznik Reserve and Zielona Gora Reserve in most cases were several times lower than average contents mentioned in literature and values characteristic for other protected areas. Therefore, chosen reserves may become a good control point for research on heavy metals content in a plant material and soil of areas influenced by a strong anthropogenic impact.

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OCENA STOPNIA ZAGROŻENIA METALAMI CIĘŻKIMI WYBRANYCH REZERWATÓW POŁUDNIOWEJ POLSKI

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Abstrakt: Badano akumulację metali ciężkich (Cd, Pb, Zn, Cu i Fe) w glebie (z głębokości 0–10 cm, 10–20 cm, 20–30 cm) oraz w szpilkach *Pinus sylvestris* L., *Picea abies* [L.] Karst., i liściach *Maianthemum bifolium* [L.] F.W. Schmidt i *Hedera helix* L. rosnących na terenach wybranych rezerwatów przyrody południowej Polski (Bukowa Kępa, Ostrężnik, Zielona Góra i Słotwina).

Materiał do analiz zbierano w okresie wegetacyjnym 2003–2004 (rezerwat Słotwina) oraz 2006–2007 (pozostałe rezerwaty). W próbkach gleby z rezerwatów Ostrężnik i Słotwina odnotowano przekroczenie normy zawartości Cd dla gleb obszarów chronionych (1 mg/kg), a z kolei ponadnormatywną zawartość Pb odnotowano w wierzchniej warstwie gleby z rezerwatu Bukowa Kępa (84,9 μg/g) i Ostrężnik (68,9 μg/g). W żadnym z badanych rezerwatów nie stwierdzono przekroczenia fitotoksycznych wartości progowych Pb i Zn. W przypadku Cd w liściach *Hedera helix* L. z rezerwatów Bukowa Kępa, Ostrężnik i Zielona Góra stwierdzono stężenia mieszczące się w dolnym zakresie wartości uznawanych za fitotoksyczne (5–10 μg/g). Odnotowane w roslinach badanych terenów chronionych stężenia Cu wskazują na niedobór tego pierwiastka i zły stan odżywienia roślin.

Słowa kluczowe: metale ciężkie, rezerwaty południowej Polski, *Pinus sylvestris* L., *Picea abies* [L.] Karst., *Maianthemum bifolium* [L.] F.W. Schmidt and *Hedera helix* L.

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OPTIMIZATION OF LAWN FERTILIZATION WITH NITROGEN. PART I. SOIL RESOURCES, YIELD AND ORNAMETNAL VALUES OF TURF

OPTYMALIZACJA NAWOŻENIA TRAWNIKÓW AZOTEM. CZ. I. ZASOBNOŚĆ GLEBY, PLON I WALORY DEKORACYJNE MURAWY

Abstract: Study objective was the effect of increasing levels of nitrogen: 0, 50, 100, 150 and 200 mg N · dm^{-3} in the soil on changes in the content of components in the soil, on grass yielding, water content in plant aboveground parts and on ornamental values of turf. With the increase of nitrogen fertilization, with the doses of 150–200 mg N · dm^{-3}, there followed a significant decrease in the content of calcium, magnesium, copper and zinc in the soil, while the content of phosphorus and sulphates increased. With a high level of nitrogen (150 mg N · dm^{-3}), the salinity of soil was increasing. No effect of nitrogen fertilization was exerted on soil pH reaction and on the contents of nitrogen, potassium, iron, manganese, chlorides and sodium. Nitrogen fertilization exerted a significant effect on the growth of fresh and dry matter and on water content in the aboveground parts of grasses. Nitrogen fertilization exerted also a significant effect on the ornamental values of lawn, such as the general appearance, sodding and weeding. The general appearance determining visual attractiveness and sodding (soil covered by grass leaf blades) were the most attractive when nitrogen content was maintained within 150–200 mg N · dm^{-3}, while a significantly smallest number of weeds was shown by the dose of 200 mg N · dm^{-3}. After soil analysis by the universal method (extract of 0.03 M CH_3COOH), the doses of 100–200 mg N · dm^{-3} can be recommended as provisional standards of nitrogen content in soil being the optimal ones for grasses grown in green belts.

Keywords: lawn, nitrogen fertilization, ornamental values, yielding

Lawns are regarded as the basic elements of green areas and their cultivation is a testimony of the awareness that green areas exert a positive influence on human nature [1]. Thanks to their specific biological properties, they can play the main role in the

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management of degraded soils causing a positive effect on soil improvement and on anticorrosive soil-creating and decorative function [2]. Fertilization adjusted to the lawn nutritional requirement contributes to a better grass propagation, it improves the durability and density of turf and at the same time it prevents weeding. Furthermore, it stimulates the development of the root zone, increases the activity of soil processes and the number of microorganisms [3].

The objective of the presented studies was the optimization of lawn fertilization with nitrogen through the determination of the effect of an increasing N nutrition on changes in the soil resources, growth and development of turf, on lawn decorative values such as general appearance, sodding, weeding and yielding (yield of fresh and dry matter and water content in the plant aboveground parts).

Material and methods

Vegetation experiments were carried out in the years 2007–2008 in the area of the Experimental Farm of the Horticultural Department "Marcelin", University of Life Sciences in Poznan. Five increasing levels of nitrogen fertilization [$mg\ N \cdot dm^{-3}$]: 0, 50, 100, 150, 200 corresponding to nitrogen doses of: 0, 10, 20, 30, 40 $g\ N \cdot m^{-2}$ were studied. Contents of the remaining macroelements in all tested combinations were supplemented to the standard levels [$mg \cdot dm^{-3}$]: P 100, K 200, Mg 180 (year 2007) and 300 (year 2008). In the period from April to July (in midmonth), on the basis of chemical analyses, top dressing was applied using ammonium nitrate (34 % N), double superphosphate (40 % P_2O_5), potassium sulphate (50 % K_2O) and magnesium sulphate (16 % MgO). Fertilization diagram and the applied doses of nutritive components are shown in Table 1.

Experiment was established in a systematic design. Each level of N fertilization was represented by four plots of 24 m^2 surface area (4 × 6 m). Studies were carried out on a 2-year old lawn, where a mixture of lawn grasses was sown in the amount of 25 $g \cdot m^2$ composed of: perennial ryegrass (*Lolium perenne* L.) 'Grasslands Nui' (45 %), tall fescue (*Festuca arundinacea* Schreb) 'Finelawn' (25 %), red fescue (*Festuca rubra* Hack.) 'Olivia' (10 %), red fescue (*Festuca rubra* Hack.) 'Boreal' (15 %), kentucky bluegrass (*Poa pratensis* L.) 'Balin' (5 %).

During the vegetation experiment, according to the needs, the lawn was irrigated with a dose of about 10 mm of water. The lawn was systematically mown in 10–12 day intervals. Each time, the fresh matter of the aboveground plant parts defined as plant fresh matter yield was weighed. After water content measurement, the dry matter content was determined.

Before experiment establishment, using the universal method, the content of macro- and microelements in the soil was measured [$mg \cdot dm^{-3}$] which showed the following values: NH_4 trace; N-NO_3 4.0; P 35.0; K 132.0; Ca 2756.0; Mg 143.0; S-SO_4 2.0; Fe 115.9; Zn 23.2; Mn 7.7; Cu 3.0; B 0.36; Na 15.0; Cl 22.0; pH 7.32 and EC 0.11 [$mS \cdot cm^{-1}$]. Soil samples for analyses were taken three times in the year 2007 (on 25.04, 24.05 and 17.07) while in 2008 – 5 times (on 15.03, 15.04, 12.06, 08.07, 19.08). Each time, from the given combination, 14–18 individual samples were taken from the

Table 1

Applied doses of components [$g \cdot m^{-2}$]

Component	N level														
	N-0			N-50			N-100			N-150			N-200		
	2007	2008	Σ	2007	2008	Σ	2007	2008	Σ	2007	2008	Σ	2007	2008	Σ
N	0.0	0.0	0.0	10.0	10.0	20.0	20.0	20.0	40.0	30.0	30.0	60.0	40.0	40.0	80.0
P	39.0	11.6	50.6	39.0	11.6	50.6	39.0	11.6	50.6	39.0	11.6	50.6	39.0	11.6	50.6
K	14.0	1.6	15.6	14.0	17.2	31.2	14.0	19.4	33.4	14.0	22.2	36.2	14.0	25.2	39.2
Mg	8.0	0.0	8.0	8.0	18.6	26.6	8.0	18.6	26.6	8.0	24.8	32.8	8.0	24.8	32.8
S	17.2	0.7	17.9	17.2	31.2	48.4	17.4	32.1	49.5	17.2	40.2	57.4	17.2	42.6	59.8

layer of 0–20 cm, and after mixing, a representative mixed sample (0.4–0.5 dm^3) was obtained. Available macroelement forms of sodium and chlorides were determined by universal method in 0.03 M CH_3COOH, while microelements were determined in Lindsay solution [4, 5]. Determinations were made using the following methods: N-NO_3, N-NH_4 – by microdistillation method (according to Bremner in Starck's modification), P – was measured colorimetrically with ammonium vanadomolibdate, K, Ca, Na – by flame photometry; Cl – nephelometrically with $AgNO_3$; S-SO_4 – nephelometrically with $BaCl_2$; B – by colorimetry with curcumin; Mg, Fe, Mn, Zn, Cu – by atomic absorption spectrometry (AAS – on Carl Zeiss-Jena apparatus), salinity [EC units] – conductometrically, at soil:water relation = 1:2 (v/v); pH – by potentiometry at soil:water relation = 1:2 (v/v).

Exact estimation of the ornamental values of turf was carried out in the midmonth of July, August and September of each year of studies. The following parameters were estimated: general appearance of the turf (in a 9-degree scale, where 1^o denoted a negative appearance devoid of any decorative values, while 9^o – showed a very good and attractive appearance [6, modified], sodding, ie soil covered by the aboveground parts of grasses in a 9-degree evaluation scale, where 1^o indicated surface cover [%]: 1^o – 0–5; 2^o – 6–15; 3^o – 16–25; 4^o – 26–40; 5^o – 41–60; 6^o – 61–75; 7^o – 76–85; 8^o – 86–95; 9^o – 96–100 and weeding of turf.

Changes in the component content in soil, yielding of plants, water content in the aboveground parts of grasses and ornamental values of turf were subject to statistical analysis using Duncan's test (α = 0.05).

Results and discusion

Content of nutritive components in soil

No significant effect of nitrogen fertilization on the mean content in soil of ammonium nitrogen, nitrate(V) nitrogen and potassium were found (Table 2). However, a significant effect was shown by the levels of 50–200 mg N-NO_3 · dm^{-3} on the increase of phosphorus in soil. In contrast to phosphorus, there was a significant effect of nitrogen fertilization on the decrease of calcium and magnesium contents particularly visible in the range of 150–200 mg N-NO_3 · dm^{-3} of soil. Content of sulphates increased under the influence of nitrogen fertilization, whereby at the levels of N-50 and N-150, the effect was significant.

Studies of microelements did not show any significant effect of increasing nitrogen fertilization on the contents of iron, manganese and chlorides in soil, or of the ballast ion represented by sodium (Table 3). On the other hand, it was found that the soil became poorer in zinc and copper content.

Fertilization with nitrogen on the levels of 100–200 mg N-NO_3 · dm^{-3} increased soil salinity, whereby, at the level of 150 mg N-NO_3, it was statistically proven (Table 3). Nitrogen fertilization did not cause any significant changes in the pH of soil. Soil reaction in the studied levels of nitrogen fertilization was alkaline – $pH_{(H2O)}$ 7.19–7.37.

Table 2

Effect of nitrogen fertilization on the content of macroelements in soil [mg · dm^{-3}]

N level	[mg · dm^{-3}]																				
	N-NH_4			N-NO_3			P			K			Ca			Mg			S-SO_4		
	Year																				
	2007	2008	$\bar{x}$	2007	2008	$\bar{x}$	2007	2008	$\bar{x}$	2007	2008	$\bar{x}$	2007	2008	$\bar{x}$	2007	2008	$\bar{x}$	2007	2008	$\bar{x}$
N-0	7.0a	10.5a	8.8a	0.0b	9.8a	4.9b	54.7b	57.7a	56.2ab	151.7a	115.0a	133.4a	2957.0a	4152.2a	3554.6a	190.0a	223.7a	206.9a	66.7b	22.4b	44.6b
N-50	8.3a	10.5a	9.4a	4.7a	11.2a	8.0ab	55.0b	38.3b	46.7b	172.7a	88.5a	130.6a	2839.0ab	4322.5a	3580.8a	188.3a	275.1a	231.7a	121.3a	111.0a	116.2a
N-100	6.0a	11.2a	8.6a	0.0b	9.1a	4.6b	60.7ab	55.5a	58.1ab	135.0a	103.5a	119.3a	2580.3ab	3534.1ab	3057.2ab	163.0a	264.8a	213.9a	68.3b	79.6a	74.0ab
N-150	8.3a	11.2a	9.8a	8.3a	14.0a	11.2a	71.7a	67.5a	69.6a	148.3a	95.5a	121.9a	2234.7b	2893.0b	2563.9b	159.0a	231.4a	195.2ab	148.3a	81.7a	115.0a
N-200	8.3a	10.5a	9.4a	0.0b	16.1a	8.1ab	74.7a	64.6a	69.7a	153.0a	82.6a	117.8a	2251.0b	2624.6b	2437.8b	150.3a	217.4a	183.9b	86.3ab	77.9a	82.1ab
$\bar{x}$	7.6	10.8		2.6	12.0		63.4	56.7		152.1	97.0		2572.4	3505.3		170.1	242.5		98.2	74.5	

Table 3

Effect of nitrogen fertilization on the content of microelements and sodium in soil [mg · dm^{-3}] and on pH and salinity

N level (A)	[mg · dm^{-3}]																		Salinity			$pH_{(H_2O)}$		
	Fe			Mn			Zn			Cu			Cl			Na			[mS · cm^{-1}]			[-]		
	Year (B)																							
	2007	2008	$\bar{x}$ (A)	2007	2008	$\bar{x}$ (A)	2007	2008	$\bar{x}$ (A)	2007	2008	$\bar{x}$ (A)	2007	2008	$\bar{x}$ (A)	2007	2008	$\bar{x}$ (A)	2007	2008	$\bar{x}$ (A)	2007	2008	$\bar{x}$ (A)
N-0	75.5a	88.4a	82.0a	5.2a	15.8a	10.5a	31.3a	42.2a	36.8a	2.30a	4.75a	3.53a	19.3a	37.8a	28.6a	21.0a	33.0a	27.0a	0.18a	0.23a	0.21a	7.08a	7.35a	7.19a
N-50	73.4a	73.7a	73.6a	6.2a	15.7a	11.0a	19.0b	21.6b	20.3b	1.90b	2.75b	2.33b	18.0a	26.4a	22.2a	20.3a	33.7a	27.0a	0.20a	0.21a	0.21a	7.21a	7.65a	7.37a
N-100	79.6a	90.7a	85.2a	6.8a	19.2a	13.0a	16.5b	22.0b	19.3b	1.90b	2.92b	2.41b	28.6a	36.5a	32.6a	20.0a	29.8a	24.9a	0.20a	0.27a	0.24a	7.19a	7.57a	7.30a
N-150	81.2a	89.0a	85.1a	6.2a	17.7a	12.0a	16.1b	20.8b	18.5b	1.80b	3.04b	2.42b	19.7a	35.8a	27.8a	19.3a	29.7a	24.5a	0.26a	0.33a	0.30b	7.08a	7.42a	7.21a
N-200	92.4a	88.2a	90.3a	7.3a	17.2a	12.3a	20.0b	21.9b	21.0b	2.10b	3.16b	2.63b	18.0a	41.3a	29.7a	18.0a	31.7a	24.9a	0.17a	0.26a	0.22a	7.07a	7.41a	7.26a
$\bar{x}$ (B)	80.4	86.0		6.3	17.1		20.6	25.7		2.0	3.32		20.7	35.6		19.7	31.6		0.20	0.26		7.13	7.44	

Absence of any effect on the differentiation of reaction was probably the result of high calcium content in soil (2437.8–3580.8 mg Ca · dm^{-3}).

Summing up, one can conclude that nitrogen fertilization, with the maintained high levels of 150–200 mg N-NO_3 · dm^{-3}, significantly decreased the contents of calcium, magnesium, zinc and copper and it increased the content of phosphorus and sulphur. However, it did not exert any effect on the contents of nitrogen, potassium, iron and manganese. Significant decrease of the contents of calcium, magnesium, zinc and copper could have been caused by the removal of those components together with the yield of plants which was intensified by the increasing nitrogen fertilization. Increase of phosphorus content can be connected with the decrease of calcium, potassium and magnesium contents and thereby with a smaller retardation of this component. On the other hand, the increase of phosphates was the effect of potassium phosphate and magnesium phosphate application in order to maintain the correct levels of potassium and magnesium in the soil.

Fresh matter yield of the aboveground grass parts

Significant effect of nitrogen fertilization was found to be exerted on the fresh matter of aboveground grass parts (Table 4). Yielding dynamics in the successive years of studies is shown in Figure 1. The presented data indicate a strong yield-creating influence of top dressing with nitrogen on the yield of the aboveground parts of plants. Data available in literature show higher yielding of grasses with smaller doses of nitrogen in case of top dressing. It is reported the best yielding of perennial ryegrass (*Lolium perenne* L.), being the basic species in the studied mixture of grasses, in a peat-mursh soil with nitrogen fertilization by the dose of 120 kg N · ha^{-1} (corresponding to 60 mg N · dm^{-3}) [7]. The most intensive fertilization of perennial ryegrass in the first year after seeding improved the aesthetical values of plants [8]. It is reported that best yielding of red fescue (*Festuca rubra* L.) was obtained by them also with the dose of 120 kg N · ha^{-1} [9]. Those authors showed that nitrogen fertilization exerted a favourable influence on the morphological features of grasses, among others on the height and length of inflorescences. Earlier studies reported that for some species and cultivars of grasses (among others the *Festulolium* hybrids originating from the

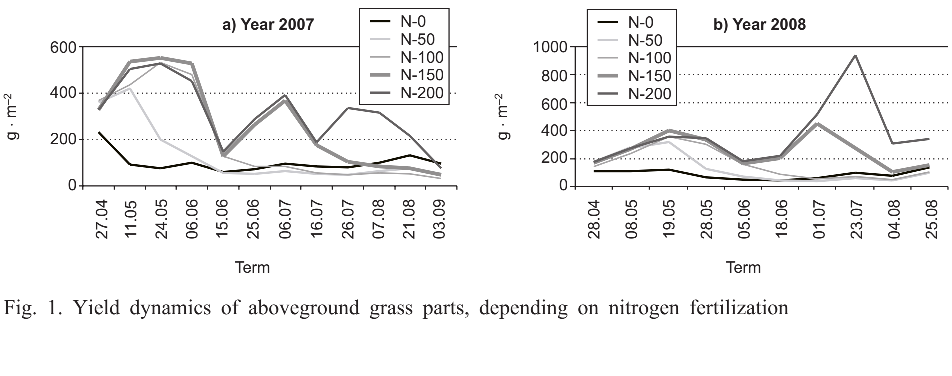

Fig. 1. Yield dynamics of aboveground grass parts, depending on nitrogen fertilization

Table 4

Effect of nitrogen fertilization on the yield of fresh and dry matter and on water content in the aboveground parts of grasses

N level	Fresh Yield [g · m^{-2}]			Dry Mass Yield [g · m^{-2}]			Water content [%]			
	Term									
	2007	2008	$\bar{x}$	2007	2008	$\bar{x}$	June	July	August	$\bar{x}$
N-0	1221.2d	871.0d	1046.1e	366.4c	261.3c	313.8c	65.4b	70.1ab	74.5a	70.0ab
N-50	1566.9c	1245.7c	1406.3d	504.5bc	401.1b	452.8b	65.5b	68.1b	69.9b	67.8b
N-100	2357.4b	1554.9c	1956.2c	711.9b	469.6b	590.8b	68.5ab	68.7b	72.3a	69.8b
N-150	3192.2ab	2529.3b	2860.8b	852.3a	675.3ab	763.8ab	74.3a	73.5a	72.0ab	73.3a
N-200	3765.1a	3659.1a	3712.1a	960.1a	933.1a	946.6a	74.1a	77.5a	72.0ab	74.5a

Mean values marked with the same letter, for the particular columns, do not differ significantly.

cross-breeding of species from *Festuca* and *Lolium*), the most effective was the nitrogen dose of 150 kg N · ha^{-1} (75 mg N · dm^{-3}) [10].

A significant effect of nitrogen fertilization on the increase of water content was found in the aboveground parts (Table 4). The smallest amount of water content was determined in the combinations N – 0; N – 50 and N – 100 (70.0; 67.8 and 69.8 %, respectively), while the significantly highest water content was found in combinations N – 150 and N – 200 (73.3 and 74.5 %, respectively)

Decorative values

A positive tendency in the improvement of the general appearance (including the colour of leaves) and the degree of turf sodding were found with the increasing nitrogen fertilization (Table 5). In the second year of studies (2008), there was a distinct improvement in the lawn condition, in comparison with the previous state (in 2007). The most favourable decorative values were shown by lawns in the combinations N-150 and N-200; less good results were shown by the combinations N-100 and the worst effect was found after the application of combinations N – 0 and N – 50. Together with the duration of the vegetation period, there appeared a tendency, typical of lawns, to a decrease of the decorative values, which was confirmed by the studies [11]. Those authors mentioned that among the factors exerting an effect on lawn attractiveness (including sodding), the species composition of grass mixtures also plays an important role. In spring, the best sodding was shown by mixtures containing 35–40 % of perennial ryegrass, 40–55 % of Kentucky bluegrass and 10–20 % of red fescue. In summer, the degree of soil cover did not depend on the composition of grass mixtures. In autumn, the worst sodding was shown by the turf containing over 50 % of perennial ryegrass. It was showed that a good quality of lawns can be obtained by the adequate selection of species and cultivars [12]. The most aesthetic appearance was represented in the spring period by mixtures containing at least 50 % of perennial ryegrass. Different conclusions than those obtained in our studies referring to the effect of nitrogen fertilization were formulated [13]. In the opinion of the mentioned author, in case of a renovation of a turf, very effective were low nitrogen doses 50 kg N · ha^{-1} (25 mg N · dm^{-3} of soil) which exerted a positive effect on the attractiveness of turf and on sodding.

One can recommend as a provisional standard the following contents of nutritive components in soil for lawns [mg · dm^{-3}]: N 100–200; P 100; K 200; Mg 180–300. The conventional maximum single application of slow release N is 73 kg N · ha^{-1} (36.5 mg N · dm^{-3}) – but some fertilizer manufacturers recommend a single season long application rate of 146 kg N ha (73 mg N · dm^{-3}) [14]. The same authors confirmed our observations that in case of the absence of N fertilization, the appearance of lawns significantly deteriorated. The best turf density is obtained with the use of an annual dose of 300 kg N · ha^{-1} (corresponding to 150 mg N · dm^{-3} of soil) [15].

A significant effect of nitrogen fetrtilization was found to be exerted on lawn weeding (Table 5). The greatest weeding was found in the control combination (N – 0), while the least number of weeds was recorded in the combinations: N – 150 and N –

Table 5

Effect of nitrogen fertilization on the decorative values of a lawns (2007, 2008)

Term	N level (A)					$\bar{x}$
	N-0	N-50	N-100	N-150	N-200	
Appearance of lawn [in degrees]						
July	6.00 (6.25)	6.50 (6.25)	6.50 (6.25)	8.75 (7.25)	8.50 (8.25)	7.15a (6.85)a
August	4.25 (4.25)	5.00 (4.75)	5.25 (5.25)	5.00 (7.25)	6.00 (7.75)	5.10b (5.85)ab
September	5.00 (5.00)	4.20 (4.20)	4.75 (4.75)	6.00 (7.00)	5.75 (7.50)	5.15b (5.69)b
$\bar{x}$	5.08b (5.17)b	5.08b (5.07)b	5.50b (5.42)b	6.58a (7.17)a	6.75a (7.83)a	
Sodding [in degrees]						
July	4.00 (4.25)	4.50 (4.75)	5.50 (5.25)	7.50 (7.50)	7.50 (8.25)	5.80a (6.00)a
August	5.50 (5.75)	5.75 (6.25)	6.00 (7.75)	5.75 (7.75)	6.50 (8.00)	5.90a (7.10)a
September	4.00 (4.50)	4.75 (5.25)	4.50 (6.25)	5.50 (7.00)	4.75 (7.25)	4.70b (6.05)a
$\bar{x}$	4.50c (4.83)c	5.00bc (5.42)b	5.33b (6.42)b	6.25a (7.42)a	6.25a (7.83)a	
Weeding [number of weeds · m^{-2}]						
July	1.29	1.17	1.13	0.58	0.33	1.29 a
August	1.25	1.25	1.08	0.42	0.17	1.25 a
September	1.17	1.21	1.08	0.42	0.17	1.17 a
$\bar{x}$	1.24 a	1.21 a	1.10 a	0.47 b	0.22 b	

Mean values marked with the same letter, for the particular years of studies, do not differ significantly. Data in brackets refer to year 2008.

200. With the nitrogen fertilization increase, the weed species composition was changed. In the control combination, there occurred: common dandelion (*Taraxacum officinale* F.H. Wigg.); ribwort plantain (*Plantago lanceolata*); white clover (*Trifolium repens* L.). In the N-50 and N-100 combinations, there occurred: common dandelion, ribwort plantain, while in the combination N-150 and N-200, only common dandelion was found. It is reported that the factor which prevents weeding is the adjustment of mineral fertilization to the nutritive requirements of plants which contributes to a better propagation of grasses, improves the durability and density of turf [3]. Significant effect on lawn degradation is exerted by water deficit and too rare lawn grass mowing [16].

On the basis of our studies, one can conclude that one of the factors causing a degradation and deterioration of lawn appearance in the green belts can be the fact that mineral fertilization of plants is not properly adjusted to the nutritional requirements of plants. In order to optimize the cultivation and to improve the appearance of lawns, a controlled fertilization based on chemical soil analyses is recommended.

Conclusions

1. A significant effect of increasing nitrogen fertilization on the levels of 150–200 mg $N\text{-}NO_3 \cdot dm^{-3}$ was found to be exerted on the increase of phosphorus and sulphur content in soil. On the other hand, the contents of calcium, magnesium, zinc and copper decreased. No significant changes were found in the contents of nitrogen, potassium, iron and manganese.

2. Nitrogen fertilization exerted a significant effect on the increase of fresh and dry matter yield and on water content in the aboveground parts of grasses.

3. The most favourable appearance and the highest sodding of turf was obtained when the nitrogen content was maintained in the range of 150–200 mg $N\text{-}NO_3 \cdot dm^{-3}$ of soil. The least weeding was found at N content of 200 mg $N\text{-}NO_3 \cdot dm^{-3}$.

4. Doses of 100–200 mg $N \cdot dm^{-3}$ can be recommended as provisional standards of nitrogen content in the soills for lawns.

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OPTYMALIZACJA NAWOŻENIA TRAWNIKÓW AZOTEM. CZ. I. ZASOBNOŚĆ GLEBY, PLON I WALORY DEKORACYJNE MURAWY

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Abstrakt: Badano wpływ wzrastających poziomów azotu: 0, 50, 100, 150 i 200 mg N $\cdot$ dm^{-3} gleby na zmiany zawartości składników w glebie, plonowanie traw, zawartość wody w częściach nadziemnych roślin i walory dekoracyjne murawy. Wraz ze wzrostem nawożenia azotem, przy poziomach 150–200 mg N $\cdot$ dm^{-3}, znacznemu obniżeniu ulegała zawartość wapnia, magnezu, miedzi i cynku w glebie, a wzrastała zawartość fosforu i siarczanów. Przy wysokim poziomie azotu (150 mg N $\cdot$ dm^{-3}) wzrastało zasolenie gleby. Nie stwierdzono wpływu nawożenia azotem na odczyn pH gleby oraz zawartość azotu, potasu, żelaza, manganu, chlorków i sodu. Stwierdzono istotny wpływ nawożenia azotem na wzrost plonu świeżej i suchej masy oraz zawartość wody w częściach nadziemnych traw. Stwierdzono istotny wpływ nawożenia azotem na walory dekoracyjne trawnika, takie jak: aspekt ogólny, zadarnienie oraz zachwaszczenie. Aspekt ogólny, określający jego atrakcyjność wizualną oraz zadarnienie (pokrycie gleby blaszkami liściowymi) były najlepsze przy utrzymywaniu zawartości azotu na poziomach 150–200 mg N $\cdot$ dm^{-3}, natomiast najmniejsze zachwaszczenie było przy zawartości 200 mg N $\cdot$ dm^{-3}. Jako tymczasowe standardowe zawartości azotu w glebie, optymalne dla wzrostu traw na terenach zieleni, można zalecać 100–200 mg N $\cdot$ dm^{-3} gleby, przy analizie gleby metodą uniwersalną (wyciąg 0,03 M CH_3COOH).

Słowa kluczowe: trawnik, nawożenie azotem, wartość dekoracyjna, plonowanie

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DO ANTIOXIDANTS COUNTERACT THE TOXIC EFFECTS OF PYRETHROIDS ON *Saccharomyces cerevisiae* YEAST?

CZY ANTYOKSYDANTY PRZECIWDZIAŁAJĄ SKUTKOM TOKSYCZNEGO ODDZIAŁYWANIA PYRETROIDÓW NA KOMÓRKI DROŻDŻY *Saccharomyces cerevisiae*?

Abstract: Pyrethroids are synthetic esters of primary or secondary alcohols containing at least one double bond and chrysanthemic acid [2,2-dimethyl-3-(2-methylpropenyl)-cyclopropanecarboxylic acid), or halogen analogues of it. These compounds have been used as insecticides. Their mechanism of toxic action on insects consists in inhibiting the activity of ion channels in nerve cells. According to data from the literature, generation of reactive forms of oxygen may be the mechanism of numerous non-specific reactions induced by these pesticides in various organisms. The aim of this study was to determine whether supplementing media with antioxidants protects *Saccharomyces cerevisiae* yeast cells from loss of viability caused by incubation with pyrethroids. The yeast cells were incubated for 2 h with selected pyrethroids and then plated on solid medium containing various antioxidants. The survival rates of yeast cells grown on control media and enriched media were compared. The antioxidants applied were not found to protect the yeast cells from the toxicity of the pyrethroids.

Keywords: pyrethroids, yeast, antioxidants

Pyrethroids are synthetic esters of primary or secondary alcohols containing at least one double bond and chrysanthemic acid [2,2-dimethyl-3-(2-methylpropenyl)-cyclopropanecarboxylic acid), or halogen analogues of this acid [1]. The literature dealing with the biological activity, mechanisms of action, and toxicity of pyrethroids is very extensive. Authors of many studies have stressed the acute toxicity of pyrethroids for insects, fish, and other aquatic organisms [2–4]. These compounds inhibit ion channel (sodium channel) function in the nerve cell membranes of insects, leading to their death [5]. Pyrethroids, like other xenobiotics, can affect the functioning of cells of all organisms and lead to potentially dangerous biochemical consequences, such as changes in the activity of enzymes, including those responsible for antioxidant protection of

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cells; atypical metabolic processes; acceleration of ageing processes; and endocrine system disturbances.

According to data from the literature, the common mechanism of these non-specific reactions of organisms to pyrethroids may be free radical generation [6]. When the toxic effects of pyrethroids on yeast cells were studied, changes characteristic of oxidative stress were observed, such as changes in catalase and superoxide dismutase activity, a decrease in reduced glutathione, and decreased concentration of thiol groups and total antioxidant capacity [7–9].

Physiological processes involved in cellular oxygen metabolism lead to generation of reactive oxygen species – (ROS). A balance between production and elimination of ROS is maintained thanks to antioxidant enzymes and antioxidants. Disruption of this homeostasis is manifested by increasing concentration of free radicals, and leads to oxidative stress [10]. Many different environmental factors are responsible for free radical generation, including pesticides.

A group of compounds with antioxidant functions, having very diverse chemical structure, occurs in the cells of all organisms. The task of these molecules is to react with free radicals and inhibit free radical reactions in the early stages of their propagation. Free radicals can react nonspecifically with antioxidants such as glutathione, cysteine, vitamins A, C, and E, uric acid, taurine, metallothioneine*,* and plant polyphenols. Vitamin E, α-lipoic and coenzyme Q are the most important antioxidants protecting the lipids of biological membranes from free radicals [10]. Ascorbic acid and glutathione prevent spreading of free radical reactions in the hydrophilic environment [11]. The literature provides numerous examples of the synergistic effects of hydrophobic and hydrophilic antioxidants (vitamins E and C, glutathione and vitamin A) [12].

Yeast cells are incapable of synthesizing tocopherols and ascorbic acid, but they easily take up these components from medium and build them into their cellular structures. *S. cerevisiae* yeast produce erythroascorbic acid, but its concentration is considerably lower than ascorbic acid concentration in the cells of other eukaryotic organisms [13].

The aim of this study was to test whether supplementing medium with antioxidants would protect *S. cerevisiae* yeast cells from death caused by incubation with pyrethroids. The study used antioxidants that act within the hydrophilic environment (ascorbic acid) and the hydrophobic environment (alpha-tocopherol) of cellular organelles, as well as their derivatives ascorbic acid 6-palmitate (which acts within the cell membrane environment) and alpha-tocopherol acid succinate (which, unlike tocopherol, acts within the hydrophilic environment of the cell).

Material and methods

Yeast strain: a wild-type strain of the yeast SP-4 phenotype Mat α leu1 arg4 [14]. Culture conditions: The yeast were grown in liquid YPG medium in standard conditions to the late logarithmic phase. The yeast cells were incubated for two hours with the following pyrethroids: cypermethrin, fenvalerate, tetramethrin, and permethrin. The pyrethroid solutions were diluted so that their final concentration in the incubation mixture was 50 and 100 μg · cm^{-3}. Following incubation with pyrethroids the yeast cell suspension was diluted to a density of about 1–5 × 10^3 cells · cm^{-3} and plated on Petri dishes with solid YPG medium + 2 % agar supplemented with antioxidants in concentrations that do not reduce the survival rate of yeast cells. The antioxidant solutions were rubbed into the solidified medium directly before plating. After plating, the Petri dishes were incubated for two days at a temperature of 28 ^{o}C and then the number of colonies was counted. The survival rates of the yeast cells were determined as percentages. 100 % was taken to be the number of colonies obtained in the control sample.

Results

The aim of the first part of the experiment was to determine the effect of selected antioxidants on the survival rate of yeast cells (Table 1).

Table 1

Survival rate [%] of wild type SP4 (*wt*) yeast cells in the presence of selected antioxidants

Type and final concentration [mM] of antioxidants	Survival rate [%] of wild type SP4 (*wt*) yeast cells
control	100
(±)-α-Tocopherol	
0.015	105.3
0.03	91.5
0.045	94.5
0.06	84.8
0.09	85.9
(+)-α-Tocopherol acid succinate	
0.006	104.8
0.012	97.8
0.025	92.5
0.036	98.4
0.125	90.7
0.25	94.8
0.375	86.1
Ascorbic acid 6-palmitate	
0.016	102.5
0.03	86.8
0.05	79.8
0.16	78.5
0.32	82.0
0.48	80.4
Ascorbic acid	
40	94

The lower survival rate of the yeast cells following application of higher doses of tocopherol, tocopherol succinate and ascorbic acid 6-palmitate indicates that these

Table 2

Survival rate [%] of wild type SP4 (*wt*) yeast cells in the late logarithmic phase of growth in the presence of selected antioxidants after previous incubation with cypermethrin or fenvalerate

Type and final concentration of antioxidants [mM]	Type and final concentration of pyrethroids [$\mu g \cdot cm^3$]							
	cypermethrin		fenvalerate		tetramethrin		permethrin	
	50	100	50	100	50	100	50	100
Survival rate after incubation with pyrethroid	78.5	47.7	100	25.3	76.2	41.3	87.4	25.2
(±)-Alpha-tocopherol 0.015	95.2	37.8	103.2	13.25	69.5	51.3	83.8	32.9
α-Tocopherol acid succinate 0.006	84.7	23.9	102.7	10.95	89.7	34.9	79.9	30.2
Ascorbic acid 6-palmitate 0.016	78.3	33.1	119	24.1	77.9	37.7	72.7	22.6
Ascorbic acid 40	85.2	36.4	97.1	29.6	75.2	40.8	86.4	23.9

antioxidants may exhibit pro-oxidant activity. Ascorbic acid concentration was determined in earlier experiments [15]. Yeast are capable of growing in the presence of high doses of vitamin C. Exceeding a dose of 80 mM lowers the survival rate of cells in air. Pyrethroids lower the survival rate of yeast cells in the late logarithmic phase of growth. When a 50 μg · cm^3 dose was applied, cypermethrin was found to be the most toxic of the pyrethroids, followed by tetramethrin and permethrin, while fenvalerate did not reduce the cell's ability to form colonies. The higher, 100 μg · cm^3 dose of pyrethroids was more toxic; permethrin and fenvalerate caused a severe reduction in survival rate.

To test whether supplementation with antioxidants protects against the toxic activity of pyrethroids, yeast cells were incubated with cypermethrin, fenvalerate, tetramethrin, or permethrin, and then plated on media containing antioxidants. However, the antioxidants were not found to significantly influence the survival rate of the yeast cells. A slight increase in survival rate in the sample with alpha-tocopherol was found only in the case of the lower dose of cypermethrin, tetramethrin and permethrin. Following incubation with 50 μg · cm^3 of cypermethrin, and subsequent plating of cells on medium enriched with alpha-tocopherol, survival rate increased about 16 %. For tetramethrin and permethrin, the survival rate increased 13 % and 10 %, respectively.

Discussion

Pyrethroids are divided into two types: type I lacks a cyano group (*cis* and *trans* permethrin), while type II contains a cyano group (cypermethrin, fenvalerate). Surrales postulates [16] that the toxicity of pyrethroids for mammal cells may be modulated by the organism's metabolic activity. Biodegration products of pyrethroids do not exhibit insecticidal activity, but are more toxic than the parent pyrethroid for mammals and aquatic organisms. The mechanism of action of many pesticides involves inhibition of mitochondrial complex I activity [17]. During metabolism of cypermethrin, cyanohydrin is formed, from which thiocyanate anions are generated [1]. Various symptoms of poisoning with type I and II pyrethroids have been noted in mammals. Rats given type I pyrethroids exhibit symptoms of poisoning known as the T-syndrome – aggressive behaviour, ataxia, muscle tremors, and convulsions, while type II pyrethroids induce the CS-syndrome, manifested by hypersensitivity, epileptic seizures, chorea, and ptyalism, as well as a burrowing reflex. Type II pyrethroids attack the central nervous system, while type I pyrethroids attack the peripheral nerves [18]. Cyanides and thiocyanates are inhibitors of mitochondrial respiratory enzymes. This toxicity mechanism of pyrethroids has marginal significance in yeast. In the case of yeast cells, inhibition of the respiratory chain does not lead to cell death, because yeast are facultative anaerobes. Nevertheless, following incubation with pyrethroids, an increase in the number of rho-mutants, which are incapable of growing on non-fermentable carbon sources, has been observed among the surviving cells [8].

Induction of oxidative stress is one of the main mechanisms of action of many pesticides. Damage to membrane lipids, protein and DNA is the endpoint biomarker of the oxidative stress-inducing effects of pesticides [19]. Data from the literature provide numerous examples demonstrating that pyrethroids cause changes in the activity of antioxidant enzymes and in the concentration of antioxidants in the cells of various organisms.

Changes in parameters characteristic of oxidative stress have also been observed in the case of yeast cells. Our previous experiments have shown that mutants lacking the main antioxidant enzyme, superoxide dismutase, are more sensitive to pyrethroids. We have also published the results of an experiment confirming that pyrethroids cause a disturbance of redox potential and reduce concentration of GSH and thiol groups [7, 8]. Decreased total antioxidant capacity in yeast cell extracts suggests that the antioxidant system is involved in the detoxification of pyrethroids [20].

Under certain conditions, antioxidants protect cells from uncontrolled free radical reactions. The literature provides many examples supporting the thesis that antioxidants are capable of inhibiting or mitigating a disease process involving free radicals [21, 22].

Mutant *sod 1* yeast lacking the main antioxidant enzyme, superoxide dismutase Cu Zn SOD, is hypersensitive to the effects of pro-oxidant factors, and unlike the wild type strain, is not capable of growing in an oxygen atmosphere. Supplementing the medium with ascorbic acid enables cells of the *sod 1* mutant to grow in an oxygen atmosphere [15]. This example confirms that the commonly used antioxidant ascorbic acid can protect cells of the *sod 1* yeast mutant from free radicals.

Ascorbate in the presence of transition metal ions can oxidize and stimulate free radical reactions. One of the most dangerous potential reactions is reduction of iron ions to Fe^{2+} (the Fenton reaction), which can enhance generation of hydroxyl radicals. The oxidized form of vitamin C, dehydroascorbate, can damage erythrocyte membranes and affect various metabolic reactions [10]. The antioxidant mechanism of alpha-tocopherol does not involve reaction with oxygen, but rather interception of the free radical chain reaction, which is perpetuated not by oxygen but by fatty acids. Alpha-tocopherol reacts with fatty acid peroxyl radicals, the primary products of lipid peroxidation, and intercepts the chain reaction [23]. The tocopherol radical generated in this reaction can react with other antioxidants, such as glutathione, and be regenerated into tocopherol. A reaction between glutathione and tocopherol is catalyzed by an enzyme that reduces the tocopherol radical [12].

The results of an experiment using antioxidants and pyrethroids was published by Kale et al [24], who found that vitamin E protects rat tissues from oxidative stress that has been increased by pyrethroids. Changes characteristic of oxidative stress were less severe in animals that had been given vitamin E and then pyrethroids than in animals treated only with pyrethroids. These changes included a high level of lipid peroxidation products and a higher level of activity of superoxide dismutase, catalase and glutathione transferase than is found under physiological conditions. Vitamin E only partially protected acetylcholinesterase from inhibition caused by pyrethroids. A histological picture of liver tissues has also confirmed the protective role of vitamin E. Cypermethrin and fenvalerate induced necrotic changes in the liver, but administration of vitamin E protected the physiological structure of hepatocytes and counteracted the destruction of cells exposed to pyrethroids.

Vitamin E protects cell membranes against lipid peroxidation, which is one of the frequently observed symptoms of pyrethroid toxicity [25]. The mechanism of action of pyrethroids on cell membranes depends on the type of molecular structure. Type I pyrethroids, which have no cyano group (eg permethrin), penetrate biological membranes and affect the activity of intracellular enzymes [18]. Type II pyrethroids, which contain a cyano group, have a more hydrophilic molecular structure and it is more difficult for them to overcome the barrier of the cell membrane. For this reason they have a stronger effect on the cell membrane structure and damage it. In the case of yeast cells, the lipid peroxidation process has only marginal significance, as about 80 % of all lipids in the plasma membranes of yeast are monounsaturated and saturated fatty acids [26]. Nevertheless, pyrethroids cause yeast cell membranes to lose integrity, which was confirmed by staining conducted in previous studies. Pyrethroids can affect the functioning of ion channels in membranes, which disorganizes transmembrane transport. Gabianelli postulates [4] that pyrethroids can act on cells in two ways: by inducing oxidative stress in the cytoplasm and, due to their hydrophobicity, by accumulating in biological membranes.

A new way of looking at the toxicity of pesticides, including pyrethroids, is postulated by Kaseras and Mantion [26]. Many studies on the effects of pyrethroids and other pesticides (organochlorines, organophosphate pesticides) have shown that they affect intracellular ion balance, particularly of calcium. Even slight changes in intracellular Ca^{2+} concentration induced by these pesticides affect the activity of constitiutive nitric(II) oxide synthetase. One of the products of the reaction catalyzed by this enzyme is nitric(II) oxide. NO is rapidly oxidized by non-enzymatic reactions to form NO_3 (nitrate). NO also reacts with glutathione to form nitrosothiol or with heme to yield heme-NO [27]. Depending on conditions, nitric(II) oxide can exhibit strong pro-oxidant activity and increase oxidative stress. It can also exhibit cytotoxic activity. Pesticides induce a number of non-specific reactions by disrupting nitric(II) oxide metabolism. This short-lived molecule can affect many metabolic processes; it acts as a free radical, a second messenger, a neurotransmitter, or a hormone. A disturbance of calcium homeostasis causes a reduction in constitutive NO production, leading to cellular excitability and impairment of immunity (chemotaxis) and affecting signal transmission pathways, which precludes a cellular response to negative environmental factors.

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CZY ANTYOKSYDANTY PRZECIWDZIAŁAJĄ SKUTKOM TOKSYCZNEGO ODDZIAŁYWANIA PYRETROIDÓW NA KOMÓRKI DROŻDŻY *Saccharomyces cerevisiae*?

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Abstrakt: Pyretroidy są syntetycznymi insektycydami, w swojej strukturze chemicznej zawierają alkohol pierwszo- lub drugorzędowych (zawierających przynajmniej jedno wiązanie podwójne) połączony estrowo z kwasem chryzantemowym [kwasu 3-(2,2-dimetylowinylo)-2,2-dimetylo cyklopropanokarboksylowego] lub analogiem tego kwasu. Związki te znalazły zastosowanie jako insektycydy. Mechanizm ich toksycznego działania na owady polega na hamowaniu aktywności kanałów jonowych w komórkach nerwowych. Według danych literaturowych te pestycydy mogą wywoływać u różnych organizmów szereg niespecyficznych reakcji, których wspólnym mechanizmem może być generowanie reaktywnych form tlenu. Badając toksyczne działanie pyretroidów na komórki drożdży, stwierdzono występowanie mian charakterystycznych dla stresu oksydacyjnego, takich jak: zmiany aktywności katalazy i dysmutazy ponadtlenkowej, zmniejszenie zredukowanego glutationu, zmniejszenie stężenia zredukowanego glutationu i grup tiolowych, obniżenie całkowitej zdolności antyoksydacyjnej. Celem prezentowawnej pracy było zbadanie, czy dodatek antyoksydantów do pożywki uchroni komórki drożdży *Saccharomyces cerevisiae* przed zabiciem powodowanym inkubacją z pyretroidami. Komórki drożdży inkubowano przez 2 h z wybranymi pyretroidami, a następnie wysiewano na pożywkę stałą zawierającą różne antyoksydanty fenolowe, a także witaminy i ich pochodne. Porównywano przeżywalność komórek drożdży na pożywkach kontrolnych i wzbogaconych o antyoksydanty. W przypadku komórek drożdży nie stwierdzono ochronnej roli zastosowanych antyoksydantów przed toksycznością pyretroidów.

Słowa kluczowe: pyretroidy, antyoksydanty, drożdże

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IONIC COMPOSITION OF LOW SEDGE EUTROPHIC MAJERZ FEN WATERS IN THE PIENINY NATIONAL PARK

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Abstract: Ionic composition of the analyzed fen waters was variable over the study period and depended on climatic and anthropogenic factors, such as temperature and precipitation and also on the use of land situated on a slope above the analyzed fen. On the basis of ionic composition Majerz fen water, according to the Szczukariew-Prikłoński classification, may be classified in the 18th hydrochemical class of hydrogen carbonate-calcium-magnesium waters.

Keywords: ionic composition, eutrophic fen

Hydrogenic habitats, in which fens may be included, fulfill very important environmental functions. They play a crucial role in surface fresh waters retention, their purification and accumulation of carbon and nitrogen compounds. They also provide a habitat of many stenotypic plant and animal species including protected species [1, 2]. Specific conditions in these habitats are connected with the type of their hydrological feeding together with chemical composition and physical properties of waters. A high level of surface waters stimulates the process of organic matter accumulation, whereas water feeding these habitats enriches them in minerals at the same time shaping their trophicity. However, the state of dynamic balance in fens may be easily disturbed because of their drainage or a change of ionic composition of their feeding waters. Stenotypic species, characterized by specific habitat requirements are particularly sensitive to changes [3, 4].

The aim of the research was to determine the impact of climatic conditions and the way of land use on chemical and physical properties of waters and soils of low sedge slope eutrophic fen.

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Materials and methods

The research comprised the soil and waters of Majerz slope fen covered by vegetation characteristic for *Valeriano-Caricetum flavae* alliance and situated on the Majerz glade in the Pieniny National Park. The analyzed fen developed on the andesite matrix [5]. Two soil pits, situated in its upper and lower part, were made in order to determine soil properties of the analyzed fen. On the basis of the organic carbon content, depth of organic horizons, degree of organic matter decomposition (Table 1) and the type of hydrological feeding, the analyzed fen soils may be counted to Rheic Histosols (Eutric) according to the WRB taxonomy [6].

Piezometres were installed in the places where the soil pits were made and water samples were collected from them from March to November 2005. In the analyzed fen soils the mineral content, pH and degree of organic matter decomposition were determined using the methodology suggested by Sapek and Sapek [7] for organic soils. The temperature, pH, electroconductivity, mineralization, oxygenation and ionic composition were determined using the methodology suggested by Dojlido [8] in the waters sampled from the piezometers placed in the fen soils.

Dependencies between the selected water properties were assessed using Spearman's rank simple correlation coefficient for non-parametric statistics.

Results and discussion

The analyzed fen was characterized by a soligenous type of hydrological feeding. In conditions of high moisture, the bog process of organic material accumulation was in progress in the investigated fen surface horizon. The surface horizons of the investigated soils revealed a high degree of organic material decomposition characteristic for low peats with amorphous-lump structure (Table 1).

Table 1

Essential properties of analyzed fen soils

Profile	Depth [cm]	Horizon symbol	Decomposition degree	Mineral content [%]	WRB 2007
Location: Majerz Glen, 640 m.a.s.l, land slope 8°					
Majerz I	0–20	Ha	H_6	35,0	Rheic Sapric Histosols (Eutric)
	20–45	He1	H_3	52,5	
	45–58	He2	H_3	53,0	
	58–(68)	Cl	—	—	
Majerz II	0–24	Ha	H_6	34,6	Rheic Sapric Histosols (Eutric)
	24–43	He1	H_3	52,3	
	43–65	He2	H_3	59,7	
	65–(75)	Cl	—	—	

The high level of decomposition should be attributed to a considerable oxygen content in the fen feeding waters, which stimulated the organic matter decomposition process. Similar dependencies between the degree of organic matter decomposition and oxygenation of fen feeding waters were registered by Nicia and Miechówka [9] in the waters of eutrophic fens developed on the lime rock matrix.

On the basic of the ionic composition the Majerz fen waters may placed in the 18th hydrochemical class of hydrogen carbonate-calcium-magnesium waters according to Szczukariew-Prikłoński classification [10]. However, ionic composition of the studied fen waters was variable throughout the research period, the state of dynamic balance was observed in them. Changes in the analyzed fen water chemistry observed during the period of research were caused by the factors such as: precipitation, temperature and the presence of animals grazed on a slope above the researched fen.

On the basis of their mineralization, the examined fen waters may be classified after Pazdro [11], to fresh waters with mineralization from 100 to 500 $mg \cdot dm^{-3}$. The analyzed fen waters were characterized by variable values of total mineralization over the period of research. The highest concentrations of mineral substance were assessed in May over the period of between two and three-weeks of rainless weather (Table 2). The mineral substance content, electroconductivity values and concentrations of Ca^{2+}, Mg^{2+} and HCO_3^- were decreasing in the periods of intensified rainfall as a result of the so-called "dilution effect". Allan [12] described a similar relationship between substance concentrations in river waters and precipitation, whereas Nicia and Miechówka [13] observed it in fen waters. The mineral content is connected with the value of electroconductivity [14]. A statistical analysis of the obtained results revealed a significant dependence between the mineral substance content and the value of electroconductivity (simple correlation coefficient $r = 0.9500$ at $p < 0.01$) (Table 3).

The other factor which apparently affected ionic composition of the examined fen water was the temperature. Changes of temperature cause a change of intensity of processes influencing water chemistry shaping. These processes include, among others, nitrification and biological sorption. Because the intensity of nitrification increases with temperature [15], the concentration of NO_3^- ions was also growing in the analyzed fen water with increasing temperature. Statistical analysis showed a significant relationship between nitrate(V) ion concentration and temperature of the studied fen waters described by a simple correlation coefficient $r = 0.6024$ at $p < 0.1$. The increase in temperature was accompanied by biological sorption involving the incorporation of biogenic components into plant and animal organisms [16]. This process may explain the phosphorus content in the investigated fen waters decreasing with raising temperature.

Changes of phosphate ion concentrations may also be connected with human activity and the presence of wild animals. In June a flock of about 800 sheep was kept on a slope above the fen and the animals left a considerable amount of excrements. Intensive rainfall in June caused leaching phosphate ions from the sheep and wild animal excrements, which enriched the fen waters.

Table 2

Properties of analyzed fen waters

Properties*		Month of sampling										
		III	IV	V	VI	VII	VIII	IX	X	XI	$\overline{X}$**	A***
Temperature	°C	5.7	10.9	12.3	13.7	13.9	15.3	14.5	11.7	6.1	11.6	3.5
pH		6.95	7.02	7.07	7.39	7.11	7.29	7.52	7.8	7.48	7.3	0.3
Conductivity	$\mu S \cdot cm^{-1}$	448	592	675	378	590	488	511	410	436	503.1	98.2
Mineraliztion	$mg \cdot dm^{-3}$	344	470	512	329	423	389	397	345	342	394.6	63.6
O_2		2.3	0.3	0.3	0.5	0.9	1.4	1.4	2.2	1.9	1.2	0.8
HCO_3^-		234.9	300.1	323.4	105.4	211.2	289.3	270.7	256.7	265.7	250.8	64.0
SO_4^{2-}		28.43	37.08	37.8	20.69	52.65	4.75	7.09	15.16	14.31	24.2	16.0
Cl^-		6.06	4.06	4.38	5.01	3.81	3.87	3.38	3.08	3.1	4.1	1.0
NO_3^-		0.93	11.98	13.68	42.79	57.2	23.17	26.8	28.8	23.75	25.5	16.8
NH_4^+		0.04	0.04	0.04	0.27	0.02	0.04	0.03	0.01	0.01	0.1	0.1
PO_4^{3-}		23.1	44.5	46	42.75	5.6	2.67	2.31	2.05	2.05	19.0	20.2
Ca^{2+}		62.05	77.42	84.93	43.51	66.31	69.45	66.03	55.73	57.21	64.7	12.2
Mg^{2+}		18.1	29.1	31.45	14.52	17.35	21.75	25.44	15.65	16.67	21.1	6.2
Na^+		6.03	4.72	4.47	4.54	6.88	6.67	6.74	6.87	6.76	6.0	1.1
K^+		5.53	3.39	2.37	0.8	4.37	1.98	1.92	0.91	0.88	2.5	1.7

* mean value from two piezometres; ** $\overline{X}$ – mean value; ***A – standard deviation.

Table 3

Correlation table

	T	pH	Conductivity	Mineralization	O_2	HCO_3^-	SO_4^{2-}	Cl^-	NO_3^-	NH_4^-	PO_4^{3-}	Ca^{2+}	Mg^{2+}	Na^+	K^+
T	1.0000	0.3333*	0.1667	0.2500*	–0.3109*	0.0667	–0.3000*	–0.1667	0.5000*	0.1399	–0.0669	0.2167	0.1167	0.1167	–0.1667
pH	0.3333*	1.0000	–0.5333*	–0.3667*	0.3025*	–0.1833	–0.6167**	–0.7833**	0.6500**	–0.5509*	–0.7699**	–0.5667*	–0.5000*	0.5000*	–0.8000***
Conductivity	0.1667	–0.5333*	1.0000	0.9500***	–0.5883**	0.6833**	0.4833*	0.1500	–0.3667*	0.0962	0.5272*	0.9500***	0.9000***	–0.2167	0.6500**
Mineralization	0.2500	–0.3667*	0.9500***	1.0000	–0.5883**	0.6833**	0.4667*	0.0000	–0.2333	0.0087	0.4519*	0.9000***	0.8333***	–0.1333	0.5667*
O_2	–0.3109*	0.3025*	–0.5883**	–0.5883**	1.0000	–0.3866	–0.4706*	–0.3109*	–0.0420	–0.4894*	–0.7342**	–0.5630*	–0.4538*	0.5462*	–0.0336
HCO_3^-	0.0667	–0.1833	0.6833**	0.6833**	–0.3866*	1.0000	–0.1000	–0.0667	–0.6000**	0.0525	0.2427	0.7833**	0.8500***	–0.3667*	0.1667
SO_4^{2-}	–0.3000*	–0.6167**	0.4833*	0.4667*	–0.4706*	–0.1000	1.0000	0.4000*	–0.0167	0.1486	0.6611**	0.3333*	0.1833	–0.1833	0.5667*
Cl^-	–0.1667	–0.7833**	0.1500	0.0000	–0.3109*	–0.0667	0.4000*	1.0000	–0.4667*	0.8919***	0.8285***	0.2000	0.2333	–0.7833**	0.4000*
NO_3^-	0.6024**	0.6500**	–0.3667*	–0.2333	–0.0420	–0.6000**	–0.0167	–0.4667*	1.0000	–0.3323*	–0.3849*	–0.4833*	–0.6333**	0.5333*	–0.4833*
NH_4^-	0.1399	–0.5509*	0.0962	0.0087	–0.4894*	0.0525	0.1486	0.8919***	–0.3323*	1.0000	0.7902**	0.2011	0.2361	–0.8569***	0.1224
PO_4^{3-}	–0.0669	–0.7699**	0.5272*	0.4519*	–0.7342**	0.2427	0.6611**	0.8285***	–0.3849*	0.7902**	1.0000	0.5272*	0.4937*	–0.8117***	0.4268
Ca^{2+}	0.2167	–0.5667*	0.9500***	0.9000***	–0.5630*	0.7833**	0.3333*	0.2000	–0.4833*	0.2011	0.5272*	1.0000	0.9167***	–0.3000*	0.6167**
Mg^{2+}	0.1167	–0.5000*	0.9000***	0.8333***	–0.4538*	0.8500***	0.1833	0.2333	–0.6333**	0.2361	0.4937*	0.9167***	1.0000	–0.4167*	0.5500*
Na^+	0.1167	0.5000*	–0.2167	–0.1333	0.5462*	–0.3667	–0.1833	–0.7833**	0.5333*	–0.8569***	–0.8117***	–0.3000*	–0.4167*	1.0000	–0.0167
K^+	–0.1667	–0.8000***	0.6500**	0.5667*	–0.0336	0.1667	0.5667*	0.4000*	–0.4833*	0.1224	0.4268*	0.6167**	0.5500*	–0.0167	1.0000

* significance level p = 0.5; ** significance level p = 0.1; *** significance level p = 0.01.

Conclusions

1. A high level of groundwaters of the analyzed fen, their mineralization and oxygenation affected the direction of the pedogenic process and the degree of organic matter decomposition in the studied fen soils,

2. On the basis of their mineralization the examined fen waters may be classified to the 18^{th} hydrochemical class of fresh hydrogen carbonate – calcium-magnesium waters.

3. Both anthropogenic and climatic factors affected the chemistry of the analyzed fen waters.

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SKŁAD JONOWY WÓD NISKOTURZYCOWEJ EUTROFICZNEJ MŁAKI MAJERZ W PIENIŃSKIM PARKU NARODOWYM

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Abstrakt: Skład jonowy wód badanej młaki był zmienny w okresie badań i zależał od czynników klimatycznych i antropogennych. Na podstawie składu jonowego, według klasyfikacji hydrochemicznej Szczukariewa-Prikłońskiego można je zaliczyć do 18 klasy hydrochemicznej wód wodorowęglanowo-wapniowo-magnezowych.

Słowa kluczowe: skład jonowy, młaki eutroficzne

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HARMFULNESS OF BIRDS IN ARABLE CROPS IN THE IMMEDIATE VICINITY OF THE SOLID WASTE LANDFILL SITE IN TARNOW

SZKODLIWOŚĆ PTAKÓW W UPRAWACH ROLNICZYCH W BEZPOŚREDNIM SĄSIEDZTWIE SKŁADOWISKA ODPADÓW STAŁYCH W TARNOWIE

Abstract: Analyses of damage caused by birds in arable crops were conducted in the neighbourhood of the municipal waste landfill site in Tarnow. Birds were the most numerous on plots in the immediate neighbourhood of the active landfill sector. They were flying to the plots with sprouting horse bean plants causing partial damage or total destruction of the plants. Losses caused by birds may be considerable, in some case even the whole plantation was destroyed. Cultivation of plants which constitute attractive food for birds is burdened with great risk in the vicinity of municipal landfill sites.

Keywords: birds, municipal landfill site, damages

Municipal landfill sites affect their natural environment [1] and conditions of agricultural production causing among others deterioration of soil quality and value of agricultural products [2]. Various microbiological and chemical pollutants [3, 4] as well as waste, eg plastic bags may move with the air to the areas adjoining landfills. The landfill sites as such may be also a habitat for many organisms or a place where they exist temporarily to find food.

Large amounts of organic waste is brought to municipal landfill sites and it may constitute food for various animal species, including rodents and birds. Many bird species come to landfills. Some are present there mainly in winter when food is hard to find elsewhere. However, many species are relatively permanent occupants of such places. Municipal waste landfill sites are most often visited by ravens and gulls. Birds are an important element of both natural environments and agrocenoses [5], yet birds

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scavenging on landfills may pose a hazard to eg airplanes, the natural environment and for arable crops [6]. It should be remembered that many bird species significantly diminish insect pest populations. However, some bird species may themselves pose a hazard to cultivated crops [7].Crops are the most threatened by birds at the sprouting and maturing stages. Birds cause the most serious damage in orchards, eg in the maturing stage of sour cherries or cherries. Maize sown areas during sprouting are most endangered among the arable crops. Damage caused by birds may also appear in horse bean or cereals.

The present paper aimed at identification of bird harmfulness in horse bean plantations localized in the zone immediately adjoining a municipal landfill site.

Material and methods

The studies were conducted in 2006 and 2007 in Tarnow Krzyz on the solid waste landfill site. The landfill area is surrounded by ploughlands, wasteland and forest. Observations were conducted on experimental sites located in the immediate neighbourhood of the landfill. The points were set up on each side of the landfill in two zones: below 250 m and 250–500 m from its boundaries. The labelling of the observations points was presented in Table 1. On each site spring wheat, potatoes and horse bean were cultivated. A single plot area was 20 m^2. The experiment was set up in four replications. The work presents the results of bird harmfulness on horse bean plots.

Table 1

Plots located in the vicinity of the municipal waste landfill site in 2006 i 2007

Observaion point	Localization of plots in reference to landfill site	
	Direction	Zone [m]
W I	West	below 250
W II	West	250–500
N I	North	below 250
N II	North	250–500
E I	East	below 250
E II	East	250–500
S I	South	below 250
S II	South	250–500
Z	Reclaimed sector	0

The degree of damage done by birds was assessed during sprouting. On the horse bean plot observations were conducted three times: at the beginning of sprouting, at full sprouting and a week later. On each plot a 1m x 1m area was designed using a wooden frame and the number of sprouted plants and plants damaged by birds was counted in this area. The extent of plant damage was also assessed on a three degree scale: 0 – undamaged plants, 1 – damaged but not uprooted plant, 3 – wholly uprooted plants.

Traces of bird feeding on sprouted horse bean are characteristic and easy to distinguish from damage caused by other animals.

Observations of birds feeding on the experimental plots were also conducted once during full sprouting of horse bean plants. Birds feeding on the plots were counted using binoculars at some distance so as not to frighten them off. The birds were counted three times a day, ie in the morning (between 6.00 and 8.00), at noon (between 12.00 and 14.00) and in the evening (between 17.00 and 19.00). The total number of observed birds was given as a result without differentiating their species affiliation.

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

Results and discussion

Damage caused by wild animals may pose a serious hazard to profitability of agricultural or garden production [7]. Conducted research confirms that birds may to a considerable degree damage agronomic plants. Arable crops are particularly exposed to bird feeding during sprouting. Life conditions are advantageous for some bird species on municipal wastes landfill sites, which provide an important source of food. Birds may gather on landfill sites or in their vicinity in very large flocks. During the period when they do not scavenge on the landfill they fly to the adjoining areas and may cause damage to arable crops.

The extent of horse bean damage due to birds depended on the place of cultivation with respect to the active landfill sector (Table 2), because birds gather on the active sector or in its vicinity. The largest number of damaged plants was observed on the plots located on the eastern and northern side of the landfill and in the reclaimed sector area. The plots on these sites were placed closest to the active sector. Sprouted horse bean plants were particularly severly damaged on the plots located in the area of the reclaimed sector (Table 3). In 2006 birds damaged all germinating and sprouting plants. In 2007 losses caused by birds were smaller and on the most exposed site almost 60 % of sprouting plants were damaged.

Table 2

Damage of horse bean plants [%] caused by birds in the zone of the effect of the solid waste landfill in Tarnow

Date development stage	Percent of damaged plants on plots									$LSD_{p < 0.05}$
	objects									
	S		E		N		W		Z	
	I	II	I	II	I	II	I	II		
2006										
Beginning of sprouting	0.0	0.0	7.0	6.0	6.0	7.0	0.0	0.0	8.0	*5.69*
Full sprouting	0.0	0.0	19.0	14.0	16.0	14.0	4.0	2.0	20.0	*6.84*
Full 7 days after sprouting	0.0	0.0	40.0	23.0	30.0	20.0	4.0	2.0	100	*7.99*

Table 2 contd.

Date development stage	Percent of damaged plants on plots									$LSD_{p<0.05}$
	objects									
	S		E		N		W		Z	
	I	II	I	II	I	II	I	II		
2007										
Beginning of sprouting	0.0	0.0	12.0	4.0	15.0	6.0	0.0	0.0	21.0	*6.53*
Full sprouting	0.0	0.0	24.0	24.0	32.0	26.0	0.0	0.0	37.0	*8.25*
Full 7 days after sprouting	0.0	0.0	49.0	35.0	51.0	41.0	0.0	0.0	59.0	*8.41*

Table 3

Degree [%] of horse bean plant damage due to birds in the zone of the effect of the solid waste landfill in Tarnow

Date/development stage	Degree of plant damage on plots									$LSD_{p<0.05}$
	objects									
	S		E		N		W		Z	
	I	II	I	II	I	II	I	II		
2006										
Beginning of sprouting	0.0	0.0	0.08	0.06	0.07	0.10	0.0	0.0	0.11	*0.073*
Full sprouting	0.0	0.0	0.23	0.15	0.19	0.16	0.04	0.02	0.3	*0.102*
Full 7 days after sprouting	0.0	0.0	0.50	0.24	0.40	0.24	0.04	0.02	1.68	*0.131*
2007										
Beginning of sprouting	0.0	0.0	0.12	0.04	0.15	0.06	0.0	0.0	0.21	*0.065*
Full sprouting	0.0	0.0	0.33	0.34	0.47	0.38	0.0	0.0	0.56	*0.130*
Full 7 days after sprouting	0.0	0.0	0.66	0.47	0.78	0.57	0.0	0.0	0.85	*0.187*

The observations evidenced numerous bird feeding on sprouted horse bean plants (Table 4). The largest number of birds were spotted on the plots on the eastern and northern side of the landfill and on the plot located on the reclaimed part, which was also next to the active sector. During the day the number of birds on individual sites changed, which was associated not only with activity of birds but also the operating personnel on the landfill. People and machines moving on the landfill frightened off birds, which, however, very quickly returned.

Solid waste landfills may also indirectly affect arable crops, among others favouring numerous bird gathering. Municipal landfill sites are eagerly visited by birds due to large share of organic waste in the total waste mass deposited on the landfill. A low level of waste sorting in Poland leads to fast filling up active landfills. An excessive quantity of organic wastes, which could have been composted, find their way to landfills as unsorted mixed municipal wastes. Birds not only scavenge on the food remnants deposited on the landfill but also spread refuse beyond the landfill boundaries.

Table 4

Bird predation on sprouted horse bean plants in the zone of the effect of the solid waste landfill in Tarnow

Date	Number of birds [heads/plot]								
	objects								
	S		E		N		W		Z
	I	II	I	II	I	II	I	II	
2006									
Morning	0.0	0.0	5	1.25	6.75	8.25	0.0	0.0	8.5
Noon	0.0	0.0	5.25	7.75	0.5	1	0.0	0.0	0
Evening	0.0	0.0	6.5	4.25	4.75	5	0.0	0.0	15.8
2007									
Morning	0.0	0.0	9.25	6.5	10.5	8.75	0.0	0.0	7.75
Noon	0.0	0.0	10	10.5	12.3	8	0.0	0.0	7.25
Evening	0.0	0.0	0	0	6.5	5.75	0.0	0.0	5

Numerous birds gathered on the landfill site in Tarnow often sat on the plots with sprouting horse bean plants, sometimes causing total destruction of the whole plantation. The plantation localisation with respect to the active sector, where the birds gathered was also important. The longer the distance from the active sector, the lesser the exposure to damage caused by birds. The plots close to the active sectors are exposed to damage and should be protected against birds by means of suitable frightening devices, such as seed dressings or wire nets [6]. Birds may be also scarred off municipal landfills by eg birds of prey.

Conclusions

1. Close localization of the municipal landfill site favours gathering of birds on arable crops.
2. During plant sprouting birds may cause very serious injuries to horse bean plants.
3. Plantations of crops exposed to damage due to birds should be established far from active municipal landfills or protected against damage.

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SZKODLIWOŚĆ PTAKÓW W UPRAWACH ROLNICZYCH W BEZPOŚREDNIM SĄSIEDZTWIE SKŁADOWISKA ODPADÓW STAŁYCH W TARNOWIE

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Abstrakt: Badania nad szkodami powodowanymi przez ptaki w uprawach rolniczy przeprowadzono w sąsiedztwie składowiska odpadów komunalnych w Tarnowie. Ptaki występowały najliczniej na poletkach znajdujących się w bezpośrednim sąsiedztwie czynnego sektora składowiska. Ze składowiska przelatywały na poletka ze wschodzącymi roślinami bobiku, powodując częściowe uszkodzenie rośliny lub całkowite jej zniszczenie. Straty powodowane przez ptaki mogą być bardzo duże, w niektórych przypadkach doszło do całkowitego zniszczenia uprawy. Uprawa roślin, które są atrakcyjnym pokarmem dla ptaków, jest obarczona dużym ryzkiem w pobliżu składowisk odpadów komunalnych.

Słowa kluczowe: ptaki, składowisko odpadów komunalnych, uszkodzenia

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POTENTIAL OF HYDROGEL APPLICATION FOR PLANT PROTECTION

MOŻLIWOŚĆ ZASTOSOWANIA HYDROŻELI W OCHRONIE ROŚLIN

Abstract: Hydrogels are more and more commonly used in agriculture, among others as preparations increasing the substratum water capacity and improving the soil structure. They may be also utilized as fertilizer and pesticide carriers. The latter possibility was the subject matter of the present research. Hydrogel was used for making a biopreparation containing *Beauveria bassiana* fungus. The gel preparation was less effective at low temperatures than an insecticide, but at the temperatures of 25 and 30^o it was acting faster.

Keywords: hydrogel, *Leptinotarsa decemlineata*, insecticides, *Beauveria bassiana*

Climate changes contribute to long lasting drought periods, which are interrupted by downpours causing water runoff from fields carrying fertilizers and plant protection chemicals. One of the methods of reducing water deficit may be the application of soil supplements causing an increase in soil retention and at the same time improving the soil structure and counteracting water and air erosion. Polymer soil supplements belong to a group of such products [1, 2].

Superabsorbents (hydrogels), characterized by extensive water absorptiveness, mixed with soil, increase its water capacity [3], counteract water stresses by ensuring moisture for plants but also by reducing water evaporation from soil. They counteract rapid changes of the soil moisture by acting as a water buffer. During irrigation or rainfall they bind water preventing its seeping into deeper soil layers and surface runoff [4]. The results mentioned above are observed already at small doses of dry supersorbent mixed with the substratum in the proportion of 0.05–0.5 %. Plants are able to use over 90 % of water retained by a supersorbent. As a result of giving up water to plants it shrinks causing empty places in the soil. Owing to its ability to swell and shrink several times, it improves soil structure causing its loosening and aeration [5–9].

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Superabsorbents contribute to protection of surface waters and the environment through utilization of their ability to immobilize fertilizer components, herbicides and pesticides. Chemical compounds trapped in a polymer net cannot be fast washed out by water from rainfalls or irrigation. Small quantities of these compounds released successively may be efficiently utilized by plants or protect the plants against pests and destroy weeds.

Positive results of hydrogen applications have been registered in the mountain areas, on escarpments and embankments, where they prevent water erosion. They are widely used for degraded areas reclamation, particularly in places where vegetation which would make revegetation, bush planting or afforestation of the area possible, was completely destroyed. They are also ingredients of natural and artificial substrata used both in gardening and reclamation [10].

Among numerous applications of hydrogels one should mention their use for coating and conditioning of seeds. They not only ensure constant moisture but are also a matrix for fertilizer component and plant protection means crucial for proper seed germination and development of plants. In this way they minimize losses of seeding material and play a part in the environmental protection. Also roots of plants planted with uncovered root systems are covered with hydrogels, which prevents their overdrying and reduces the number of seeds falling out after planting.

Hydrogels are also used as pesticide carriers. Research was conducted on Horse Chestnut Leaf-miner (*Cameraria ohridella*) control using a chemical preparation in the form of a gel. Research is conducted on hydrogel application as a carrier in fungal preparations used for combating insect pests. In this case gel is a carrier of spores or mycelium depending on the application method. A gel preparation containing *Beauveria bassiana* fungus spores was, among others, used for potato beetle (*Leptinotarsa decemlineata* Say.) larvae control. Further practical applications of agrogels still require extensive research. Ratajkiewicz et al [11] researched a potential application of Agrogel as an adiuvant at the use of Prefera and Mycotal mycoinsecticides for combating greenhouse white fly (*Trialeurodes vaporariorum*). However, the authors did not observe any positive effect of the Agrogel on the efficacy of these biopreparations. Studies have been also conducted on the use of gels for *Trichoderma* antagonistic fungi application [12].

Measurements of absorption pressure inform about the thermodynamic state of water in the hydrogel. Only a minimum part of water (about 2–4 cm^3/g) is beyond the range of the water available to plants. From the biological point of view it means that most of water stored in superabsorbents is available to plants. On the other hand, Hetman and Martyn [13] found that a supplement of Akrygel and Alcosob gels dosed 250–500 g kg^{-1} of horticultural substratum, apart from increasing usable water retention, also significantly raised unavailable water retention. Słowińska-Jurkiewicz and Jaroszuk [14] proved that in the water capacity of Hidroplus hydrogel, adsorptive water – unavailable to plants or hardly available, constituted the highest proportion.

With respect to chemistry, superabsorbents are networked, water insoluble polymers, usually based on acrylamide, acrylic acid or metacrylic acid and their derivatives. Other macromolecules, such as networked polyvinyl alcohol, polyethyleneglycols, poly-

-*N*-vinylpirolidone, copolymers of maleic anhydride and chemically modified copolymers based on starch and cellulose.

Currently acrylic polymers have the widest agricultural applications. These are compounds obtained as a result of polymerization of acrylamide, acrylic acid and its water soluble potassium, sodium and ammonium salts.

Two groups of polymers may be distinguished :

– water soluble linear polymers,

– network (mostly water insoluble) polymers.

Superabsorbents belong to network, water insoluble polymers. They are obtained through polymerization of monomers with divinyl substances, which bind single chains into a network.

Also not network (linear) polymers, water soluble polymers are produced, which find applications as flocculants in chemical and extraction industry, in sewage treatment plants and for ground sealing.

Practical applications of ionic polymers include also the use of anion polymers. Anion (carboxyl) groups increase hydrogel absorptiveness. Water is bound by hydrogen bonds between protons and oxygen atoms of carboxyl groups. A nonionic polymer group is characterized by a considerably smaller absorptivity but it is more resistant to ions in water. Water fixation happens here using hydrogen bonding between nitrogen atoms or oxygen with hydrogen.

The work aimed to study the potential of superabsorbent application to use entomopathogenic fungi against crop pests.

Material and methods

A laboratory experiment was conducted to compare the efficacy of the tested fungal preparations and chemical insecticides. Two strains of entomopathogenic fungus *Beauveria bassiana* – Bb 57 and Bb 64 were used. The fungus was cultured under laboratory conditions on a modified glucose and potato medium (instead of agar a polyacrylic gel with an addition of cellucotton was used). After four weeks of culturing at the temperature of 25 ^{o}C distilled water was poured on the Petri dishes and then the spore suspension was decanted. Spore concentration in the suspension was assessed in Bürker chamber using the methods described by Lipa and Śliżyński [15]. The tested chemical insecticide was Decis 2.5 EC in the concentration of 0.083 %, which corresponds to a dose of 0.251 dm^3/ha when 300 dm^3 of usable liquid is used. In order to prepare fungal preparation water suspension of *B. bassiana* spores was mixed with an appropriate amount of polyacrylamide gel (Agroaquagel). The gel was specially prepared for application in sprayers, finely dispersed to prevent clogging fog nozzles. The gel was added to spore suspension in the dose of 0.5 g/dm^3. The dose of *B. bassiana* spores in the preparations was – $5 \times 10^5/cm^3$. The test insects were also sprayed with a spore water suspension in the same concentrations as the gel preparation used. The control insects were sprayed with distilled water.

The test insects were larvae (L_1, L_2, L_3 and L_4) and imago of potato beetle (*Leptinotarsa decemlineata* Say.). The larvae and beetles had been earlier collected on potato fields. After their supply to the laboratory they were fed with potato leaves. The experiment was conducted in Petri dishes with the 100 mm diameter. The dishes were lined with three layers of filtration paper. 10 potato beetle specimens at respective stages of development were placed in the dishes. Decis 2.5 EC and fungal preparations were applied by means of a hand sprayer. The experiment was conducted in four replications. After the spraying, the dishes with insects were put into a thermostat and kept at the temperature of 15, 20, 25 and 30 ^{o}C. The mortality of the test insects was checked every day for 7 days. The larvae and beetles were fed potato leaves throughout the experiment.

Results and discussion

Hydrogels find numerous applications in agriculture. They are, among others, applied as a supplement to substrata to increase their water capacity and prevent fast overdrying. Hydrogels are often used for establishing lawns. Recently research has been conducted on the potential use of hydrogels for establishing osier plantations for energy generation. Superabsorbents may prove useful for application of both chemical and biological preparations for plant protection. Some research focuses on gel use for making fungal preparations for crop pest combating. Results of such studies were presented below.

The mortality rate of the L_1 potato beetle treated with fungal preparations and insecticides was presented in Table 1. In the case of Decis 2.5EC insecticide high efficiency was registered at the temperatures of 15 and 20 ^{o}C. At 30 ^{o}C the preparation did not kill all potato beetle specimens during 7 days of the experiment. Lesser efficacy of fungal preparations was observed at lower temperatures, particularly when the fungus was applied as a water spore suspension. The fungal preparation with gel addition caused the death of all L_1 larvae at the temperatures of 20–30 ^{o}C. More pronounced differences in the efficiency of tested preparations were observed while analyzing the average life span of L_1larvae. The efficacy of Decis 2.5EC insecticide was very high at the temperatures of 15 and 20 ^{o}C. On the other hand at 25 ^{o}C the test insect life was considerably prolonged. A similar relationship was observed for L_2 larvae (Table 2), L_3 (Table 3), L_4 (Table 4) and potato beetle imago (Table 5). Diminishing efficiency of synthetic pyretroids at the temperatures above 20 ^{o}C is well known. The instructions for the use of this preparation clearly recommend its application at temperatures below 20 ^{o}C. There are preparations whose activity does not depend on the ambient temperature, eg Actara, but pyretroids are still among the most commonly used plant protection means. However, fungal preparations were far more efficient at higher temperatures than at lower ones. If at the temperatures of 15 and 20 ^{o}C the insecticide killed the test insects 3–5 times faster than fungal preparations, at 30 ^{o}C it acted more slowly than fungal preparations. A comparison of the effect of *B. bassiana* spore water suspension and gel preparation containing fungus spore showed a better efficacy of the gel preparation in killing larvae and imago of potato beetles. The chemical preparation effectively combated potato beetles at all tested stages of development. Similar fungal preparations affected both the larval and imago stages. In the case of potato beetles younger larval stages should be controlled before the beetle causes considerable yield losses. The beetles may be also combated at the moment of their invasion on a potato

Table 1

Mortality rate of potato beetle L_1 larvae treated with fungal preparation containing *B. bassiana* fungus spore

Temperature [°C]	Preparation					
	Control	Decis 2,5 EC	Fungus strain			
			Bb 57		Bb 64	
			Water suspension	Gel preparation	Water suspension	Gel preparation
Test insect mortality rate [%]						
15	0.0 a*	100.0 e	75.0 b	85.0 c	77.5 b	90.0 cd
20	0.0 a	100.0 e	77.5 b	100.0 e	95.0 de	100.0 e
25	0.0 a	100.0 e	100.0 e	100.0 e	100.0 e	100.0 e
30	0.0 a	100.0 e	95.0 de	100.0 e	100.0 e	100.0 e
Average life span of test insects [days]						
15	7.0 l	1.0 a	5.4 k	4.3 i	5.35 k	4.45 i
20	7.0 l	1.0 a	4.68 j	3.1 f	4.0 h	3.35 g
25	7.0 l	2.85 de	3.05 ef	2.03 b	2.8 d	2.05 bc
30	7.0 l	4.73 j	3.9 h	2.0 b	3.2 fg	2.25 c

* means marked with the same letter do not differ significantly at p = 0.05

Table 2

Mortality rate of potato beetle L_2 larvae treated with fungal preparation containing *B. bassiana* fungus spore

Temperature [°C]	Preparation					
	Control	Decis 2,5 EC	Control			
			Bb 57		Bb 64	
			Water suspension	Gel preparation	Water suspension	Gel preparation
Test insect mortality rate [%]						
15	0.0 a*	100.0 g	67.5 b	95.0 f	75.0 c	100.0 g
20	0.0 a	100.0 g	82.5 d	100.0 g	87.5 e	100.0 g
25	0.0 a	100.0 g	100.0 g	100.0 g	100.0 g	100.0 g
30	0.0 a	100.0 g		100.0 g	100.0 g	100.0 g
Average life span of test insects [days]						
15	7.0 j	1.0 a	5.6 i	4.1 g	5.45 i	4.1 g
20	7.0 j	1.0 a	4.2 g	3.0 d	4.2 g	3.25 e
25	7.0 j	2.75 c	2.9 cd	2.2 b	2.7 c	2.3 b
30	7.0 j	4.65 h	3.5 f	2.85 cd	3.4 ef	2.4 b

* means marked with the same letter do not differ significantly at p = 0.05

Table 3

Mortality rate of potato beetle L_3 larvae treated with fungal preparation containing *B. bassiana* fungus spore

Temperature [°C]	Preparation					
	Control	Decis 2,5 EC	Fungus strain			
			Bb 57		Bb 64	
			Water suspension	Gel preparation	Water suspension	Gel preparation
Test insect mortality rate [%]						
15	0,0 a*	100.0 g	65.0 b	90.0 ef	67.5 b	95.0 fg
20	0,0 a	100.0 g	85.0 de	100.0 g	82.5 cd	100.0 g
25	0,0 a	100.0 g	100.0 g	100.0 g	100.0 g	100.0 g
30	0,0 a	77.5 c	85.0 de	100.0 g	100.0 g	100.0 g
Average life span of test insects [days]						
15	7,0 l	1.0 a	5.65 k	4.25 hi	5.6 k	4.25 hi
20	7,0 l	1.0 a	4.05 gh	3.0 de	4.38 i	3.25 ef
25	7,0 l	2.83 cd	2.9 cd	2.2 b	2.7 c	2.3 b
30	7,0 l	4.68 j	3.9 g	2.85 cd	3.4 f	2.4 b

* means marked with the same letter do not differ significantly at p = 0.05

Table 4

Mortality rate of potato beetle L_4 larvae treated with fungal preparation containing *B. bassiana* fungus spore

Temperature [°C]	Preparation					
	Control	Decis 2,5 EC	Fungus strain			
			Bb 57		Bb 64	
			Water suspension	Gel preparation	Water suspension	Gel preparation
Test insect mortality rate [%]						
15	0,0 a*	100.0 e	70.0 b	97.5 e	80.0 c	100.0 e
20	0,0 a	100.0 e	82.5 cd	100.0 e	87.5 d	100.0 e
25	0,0 a	100.0 e	100.0 e	100.0 e	100.0 e	100.0 e
30	0,0 a	80.0 c	85.0 cd	100.0 e	97.5 e	100.0 e
Average life span of test insects [days]						
15	7,0 l	1.0 a	5.55 k	4.55 i	4.95 j	4.1 gh
20	7,0 l	1.0 a	4.2 h	3.5 f	3.95 g	3.25 e
25	7,0 l	2.75 cd	2.9 cd	2.2 b	2.7 c	2.3 b
30	7,0 l	4.58 i	3.9 g	2.98 d	3.4 ef	2.4 b

* means marked with the same letter do not differ significantly at p = 0.05

Table 5

Mortality rate of potato beetle imago treated with fungal preparation containing *B. bassiana* fungus spore

Temperature [^{o}C]	Preparation					
	Control	Decis 2,5 EC	Fungus strain			
			Bb 57		Bb 64	
			Water suspension	Gel preparation	Water suspension	Gel preparation
Test insect moratlity rate [%]						
15	0,0 a*	100.0 g	60.0 b	97.5 fg	70.0 c	100.0 g
20	0,0 a	100.0 g	85.0 g	100.0 g	90.0 de	100.0 g
25	0,0 a	100.0 g	100.0 g	100.0 g	100.0 g	100.0 g
30	0,0 a	85.0 d	92.5 ef	100.0 g	100.0 g	100.0 g
Average life span of test insects [days]						
15	7,0 k	1.0 a	5.75 j	4.0 h	5.55 j	4.1 h
20	7,0 k	1.0 a	4.13 h	3.0 de	4.1 h	3.25 ef
25	7,0 k	2.85 cd	2.9 cd	2.2 b	2.7 c	2.3 h
30	7,0 k	4.43 i	3.65 g	2.85 cd	3.4 fg	2.4 b

* means marked with the same letter do not differ significantly at $p = 0.05$

plantation. Studies on agrogel application have been also undertaken in other research centres. Ratajkiewicz et al [11] used agrogel dosed 0.4 % as an adiuvant at mycoinsecticides Preferal and Mycotal application for greenhouse white fly combating. Research conducted by these authors did not corroborate a positive effect of agrogel addition to biopreapartion on their efficacy. It shows the necessity for further research aimed at developing of both the technique of hydrogel application and new hydrogels.

The potato beetle remains one of the most serious potato pests [16], therefore seeking new methods of its combating is fully justified. According to Pruszyński and Węgorek [17] chemical protection plays and will play the main role in potato beetle control. However, the obtained results point to potential practical application of fungal preparations for this pest combating.

Conclusions

1. The efficacy of synthetic Decis 2.5EC insecticide preparation is much stronger than *B. bassiana* fungal preparations at the temperatures of 15 and 20 ^{o}C.
2. At the temperatures of 25 and 30 ^{o}C *B. bassiana* fungal preparations revealed better efficiency in killing potato beetle.
3. Gel formula of the fungal preparation was more efficacious in comparison with *B. bassiana* spore water suspension.

Acknowledgement

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MOŻLIWOŚĆ ZASTOSOWANIA HYDROŻELI W OCHRONIE ROŚLIN

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Abstrakt: Hydrożele są coraz powszechniej stosowane w rolnictwie m.in. jako preparaty zwiększające pojemność wodną podłoża i poprawiające strukturę gleby. Można je również wykorzystywać jako nośnik nawozów i pestycydów. Ta ostatnia możliwość była przedmiotem niniejszych badań. Hydrożel wykorzystano do przygotowania biopreparatu zawierającego grzyba *Beauveria bassiana*. Preparat żelowy był w niskich temperaturach mniej skuteczny niż insektycyd, ale w temperaturach 25 i 30 °C działał szybciej.

Słowa kluczowe: hydrożel, *Leptinotarsa decemlineata*, insektycydy, *Beauveria bassiana*

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Magdalena SENZE[1] and Monika KOWALSKA-GÓRALSKA[1]

NICKEL AND CADMIUM IN BOTTOM SEDIMENTS OF THE SLUP AND LUBACHOW DAM RESERVOIRS (LOWER SILESIA PROVINCE)

NIKIEL I KADM W OSADACH DENNYCH ZBIORNIKÓW ZAPOROWYCH SŁUP I LUBACHÓW (WOJEWÓDZTWO DOLNOŚLĄSKIE)

Abstract: Bottom sediments from dam reservoirs: Slup and Lubachow were studied. The concentration of nickel and cadmium was determined. The bottom sediments in the Lubachow reservoir were found to have accumulated more nickel (k = 23373) and in the Slup reservoir – cadmium (k = 2259). As a general rule, metal concentrations were the lowest in the central parts of the reservoirs.

Keywords: nickel, cadmium, dam reservoirs, bottom sediments, water

The composition of bottom sediments is a resultant not only of the catchment basin structure, but also local meteorological conditions (dry and wet deposition), natural biological, chemical and physical processes, and anthropogenic factors. Metals present in dam reservoirs come from atmospheric precipitation and from inflows from direct and indirect catchment basins. In the so-called "working" dam reservoirs, where due to demand for water its flow is regulated, the bottom (bottom sediments) often comes into direct contact with atmospheric air, ie with the pollutants present in it. Water level regulation in reservoirs provided with a bottom discharge spout (the reservoirs at Slup and Lubachow) results in the outflow of the active, surface layer of the sediment and its accumulation while the reservoir is being filled. In such circumstances the quantitative composition of the sediments may be subject to change, and the quantity and the nature of its chemical compounds may vary [1].

The two reservoirs Slup and Lubachow in question are used as a source of drinking water and water used for household purposes. This was reason of chemical analysis of their bottom sediments. An attempt was also made to determine the accumulation rates for Ni and Cd in the bottom sediments.

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Materials and methods

The Lubachow dam reservoir was built in 1917 on the Bystrzyca River, 75 km from its source. The reservoir and its engineering structures are not subject to direct protection, although drinking water is drawn from here for the towns of Dzierzoniow, Bielawa and Pieszyce. The facility is also used for flood protection, power generation and recreation [2]. Its direct catchment is made up by mountains, rocky terrain, afforested areas, and recreational areas.

The Slup reservoir was built in 1986 across the Nysa Szalona river valley 8.2 km from the river source. The reservoir bowl and all of the hydro-engineering structures lie within a direct protection zone, because they are used to gather drinking water for the city of Legnica. The reservoir also performs a flood control function [3]. Its direct catchment is constituted by arable land, meadows, pastures and a small forest area. The morphometric features of reservoirs are in Table 1.

Table 1

Morphometric characteristics of the Slup and Lubachow dam reservoirs

Reservoir parameter	Slup reservoir	Lubachow reservoir
Average depth [m]	8.00	15.70
Maximum depth [m]	18.05	36.00
Length [km]	2.90	3.50
Volume [million m^3]	31.52	8.00
Area ha	408.00	51.00

Results and discussion

Nickel concentrations in the bottom sediments of the Slup reservoir ranged from 22.52 mgNi · kg^{-1} at site 3 to 66.32 mgNi · kg^{-1} also at site 3 (Table 2). The minimum concentration recorded at Lubachow amounted to 29.62 mgNi · kg^{-1} (site 2) and the maximum to 36.99 mgNi · kg^{-1} (site 1) (Table 2). The horizontal profile of the Slup reservoir showed, just like in the case of copper, an increase in nickel concentration (from 40.91 mgNi · kg^{-1} at site 1 to 51.38 mgNi · kg^{-1} at site 3), accompanied by a rising accumulation rate (k). Such trend was not noticed for the bottom sediments at Lubachow. Here the average values at individual sites were similar, and even a slight falling tendency in respect of the horizontal profile was observed. The highest accumulation rate for nickel at Lubachow was at site 1 (k = 23373); it dropped further along the water course to k = 10010 in the centre, and then grew to 18678 near the dam.

Nickel concentration in the bottom sediments of the Goczalkowice reservoir was from 3.00 mgNi · kg^{-1} to 170.00 mgNi · kg^{-1} [4–6]. A slightly narrower range was found for the Kozlowa Gora reservoir, which was studied by Pasternak and Glinski [4] and Reczynska-Dutka [7], where nickel concentration fell within the range: 5.00–28.40 mgNi · kg^{-1}. The corresponding figures for the Rybnicki reservoir were: 19.90–38.37

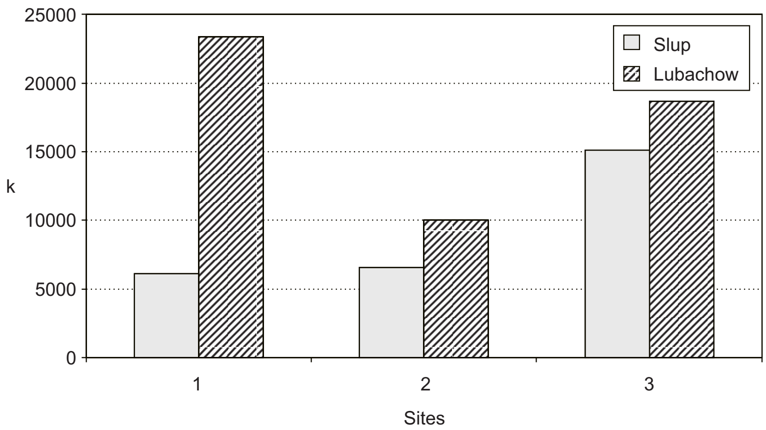

Fig. 1. Rate of accumulation nickel in bottom sediments

mgNi · kg^{-1} [8, 9]. The values were similar to those given for many years for bottom sediments from various lakes [10–12].

Table 2

Content of nickel and cadmium in bottom sediments

Sites (number)		Slup		Lubachow	
		Ni	Cd	Ni	Cd
1	x_o	40.91	0.52	35.06	1.08
	min	30.45	0.18	31.59	0.79
	max	61.52	1.06	36.99	1.54
	x_w	0.0067	0.0004	0.0015	0.0009
2	x_o	40.82	0.54	31.03	1.31
	min	34.50	0.05	29.62	1.25
	max	49.78	0.96	31.80	1.39
	x_w	0.0062	0.0004	0.0031	0.0053
3	x_o	51.38	0.68	33.62	1.16
	min	22.52	0	29.96	0.98
	max	66.32	1.31	36.41	1.39
	x_w	0.0034	0.0003	0.0018	0.0008

x_o – mean content in bottom sediments [mg · kg^{-1}]; x_w – mean content in above bottom water [mg · dm^{-1}].

Cadmium concentrations in the bottom sediments of the Slup reservoir were from below the determination threshold at site 3 up to 1.31 mgCd · kg^{-1} also at site 3 (Table 2). Very similar figures were found at Lubachow (from 0.79 mgCd · kg^{-1} at site 1 to 1.54 mgCd · kg^{-1} also at site 1). In the Slup reservoir cadmium concentrations rose in the horizontal profile from site 1 (on average 0.52 mgCd · kg^{-1}) to site 3 (on average 0.68 mgCd · kg^{-1}). Accumulation rate also rose: from k = 1301 (site 1) to

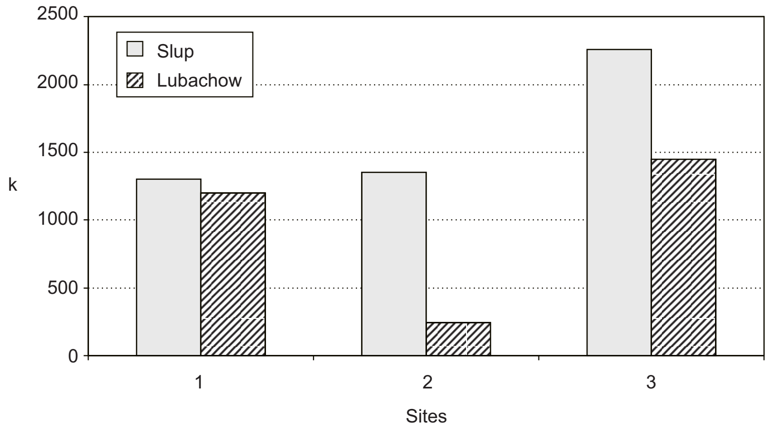

Fig. 2. Rate of accumulation cadmium in bottom sediments

k = 2259 (site 3). The changes in cadmium concentration at Lubachow, similarly to nickel, were quite small, and along the main water flow cadmium accumulated unevenly. The lowest accumulation rate (k = 247) was found in the central part of the reservoir (site 2). Of note is the fact that in both reservoirs Cd was the weakest accumulator of nickel at all sites and its accumulation rate at site 2 at Lubachow was the lowest for any metal recorded during the research.

The cadmium concentrations found by Szarek-Gwiazda [13] in the Dobczycki reservoir ranged from 0.50 mgCd $\cdot$ kg^{-1} to 1.20 mgCd $\cdot$ kg^{-1}. Cadmium concentrations at Kozlowa Gora were much higher, from 3.20 mgCd $\cdot$ kg^{-1} to 22.70 mgCd $\cdot$ kg^{-1} [7], and at Goczalkowice from 0.00 mgCd $\cdot$ kg^{-1} to 18.00 mgCd $\cdot$ kg^{-1} [5]. Similar concentrations were recorded for cadmium in the bottom sediments of the Rybnicki reservoir (from 2.75 mgCd $\cdot$ kg^{-1} to 13.10 mgCd $\cdot$ kg^{-1}) [9]. Bottom sediments sampled from numerous stagnant water reservoirs throughout Poland are characterized by concentrations similar to those found at Slup and Lubachow [7, 10, 11, 14].

Conclusions

Nickel accumulated more intensely in the Lubachow dam reservoir, whereas cadmium – at Slup. At Slup nickel and cadmium accumulated more intensely at site No. 3 in front of the dam . At Lubachow cadmium accumulated more intensely at site No. 3 and nickel at No. 1 the backwater area. Metals accumulated most weakly (the lowest accumulation rate k) in the central part of the reservoir bowls.

It should be made a regular control of level metals in bottom sediment because reservoirs are used as a source of drinking water.

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NIKIEL I KADM W OSADACH DENNYCH ZBIORNIKÓW ZAPOROWYCH SŁUP I LUBACHÓW (WOJ. DOLNOŚLĄSKIE)

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Abstrakt: Przeprowadzono badania osadów dennych zbiorników zaporowych Słup i Lubachów. Określono zawartość niklu i kadmu. Zaobserwowano większą kumulację niklu w osadach dennych ze zbiornika zaporowego Lubachów (k = 23373), a kadmu w osadach dennych zbiornika Słup (k = 2259).

Generalnie metale najsłabiej kumulowały w centralnej części zbiorników.

Słowa kluczowe: nikiel, kadm, zbiorniki zaporowe, osady denne, woda

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Paweł ZADROŻNY[1] and Paweł NICIA[1]

HEAVY METALS IN THE PEAT SOILS OF THE KONECKI COUNTY

METALE CIĘŻKIE W GLEBACH TORFOWYCH POWIATU KONECKIEGO

Abstract: The peat soils, like other hydrogenic soils, accumulate heavy metals in a much higher amount than mineral soils. Hight organic matter content in peat soils favours accumulation of heavy metals. The research aimed at determining the degree of accumulation of selected heavy metals (Cd, Pb and Zn) in peat soils located in the area of the Konecki county (Staropolski Industrial Region). The research results demonstrated that location of the analyzed peat bogs influenced heavy metal concentrations. The lowest contents of the analyzed heavy metals were characteristic of the soils of the peat bog situated at the longest distance from potential sources of pollution.

Keywords: heavy metals, peat bog soils

Natural environmental conditionings of the present Konecki county area – extensive forests and shallow lying iron ores caused that from the 2nd century BC this area constituted an important part of a huge iron metallurgy basin [1]. Technological progress, particularly the use of the water wheel dated back to the 12th century AD, caused rapid development of metallurgy and iron manufacturing. The manufactures, ironworks established at that time in this area led to a considerable development of the Staropolski Industrial Region, so that in the second half of the 16th century this region became the greatest metallurgical center on the Polish territories. Out of 320 ironworks operating at that time on the territory of Poland, as many as 142 operated in the sandomierskie province, to which the areas of the present Konecki county belonged. Moreover, a large center of glass manufacturing existed at the turn of the 16th and 17th century in the region of the Koneckie Hills [2].

Accumulation of heavy metals in soils is determined to a great extent by organic matter presence in them [3]. In hydrogenic soils, among which peat soils are counted, accumulation of these components is much higher than in mineral soils [4–7].

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Rapid development of ironworks and metallurgy in the 19th and the 20th century contributed to a considerable increase in the emission of pollutants supplying to the environment substantial quantities of heavy metals, of which a major part is deposited in soils [8, 9].

The research aimed at determining the degree of accumulation of selected heavy metals (Cd, Pb and Zn) in peat soils located in the area of the Staropolski Industrial Region.

Material and methods

The analyses comprised five peat bogs situated in the area of the Konecki county. The following peat bogs were considered: Pod Stawami Rybnymi, Las Zbojno, Obok Lasu Zbojno, Kolonia Deba i Lesnictwo Zalesie. Soil material representing all determined genetic horizons of individual soils was collected from one soil pit on each peat bog.

The following assessments were made in the soil material: the total content of carbon (C_{tot}) and nitrogen (with the calcination method at 550 ^oC, in the TOC 1200C/N apparatus), reaction (pH) determined in H_2O and KCl (using an electrometric method by the CP-135 pH-meter), the contents of cadmium, lead and zinc after former soil mineralization in a mixture of concentrated nitric(V) and chloric(VII) acids (2:1), using the AAS method (PU910 atomic absorption spectrophotometer).

Results and discussion

The analyzed soils were classified as transitory peat soils (Las Zbojno, Kolonia Dęba), low peat soils (Obok Lasu Zbojno) and peat-muck soils (Pod Stawami Rybnymi and Leśnictwo Zalesie) [10].

The researched soils revealed a very acid and acid reaction. The pH values assessed in H_2O fluctuated from 3.3 to 5.8 and in KCl from 2.4 to 5.6. In most of the analyzed soils the highest pH values were determined in the horizons situated within the reach of groundwater, which may be the result of mineral components (Ca^{2+} and Mg^{2+}) presence, which neutralize acid products of organic matter decomposition.

Total contents of carbon and nitrogen in the organic horizons of the investigated soils ranged from 101.3 to 511.5 $g \cdot kg^{-1}$ (C_{tot}) and 2.2–9.2 (N_{tot}). In the underlying mineral levels the amounts of these elements were very high (C_{tot} – 21.9–31.2 $g \cdot kg^{-1}$, N_{tot} – 0.6–0.7 $g \cdot kg^{-1}$. The computed C_{tot}/N_{tot} ratio reached high values from 33 to 97.

Concentrations of individual heavy metals in the analyzed soils ranged as follows: Cd – from trace quantities to 2.00 $mg \cdot kg^{-1}$, Pb – 1.86–49.85 $mg \cdot kg^{-1}$, Zn – 4.50–205.35 $mg \cdot kg^{-1}$. The amounts of these elements revealed considerable diversification within the analyzed soil profile. In a majority of the researched soils the highest content of the analyzed elements was assessed in the surface horizons (Table 1). These values are only in some cases slightly higher than those stated by Gorlach and Gambus [9] as the “natural” content in soil.

Table 1

Changes of heavy metal concentrations in the surface and underlying horizons of the analyzed soils and accumulation coefficients

Horizons	Cd	Pb	Zn
	mg · kg^{-1}		
Surface organic	0.45–1.25	17.51–46.20	29.01–99.65
Organic lying on mineral substratum	0.11–0.31	2.30–20.65	13.55–205.35
Underlying mineral	tr.–0.15	2.30–4.91	4.50–14.60
WA	1.45–11.36	(0.85) 3.66–6.76	(0.22) 2.24–5.46

The assessment of the amount of pollution with the analyzed heavy metals conducted on the basis of their allowable values in soil surface horizons [11] revealed that the analyzed soils meet the requirements for the soils in protected areas, classified in group A.

The contents of the analyzed elements in the surface horizons of the studied soils were definitely lower than the contents assessed in the analogous horizons of organic eutrophic [4, 6, 7] and oligotrophic [5] mountain fen soils.

Accumulation coefficients (WA) computed on the basis of individual heavy metal quantities in the surface horizons and the lowest organic horizons revealed that surface enrichment in these elements occurred in a vast majority of the studied profiles (Table 1). This enrichment, doubtlessly of anthropogenic character, most likely results from deposition of the analyzed elements from the polluted atmosphere. Only the Pb content in the soil surface horizons of Kolonia Deba peat bog (WA = 0.85) and Pb concentration in the soils of Lesnictwo Zalesie peat bog (WA = 0.22) were lower than the quantities of these elements in organic horizons lying immediately on the underlying mineral substratum.

The lowest contents of all analyzed heavy metals in the whole profiles were assessed in the peat bog Pod Stawami Rybnymi, which may result from the longest (of all analyzed objects) distance from potential pollutant emission sources.

Conclusions

1. The contents of the analyzed heavy metals in the studied peat soils of the Konecki county varies from the amount of these elements considered as natural contents only to a slight degree.

2. Soils of the majority of the investigated peat bogs are subjected to anthropogenic enrichment in Cd, Pb and Zn originating from pollutant deposition from the atmosphere, as it has been demonstrated by computed accumulation coefficients.

3. Location of the analyzed peat bogs influenced heavy metal concentrations. The lowest contents of the analyzed heavy metals were characteristic of the soils of the peat bog situated at the longest distance from potential sources of pollution.

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METALE CIĘŻKIE W GLEBACH TORFOWYCH POWIATU KONECKIEGO

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Abstrakt: Gleby torfowisk, podobnie jak inne gleby organiczne, są w większym stopniu narażone na zanieczyszczenie metalami ciężkimi niż gleby mineralne. Akumulacji metali ciężkich w glebach torfowych sprzyja duża zawartość materii organicznej. Celem przeprowadzonych badań była ocena zanieczyszczenia metalami ciężkimi (Cd, Pb, Zn) gleb torfowisk powiatu koneckiego. Przeprowadzone badania wykazały, że wpływ na zawartość metali ciężkich miała lokalizacja badanych torfowisk. Najmniejszymi zawartościami badanych metali ciężkich charakteryzowały się gleby torfowiska położonego w największej odległości od potencjalnych źródeł zanieczyszczeń.

Słowa kluczowe: metale ciężkie, gleby torfowe

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GERMINATION OF OAT SEEDS, CHARACTERIZED BY DECREASED VIGOUR, UNDER DROUGHT CONDITIONS

KIEŁKOWANIE ZIARNIAKÓW OWSA O OBNIŻONYM WIGORZE W WARUNKACH SUSZY

Abstract: In three series of experiments the effects of temperature and drought stress, the drought being simulated with polyethylene glycol (PEG) solution (8000) (–1.5 MPa), on the capacity and dynamics of germination of two oat cultivars – naked Akt and husked Bajka, were determined. The results for imbibition evaluated at 5 °C and 10 °C indicated a greater rate of water uptake in the naked form, under the conditions of both drought and the control. Under the conditions of the control, at varied temperatures of 5, 10, 15 and 20 °C, germinability was found to be significantly lower, by 15 % on average, in the naked form. Under drought conditions no germination was observed, and the seeds kept subsequently at a temperature of 20 °C for 20 days showed normal germination only in the case of the naked form (4 %). The dynamics of germination, evaluated under drought conditions at 5 °C and 10 °C, as well as after placing the seeds between layers of blotting paper moistened with water, the temperature being 10, 15 and 20 °C, was significantly higher in naked seeds, which may indicate higher resistance of that oat form to water deficiency in the substrate at the initial phase of germination.

Keywords: naked oat, husked oat, drought stress (PEG), impact of temperature, germinability, dynamics of germination

Germination modifies the potential for growth and development of a plant, and its course is strongly stimulated by external factors and seed characteristics. The start of the imbibition process, essential for activating the enzymes responsible for growth and development of the seed embryo, is conditioned, on the one hand, by the amount of water available from the substrate, and on the other – by the temperature range, most favourable for the course of the germination process [1]. Many plants, including oat, seem to be especially sensitive to water deficiency, during germination in early spring, when a low temperature of soil makes it impossible for them to use fully the over-winter

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soil water. Water stress occurring at that time may delay and decrease germination or totally stop that process [2]. In further stages of development the fibrous root system often suffers from water deficiency when spring frost occurs. Besides, cereal cultivars with husked and naked seeds differ significantly in the course of germination, which results from varied swelling rate [3]. In husked seeds the swelling phase is longer and germination, compared to naked seeds, is delayed. However, naked seeds are characterized by considerable susceptibility to mechanical damage during threshing and ennobling of the sowable material, the result of which is decreased germinability and accelerated ageing [4, 5].

The objective of the study was to determine the modifying effect of temperature and drought stress, induced by polyethylene glycol (PEG 8000), on germination of seeds of various botanical forms of oat, characterized by decreased vigour.

Material and methods

The material for the study included seeds of two oat (*Avena sativa* L.) cultivars: husked Bajka and naked Akt multiplied in observation experiments carried out at the Experimental Station of the Department of Plant Breeding and Seed Science in Prusy, in the year 2005. Harvest was performed when the seed moisture level was 15 % on average, Next, threshing was done, using a laboratory threshing machine, its rotary speed being set at 1.6 m/s. The seeds were stored under warehouse conditions.

Twenty-four months after harvest three series of experiments were carried out, during which drought conditions were simulated with the use of polyethylene glycol solution (PEG 8000), its concentration resulting in the osmotic potential of –1.5 MPa, determined according to Michel's formula [6]. In each experiment seeds were placed in Petri dishes (50 seeds per dish), between layers of blotting paper, and then 16 ml of PEG solution were added. Seed samples sown onto blotting paper moistened with water were the control. The determinations were done with three replications.

In the first experiments seeds were incubated at 5 ^{o}C and 10 ^{o}C for 6 days in the case of the control, and for 10 days in the case of seeds germinating under drought conditions. During that period, at 24-h intervals, the moisture content was determined using the drier method, and the start of the germination process, ie the occurrence of radicules above 2 mm long, was established.

In the second experiment seeds germinated under drought conditions at 5 ^{o}C, 10 ^{o}C, 15 ^{o}C and 20 ^{o}C. On the 10th day seed germinability was assessed according to ISTA methodology [7]. Then, after transferring the dishes with the seeds to 20 ^{o}C, for successive 20 days observations were done in respect of normally germinating seeds, abnormally germinating seeds, healthy but not germinating seeds, as well as moulding and rotting seeds.

In the third experiment, seeds, under drought conditions, were exposed to temperatures of 5 ^{o}C and 10 ^{o}C for 5 days, then they were rinsed with water and placed in Petri dishes onto a substrate containing water and kept at temperatures of 10 ^{o}C, 15 ^{o}C and 20 ^{o}C. For 5 successive days the dynamics of germination was evaluated using

Maguire's equation [8], the normally germinating seeds being removed from the substrate.

The results of the observation made a basis for carrying out three-factor variance analyses using the independent system. The testing was performed according to the fixed model. For statistical analysis the values expressed in percentage terms were transformed into Bliss's angle values, according to the formula $y = \arcsin\sqrt{x}$. In order to estimate the contribution of the specified sources of variability in the total variability of the investigated characteristics, the components of variance were estimated [9] and their percentage was given. The correlations between the characteristics were evaluated on the basis of the Pearson correlation coefficient or linear regression, or square regression. The values presented in Figures, expressed in percentage terms, were retransformed. For statistical calculations and diagrams Statistica 8.0 was used.

Results and discussion

The results for imbibition, evaluated in the first experiment, show that under the conditions of the control the rate of water uptake and the start of germination depended on temperature and the presence or absence of a seed husk. In the naked cultivar Akt the moisture content increased from 31 % to 43 % at a temperature of 5 °C, and from 34 % to 57 % at 10 °C (Fig. 1). In the husked cultivar Bajka, at a lower temperature the moisture content reached 33–44 %, while at 10 °C it ranged from 34 % to 52 %. The values of the direction components of regression equations show that in the naked form prolonged by 24 h incubation resulted in a faster increase in the moisture content (by

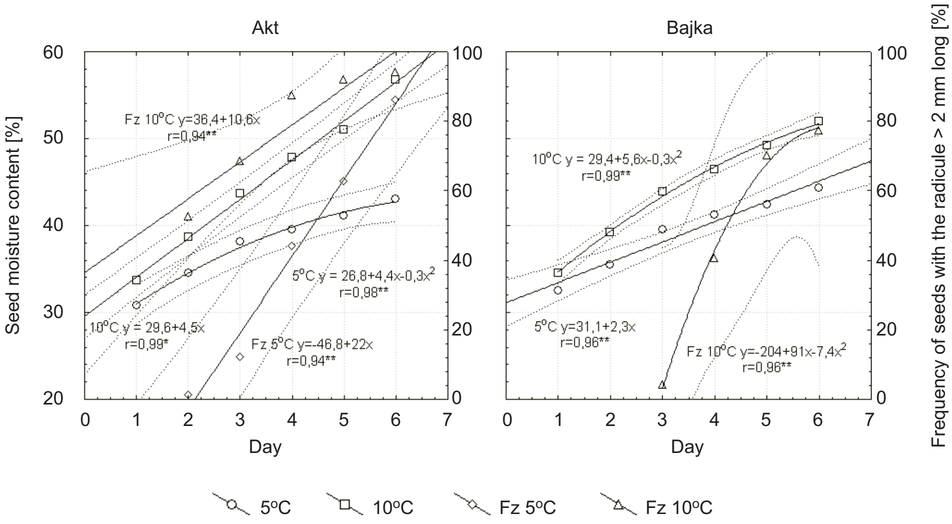

Fig. 1. Moisture content of Akt and Bajka seeds and the number of seeds with the radicule > 2 mm long subjected to imbibition at 5 °C and 10 °C under the conditions of the control (Fz – seed frequency) */** – significant at p = 0.05 and p = 0.01, respectively

4.3 % on average) compared with the husked form (3.8 %). Under the conditions of the control, first radicules, more than 2 mm long, appeared at 10 °C in the naked cultivar Akt (53 %), on the second day of the experiment, at the moisture content of 38 %, and in the husked cultivar Bajka (4 %) 24 h later, at the moisture content of 45 %. The prolonged, by 24 h, incubation caused a linear increase in the number of seeds with the radicule, its length exceeding 2 mm, which in the naked cultivar was higher at 5 °C (about 22 %). However, in the husked form the curvilinear increase in the number of such seeds was observed only at 10 °C. Thus, it seems that a naked form is better adapted to drought, at least at the beginning of the vegetation period, because water contained in the substrate, even at small amounts, has easier access to a seed and encounters no additional barrier, such as a husk. This is confirmed by the results obtained under the conditions of drought when the seed moisture content in the naked cultivar Akt changed from 25 % to 30 % at 5 °C and from 26 % to 31 % at 10 °C (Fig. 2.). In the husked cultivar, at a lower temperature the seed moisture content ranged from 26 % to 32 %, and at a higher temperature – from 27 % to 34 %, and it was of a rectilinear character. Under drought conditions the rate of water uptake in the naked cultivar, determined by the regression equation, was higher by 49 % on average, at a temperature of 10 °C. In the naked cultivar, in the presence of PEG, germination started on the sixth day (5 %) at a temperature of 10 °C and the moisture content of 29 %. The daily increase in the number of germinating seeds, in the cultivar Akt, was 9.7 %, and on the tenth day it finally reached 44 %. In the husked form single seeds with the radicule more than 2 mm long did not appear until the ninth day, at the temperature of 10 °C and the moisture content of 33 %. Similar results concerning the start of germination were observed by Mos et al [10] when studying the reaction of naked and

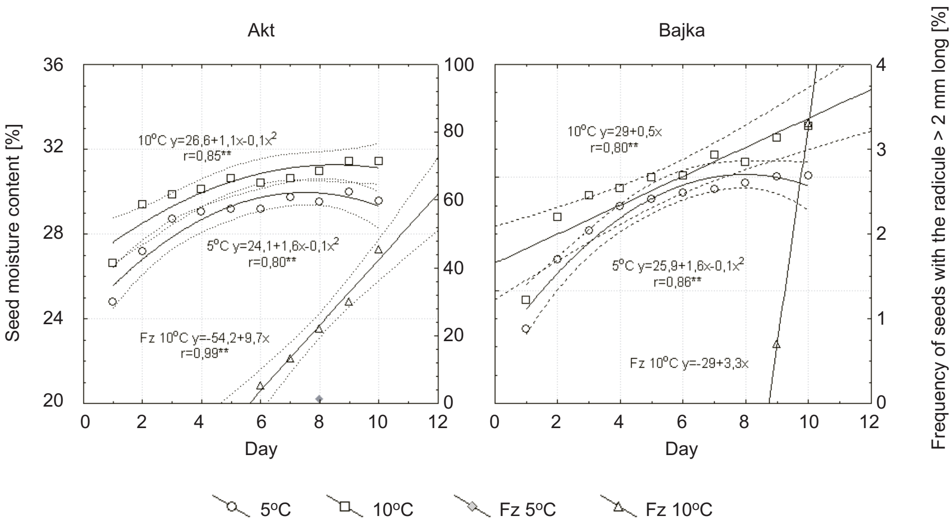

Fig. 2. Moisture content of Akt and Bajka seeds and the number of seeds with the radicule > 2 mm long subjected to imbibition at 5 °C and 10 °C under drought conditions (Fz – seed frequency). */** – significant at p = 0.05 and p = 0.01, respectively

husked oat cultivars subjected to the accelerated ageing test under the conditions of drought.

The components of variance calculated for the sources of variability determined in the second experiment showed that the greatest effect on the studied factors was exerted by drought conditions (more than 52 %) (Table 1).

Table 1

Significance of differentiation and the percentage of variance components for germinability, as well as the number of normally germinating, moulding and rotting seeds after 30 days of the experiment

Source of variability	Degrees of freedom	Germinability	Percentage of normally germinating seeds after 30 days	Percentage of moulding and rotting seeds
Temperature (A)	3	20.4**	1.3 ns	4.5**
Drought conditions (B)	1	52.8**	85.0**	26.3**
Cultivars (C)	1	0.6**	0.2*	29.6**
Interaction				
A × B	3	20.4**	0.7**	1.5*
A × C	3	0.2*	0.2 ns	0 ns
B × C	1	0.6**	4.2**	7.3**
A × B × C	3	0.2*	0 ns	2.8**
Error	32	4.7	8.3	28.0

* significant at $p < 0.05$; ** significant at $p < 0.01$; ns – insignificant.

The temperature of initial incubation, as well as the combined effect of drought conditions and temperature, had a considerable modifying share in the total variability for germinability (20.4 %). The percentage of moulding and rotting seeds depended most on the cultivar (29.6 %). Seeds stored for 24 month under warehouse conditions were characterized by decreased germinability which at an optimal temperature of 20 ^{o}C ranged from 58 % in the naked cultivar Akt to 76 % in the husked Bajka. In the seeds samples subjected to the action of PEG no germination in any of the cultivars was observed. Decreased germinability as a result of drought stress had already been observed by Hosnedl and Honsova [11] in barley, by Michalek and Borowski [2] in soybean, by Yildirim et al [13] in vegetables, by Dhanda et al [14] in wheat, and by Zurek [15] in grasses. The maximum osmotic potential used in those research works was –2.05 MPa. Mos et al [16] found that PEG 8000 concentration resulting in the osmotic potential of –1.5 MPa brings about the greatest differentiation in germination of oat genotypes characterized by high initial germinability. The results obtained in the present research work show that PEG concentration resulting in the osmotic potential of –1.5 MPa inhibited germination in both of the investigated oat forms characterized by decreased germinability. Normally germinating seeds could be noted only under the conditions of the control at a temperature exceeding 5 ^{o}C (Fig. 3). Germinability of the cultivar Akt was lower by 16 %, on average, as compared with the cultivar Bajka, irrespective of the temperature used. A rise in temperature of additional 5 degrees resulted in an increase in germinability of about 9 % in the husked form. Thus, the

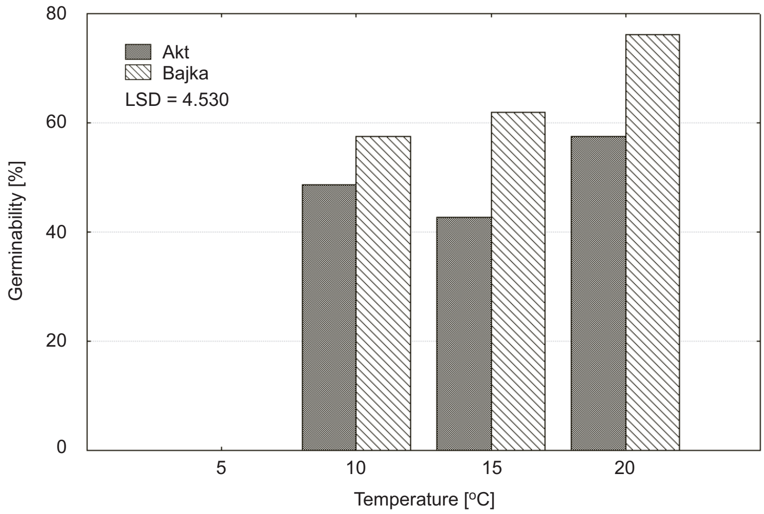

Fig. 3. Germinability of Akt and Bajka seeds under the conditions of the control, depending on temperature

decreased vigour of seeds of the investigated cultivars became apparent, especially in the naked form, which had already been observed in earlier experiments. The decreased vigour, according to Zurek [15], is an important factor limiting seed germination under drought conditions. In the case of the husked form, under the conditions of the control, transferring the seeds for successive 20 days to a temperature of 20 °C resulted in a significant, 20 % increase in the number of normally germinating seeds (Fig. 4). However, further incubation under drought conditions resulted in the occurrence of such

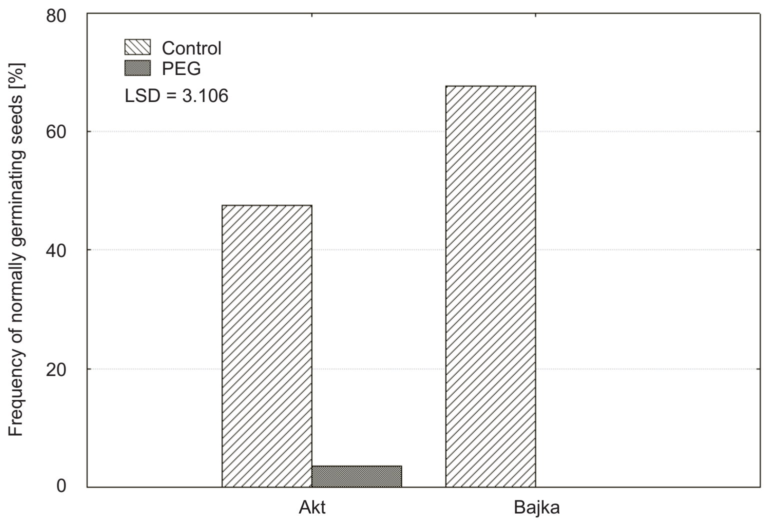

Fig. 4. The percentage of normally germinating seeds after 30 days, depending on drought conditions

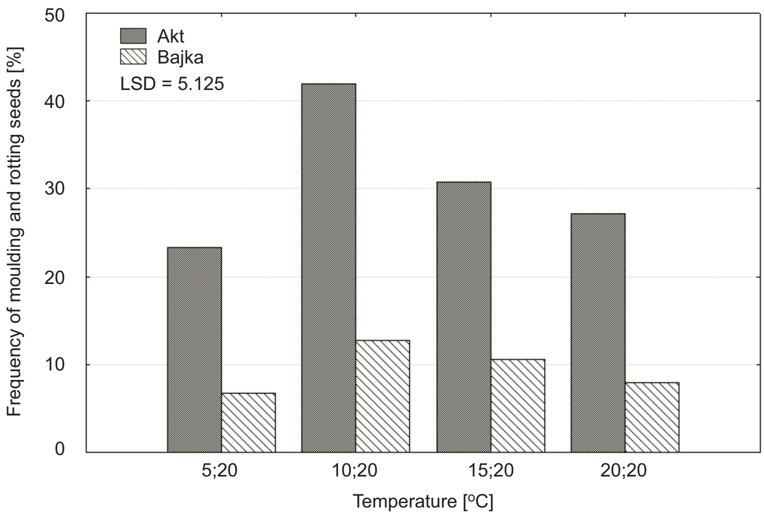

Fig. 5. The percentage of moulding and rotting seeds of the investigated cultivars after 30 days of germination, depending on temperature

seeds only in the naked form (4 %). The difference between the naked form and the husked one was also confirmed by the significantly higher, by 21 % on average, number of moulding and rotting seeds in the naked cultivar. The highest frequency of such seeds (43 %) was noted after initial incubation at a temperature of 10 ^{o}C (Fig. 5).

The germination dynamics index [8] can be used for the evaluation of many stress factors, including the effect of water deficiency on seed germination (Michalek and Borowski 2002). The variance analysis performed for that index in the third experiment showed a significant effect of incubation temperature under drought conditions and the share of this factor in the total variability reached 30 %. Besides, germination dynamics was modified by the temperature applied after transferring the seeds to the substrate containing water (16.4 %), as well as by differentiation between the cultivars (18.7 %) (Table 2). A higher temperature at the initial stage of incubation affected a rise (of about 14 %) in the rate of germination of the investigated cultivars (Table 3). Transferring the seeds, after five days, from drought conditions to the substrate containing water and keeping them at three different temperatures (10 ^{o}C, 15 ^{o}C and 20 ^{o}C) resulted in significant differences in Maguire's coefficient between the two investigated forms. The greatest value (5.9) was found for the seeds exposed to the temperature recommended for cereal seed germination (20 ^{o}C). During the whole incubation period the water deficiency in the substrate, in the case of seeds with decreased germinability, delayed germination dynamics in a different way in both the oat forms. The naked cultivar was characterized by a higher, by 14 %, value of germination dynamics (Table 4). The significance of differentiation of individual effects shows that after transferring the seeds to the substrate containing water and keeping them at different temperatures, greater, by 23 % on average, values of Maguire's coefficient were found for the naked cultivar. The naked cultivar seems to be more resistant to water deficiency in

Table 2

Significance of differentiation and the percentage of variance components for germination dynamics calculated for seeds stored at varied temperatures during and after incubation in PEG (–1.5 MPa)

Sources of variability	Degrees of freedom	Germination dynamics according to Maguire
Cultivars (A)	1	18.7*
Temperature during incubation in PEG (B)	1	29.9**
Temperature after incubation in PEG (C)	2	16.4*
Interaction		
A × B	2	8.0 ns
A × C	1	8.6 ns
B × C	2	0.0 ns
A × B × C	2	0.0 ns
Error	24	18.5

* significant at $p < 0.05$; ** significant at $p < 0.01$; ns – insignificant.

Table 3

Germination dynamics depending on the temperature applied during incubation in PEG (–1.5 MPa) and after transferring the seeds to the substrate containing water

Incubation in PEG (days 1–5)		
Temperature [°C]	Germination dynamics according to Maguire	LSD
5	4.8	0.771
10	5.6	
After incubation in PEG and transferring the seeds to the substrate containing water (days 6–10)		
10	5.0	0.901
15	4.6	
20	5.9	

Table 4

Germination dynamics of oat cultivars and the differentiation of individual effects after incubation in PEG (–1.5 MPa) and transferring the seeds to the substrate containing water

Cultivar	Germination dynamics according to Maguire			
	Total effect	Individual effects at a temperature of [°C]		
		10	15	20
Akt	5.5	5.5	5.4	5.8
Bajka	4.9	4.5	3.9	5.9
LSD	ns	0.806		

a substrate, occurring at the initial phase of the germination process, and its greater resistance to PEG 8000-induced drought should give it, during that critical period, more time for further stages connected with growth and development of seedlings. This is confirmed by the results obtained by Mos et al [10] showing that tolerance to drought in naked cultivars occurs only under the conditions of shortterm osmotic stress, and its prolongation causes in naked oat seeds a greater decrease in vigour indices, as compared with husked seeds.

Conclusions

1. The study has shown a significant effect of drought stress triggered by polyethylene glycol solution, as well as the temperatures applied, on the rate of water uptake and the start of germination.

2. The seeds of the naked cultivar Akt were characterized by a greater rate of water uptake, under the conditions of both the control and drought, and radicules more than 2 mm long were observed in that form earlier – on the second day and the sixth day respectively.

3. Seeds stored for 24 months under warehouse conditions were characterized by decreased germinability, which at an optimal temperature of 20 ^{o}C and under the conditions of the control reached 58 % in the naked cultivar Akt and 76 % in the husked cultivar Bajka.

4. Water deficiency in a substrate, in cultivars characterized by decreased germinability, delayed the germination process in a different way in both the oat forms – the husked cultivar and the naked cultivar.

5. Under drought stress lasting for five days, at 10 ^{o}C and 15 ^{o}C significantly higher germination dynamics was found in seeds of the naked cultivar Akt, which may be proof of higher resistance of this oat form to water deficiency in a substrate at the initial germination phase.

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KIEŁKOWANIE ZIARNIAKÓW OWSA O OBNIŻONYM WIGORZE W WARUNKACH SUSZY

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Abstrakt: W trzech seriach doświadczeń określono wpływ temperatury i stresu suszy symulowanej roztworem PEG (8000) (–1,5MPa) na zdolność oraz dynamikę kiełkowania dwóch odmian owsa – nagoziarnistej Akt oraz oplewionej Bajka. Ocena imbibicji wykonana dla temperatury 5 i 10 °C wykazała szybsze tempo pobierania wody u formy nagoziarnistej zarówno w warunkach suszy, jak i kontroli. Zdolność kiełkowania w zróżnicowanych temperaturach 5, 10, 15 i 20 °C osiągnęła w warunkach kontroli statystycznie istotnie mniejsze przeciętnie o 15 % wartości u formy nagoziarnistej. W warunkach suszy nie zaobserwowano kiełkowania, a przeniesienie nasion do temperatury 20 °C na kolejne 20 dni przyczyniło się do wystąpienia normlanie kiełkujących nasion tylko u formy nagoziarnistej (4 %). Dynamika kiełkowania oceniona w warunkach suszy dla temperatury 5 i 10 °C, a także po przeniesieniu na podłoże z wodą do temperatury 10, 15 i 20 °C wskazuje na statystycznie istotnie większe wartości u ziarniaków nieoplewionych, co może świadczyć o większej odporności tej formy owsa na niedobór wody w podłożu w początkowej fazie kiełkowania.

Słowa kluczowe: owies nagoziarnisty, owies oplewiony, stres suszy (PEG), wpływ temperatury, zdolność kiełkowania, dynamika kiełkowania

Varia

INVITATION FOR ECOPOLE '09 CONFERENCE

CHEMICAL SUBSTANCES IN ENVIRONMENT

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– **SI Chemical Pollution of Natural Environment and its Monitoring;**
– **SII Environment Friendly Production and Use of Energy;**
– **SIII Risk, Crisis and Security Management**;
– **SIV Forum of Young Scientists and Environmental Education;**
– **SV Impact of Environment Pollution on Food and Human Health.**

On the first day the debates of sections SI and SII will take place. The second day will be started with an ecological excursion. Afterwards the plenary Session with lectures on **environmental education** as well as **grants within the EU Programmes** and presentation of **EU Centres of Excellence** will be held. Then the **Forum of Young Scientists** – the presentation (lectures and posters) of young scientists work will take place.

The main topic of the third day is the influence of environment quality on the **human health**.

During the Conference an exhibition of publications concerned with conference topics will be also organised.

The Conference language is English.

Contributions to the Conference will be published as:

– abstracts on the CD-ROM (0.5 page of A4 paper sheet format)
– extended abstracts (4–6 pages) in the semi-annual *Proceedings of ECOpole*;

full papers will be published in successive issues of the *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (Ecol. Chem. Eng.).

Additional information one could find on the Conference website:

ecopole.uni.opole.pl

The deadline for sending the Abstracts is **31.08.2009** and for the Extended Abstracts: **1.10.2009**. The actualised list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one will find (starting from 15.07.2009) on the Conference website.

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

The Conference fee is 300 € (covering hotel, meals and transportation during the Conference). It could be reduced (to 170 €) for young people actively participating in the Forum of Young Scientists. But the colleague has to deliver earlier the Extended Abstract (4-6 pages) of his/her contribution (deadline is on 15.08.2009), and a recommendation of his/her Professor.

Fees transferred after 15.09.2009 are 10% higher.

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

Further information is available from:
Dr hab. Maria Wacławek, prof. UO
Chairperson of the Organising Committee
of ECOpole '09 Conference
University of Opole
email: Maria.Waclawek@uni.opole.pl
and mrajfur@o2.pl
tel. +48 77 455 91 49 and +48 77 401 60 42
fax +48 77 401 60 51

Conference series

1. 1992 Monitoring ’92 Opole
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5. 1996 EKO-Opole ’96 Kędzierzyn Koźle
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17. 2008 CEC ECOpole ’08 Piechowice

REGISTRATION FORM FOR THE ECOpole '09 CONFERENCE

Surname and First Name . . . .

Scientific Title/Position . . . .

Affiliation . . . .

Address . . . .

Tel./fax . . . . email . . . .

Title of presentation . . . .

. . . .

KIND OF PRESENTATION

	YES	NO
Oral		
Poster		
Taking part in discussion		

ACCOMMODATION

14/15 X		15/16 X		16/17 X	
YES	NO	YES	NO	YES	NO

MEALS

Date	Breakfast	Lunch	Dinner
14 X	—	—	
15 X			
16 X			
17 X			—

✂ -

ZAPRASZAMY
DO UDZIAŁU W ŚRODKOWOEUROPEJSKIEJ KONFERENCJI
ECOpole '09
w dniach 14–17 X 2009

SUBSTANCJE CHEMICZNE W ŚRODOWISKU PRZYRODNICZYM

Będzie to osiemnasta z rzędu konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotycząca różnych aspektów ochrony środowiska przyrodniczego. Odbędzie się ona w ośrodku „Uroczysko" na Wzgórzu Wilhelma w Piechowicach. Doroczne konferencje ECOpole mają charakter międzynarodowy i za takie są uznane przez Ministerstwo Nauki i Szkolnictwa Wyższego.

Obrady konferencji ECOpole '09 będą zgrupowane w pięciu sekcjach:

- **SI Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring;**
- **SII Odnawialne źródła energii i jej oszczędne pozyskiwanie oraz użytkowanie;**
- **SIII Zarządzanie środowiskiem w warunkach kryzysowych**;
- **SIV Forum Młodych (FM) i Edukacja prośrodowiskowa**;
- **SV Wpływ zanieczyszczeń środowiska oraz żywności na zdrowie ludzi**.

Materiały konferencyjne będą opublikowane w postaci:

- abstraktów (0,5 strony formatu A4) na CD-ROM-ie;
- rozszerzonych streszczeń o objętości 4-6 stron w półroczniku *Proceedings of ECOpole*;
- artykułów: w abstraktowanych czasopismach: *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (*Ecol. Chem. Eng.*) oraz niektórych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*.

Termin nadsyłania angielskiego i polskiego streszczenia o objętości 0,5–1,0 strony (wersja cyfrowa + wydruk) planowanych wystąpień upływa w dniu 31 sierpnia 2009 r. Lista prac zakwalifikowanych przez Radę Naukową Konferencji do prezentacji będzie sukcesywnie publikowana od 15 lipca 2009 r. na stronie webowej konferencji

ecopole.uni.opole.pl

Aby praca (dotyczy to także streszczenia, które powinno mieć tytuł w języku polskim i angielskim, słowa kluczowe w obydwu językach) przedstawiona w czasie konferencji mogła być opublikowana, jej tekst winien być przygotowany zgodnie z wymaganiami stawianymi artykułom drukowanym w czasopiśmie *Ecological Chemistry and Engineering*, które jest dostępne w wielu bibliotekach naukowych w Polsce i zagranicą. Są one takie same dla prac drukowanych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*. Zalecenia te są również umieszczone na stronie webowej konferencji.

Koszt uczestnictwa w całej konferencji wynosi 1000 zł i pokrywa opłatę za udział, koszt noclegów i wyżywienia oraz rocznej prenumeraty Ecol. Chem. Eng. (razem blisko 2000 ss.) łącznie z materiałami Konferencji. Jest możliwość udziału tylko w jednym wybranym przez siebie dniu, wówczas opłata wyniesie 650 zł i będzie upoważniała do uzyskania wszystkich materiałów konferencyjnych, jednego noclegu i trzech posiłków (śniadanie, obiad, kolacja), natomiast osoby zainteresowane udziałem w dwóch dniach, tj. w pierwszym i drugim lub drugim i trzecim, winny wnieść opłatę w wysokości 800 zł. Opłata dla magistrantów i doktorantów oraz młodych doktorów biorących aktywny udział w Forum Młodych może być zmniejszona do 600 zł, przy zachowaniu takich samych świadczeń. Osoby te winny dodatkowo dostarczyć: rozszerzone streszczenia (4–6 stron) swoich wystąpień (**do 15.08.2009 r.**). Jest także wymagana opinia opiekuna naukowego. Sprawy te będą rozpatrywane indywidualnie przez Radę Naukową oraz Komitet Organizacyjny Konferencji. Członkowie Towarzystwa Chemii i Inżynierii Ekologicznej i Polskiego Towarzystwa Chemicznego (z opłaconymi na bieżąco składkami) mają prawo do obniżonej opłaty konferencyjnej o 25 zł. Opłaty wnoszone po dniu 15 września 2009 r. są większe o 10% od kwot podanych powyżej. Wszystkie wpłaty winne być dokonane na konto w Banku Śląskim:

BSK O/Opole Nr 65 1050 1504 1000 0005 0044 3825

i mieć dopisek ECOpole ’09 oraz nazwisko uczestnika konferencji.

Po konferencji zostaną wydane 4–6-stronicowe rozszerzone streszczenia wystąpień w półroczniku *Proceedings of ECOpole*. Artykuły te winny być przesłane do **1 października 2009 r**. Wszystkie nadsyłane prace podlegają zwykłej procedurze recenzyjnej. Wszystkie streszczenia oraz program Konferencji zostaną wydane na CD-ROM-ie, który otrzyma każdy z uczestników podczas rejestracji. Program będzie także umieszczony na stronie webowej konferencji.

Za Komitet Organizacyjny
dr hab. inż. Maria Wacławek, prof. UO
Wszelkie uwagi i zapytania można kierować na adres:
Maria.Waclawek@uni.opole.pl
lub mrajfur@o2.pl
tel. 077 401 60 42
tel. 077 455 91 49
fax 077 401 60 51

Kalendarium

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ZGŁASZAM UCZESTNICTWO W KONFERENCJI ECOpole ’09
(Prosimy o wypełnienie zgłoszenia drukowanymi literami)

Nazwisko i imię . . . .

Tytuł (stopień) naukowy/stanowisko . . . .

Miejsce pracy . . . .

Adres . . . .

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Dane instytucji (nazwa, adres, NIP), dla której ma być wystawiona faktura: . . . .

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RODZAJ PRZEWIDYWANEGO WYSTĄPIENIA:

	TAK	NIE
Referat		
Poster		
Głos w dyskusji		

TYTUŁ WYSTĄPIENIA . . . .

. . . .

ZAMAWIAM NOCLEG

14/15 X		15/16 X		16/17 X	
TAK	NIE	TAK	NIE	TAK	NIE

ZAMAWIAM POSIŁKI

Data	Śniadanie	Obiad	Kolacja
14 X	—	—	
15 X			
16 X			
17 X			—

15th ICHMET

15th International Conference on Heavy Metals in the Environment
September 19–23, 2010
Gdańsk, Poland

Organized by
Chemical Faculty, Gdańsk University of Technology (GUT)
together with
Committee on Analytical Chemistry of the Polish Academy Sciences (PAS)

15th ICHMET – is a continuation of a series of highly successful conferences that have been held in major cities of the world since 1975. These conferences typically draw 500–1000 participants from countries in many parts of the world. Well over 5000 scientists have taken part in this series of conferences including most leaders in the field. Apart from the city's natural beauty, Gdansk is logical choice for the 15th Conference to highlight the outstanding work that is being done on heavy metals in central Europe. The venue for the meeting will be the Gdansk University of Technology (GUT) which features many tourist attractions.

The Conference will include a number of invited lectures treating frontier topics prepared by specialist with international reputation, oral presentation and poster sessions. ICHMET welcomes contributions on all aspects of any heavy metal in the environment. All presentation will be connected with such topics as:

- Risk assessment and risk management pertaining to toxic metals in the environment
- Susceptibility and protection of children from toxic metals in their environment
- Measurement and exposure assessment
- Biomarkers of exposure and effects of heavy metals
- Gene-environment-metal interactions
- Trend tracking/analysis of heavy metal data – spatial and temporal
- Risk communication pertaining to heavy metals
- Life cycle analysis for metalliferous consumer products
- Soil quality criteria
- Remediation technologies
- Control strategies for heavy metal emissions and deposition
- Metal mixtures – mechanistic and epidemiological studies
- Nutrient-metal interactions
- Advancements in analytical tools (procedures and measurement devices)

- Toxicology of heavy metals, from cellular and genomic to ecosystem levels
- Heavy metals in foods
- Impact of global change on heavy metal cycle

For further information on the conference, please contact:

Professor Jacek Namieśnik (Conference Chairman)
Gdansk University of Technology,
Chemical Faculty, Department of Analytical Chemistry
G. Narutowicza 11/12, 80–233 Gdansk, (Poland),
e-mail: chemanal@pg.gda.pl
homepage: http://www.pg.gda.pl/chem/ichmet/

GUIDE FOR AUTHORS
ON SUBMISSION OF MANUSCRIPTS

A digital version of the Manuscript addressed –

Professor Witold Wacławek
Editorial Office of monthly *Ecological Chemistry and Engineering*
(Ecol. Chem. Eng.)
Uniwersytet Opolski
ul. Oleska 48, 45–951 Opole, Poland
Tel. +48 77 452 71 34, fax +48 77 455 91 49,
Email – waclawek@uni.opole.pl

should be sent by email to the Editorial Office Secretariat – mrajfur@o2.pl

The Editor assumes, that an author submitting a paper for publication has been authorised to do that. It is understood the paper submitted to be original and unpublished work, and is not being considered for publication by another journal. After printing, the copyright of the paper is transferred to *Towarzystwo Chemii i Inżynierii Ekologicznej* (*Society for Ecological Chemistry and Engineering*). In preparation of the manuscript please follow the general outline of papers published in the most recent issues of *Ecol. Chem. Eng*., a sample copy can be sent, if requested.

Papers submitted are supposed to be written in English language and should include a summary and keywords, if possible also in Polish language. If not then the Polish summary and keywords will be provided by the Editorial Office. All authors are requested to inform of their current addresses, phone and fax numbers and their email addresses.

It is urged to follow the units recommended by the *Systéme Internationale d'Unites* (SI). Graph axis labels and table captions must include the quantity units. The use of the following commonly applied expressions is recommended: mass – m/kg, time – t/s or t/min, current intensity – I/A; thermodynamic temperature – T/K, Celsius scale temperature – t/°C or θ/°C (if both time and Celsius scale units need to be used, the symbol θ/°C for temperature is to be taken) etc.

Symbols recommended by the International Union of Pure and Applied Chemistry (Pure and Appl. Chem., 1979, **51**, 1–41) are to be followed.

Graphics (drawings, plots) should also be supplied in the form of digital vector – type files, *e.g*. Corel-Draw, Grapher for Windows or at least in a bitmap format (TIF, PCK, BMP). In the case of any query please feel free to contact with the Editorial Office.

Footnotes, tables and graphs should be prepared as separate files.

References cited chronologically should follow the examples given below:

[l] Kowalski J. and Malinowski A.: Polish J. Chem. 1990, **40**(3), 2080–2085.

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

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

Journal titles should preferably follow the Chem. Abst. Service recommended abbreviations.

Receipt of a paper submitted for publication will be acknowledged by email. If no acknowledgement has been received, please check it with the Editorial Office by email, fax, letter or phone.

ZALECENIA DOTYCZĄCE PRZYGOTOWANIA MANUSKRYPTÓW

Praca przeznaczona do druku w miesięczniku *Ecological Chemistry and Engineering A/Chemia i Inżynieria Ekologiczna A* powinna być przesłana na adres Redakcji:

Profesor Witold Wacławek
Redakcja Ecological Chemistry and Engineering
Uniwersytet Opolski
ul. Oleska 48, 45–951 Opole
tel. 077 401 60 42, fax 077 455 91 49
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Przypisy i tabele podobnie jak rysunki zapisujemy jako osobne pliki.

Literaturę prosimy zamieszczać wg poniższych przykładów:

[1] Kowalski J. and Malinowski A.: Polish J. Chem. 1990, **40**, 2080–2085.

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

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

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