SOCIETY OF ECOLOGICAL CHEMISTRY AND ENGINEERING

ECOLOGICAL CHEMISTRY AND ENGINEERING A

CHEMIA I INŻYNIERIA EKOLOGICZNA A

Vol. 16 No. 7

OPOLE 2009

EDITORIAL OFFICE

Opole University, Chair of Chemical Physics
POB 313, ul. Oleska 48, 45-951 Opole
tel./fax +48 77 455 91 49
email: waclawek@uni.opole.pl
http://tchie.uni.opole.pl

SECRETARIES

Agnieszka Dołhańczuk-Śródka, tel. +48 77 401 60 45, email: agna@.uni.opole.pl
Małgorzata Rajfur, tel. +48 77 401 60 42, email: mrajfur@o2.pl

SECRETARIES' OFFICE

tel. +48 77 401 60 42
email: mrajfur@o2.pl

Ecological Chemistry and Engineering
is partly financed by Ministry of Science and Higher Education, Warszawa
and Provincial Found for Environmental Protection and Water Management, Opole

ISSN 1898-6188

CONTENTS

VARIA

SPIS TREŚCI

VARIA

Artykuły publikowane w tym zeszycie były przedstawione w czasie XIII Międzynarodowej Konferencji Naukowej METAL IONS AND OTHER ABIOTIC FACTORS IN THE ENVIRONMENT, Kraków, 12–13 maja 2008 r.

Spotkania te są corocznie organizowane przez Katedrę Ochrony Środowiska Rolniczego Uniwersytetu Rolniczego w Krakowie, kierowaną przez Panią Profesor Magdalenę Jaworską.

Prezentowane artykuły przeszły normalną procedurę recenzyjną i redakcyjną.

Papers published in the issue have been presented during the 13th Scientific Conference on METAL IONS AND OTHER ABIOTIC FACTORS IN THE ENVIRONMENT, Krakow, 12–13 May 2008.

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Magdalena JAWORSKA[1]

SOME RESEARCH PROBLEMS IN MODERN PESTICIDE MANUFACTURING

NIEKTÓRE PROBLEMY BADAWCZE W PRODUKCJI NOWOCZESNYCH PESTYCYDÓW

Abstract: Efficient plant protection involves the application of all methods available within the IPM (Integrated Pest Manegement) programme. The chemical plant protection method is undergoing considerable transformations since not only new efficient biologically active substances are sought but the requirements concerning their biological safety for non-target organisms and the whole environment have increased. No success is possible without a proper functional form. New formulations and microcapsules, oil dispersions or agrogels should be biodegradable and cannot pollute the environment. Scientific research aimed at seeking substances enhancing the pesticide effect, such as capsaicine, which is a natural component of pepper (paprika), increasing toxicity of some insecticides. Environment-friendly preparations are products based on chitosan destruction, useful particularly for the protection of organic crops and valuable ornamental plants against diseases. Biotechnological methods are used in Poland and in the world to develop biopesticides, such as preparations containing new species and strains of live entomopathogenic nematodes for plant protection against pests.

Keywords: chemical pesticides, biopesticides, environment protection

Insects (*Insecta*) constitute the most numerous phylum among the anthropods (*Anthropoda*). To this day over 2 million insect species have been identified and new ones are discovered and described every year.

Simultaneous evolution of insects and plants, lasting for over 300 million years formed a strict interdependence between these organisms. It is particularly apparent between flower plants and pollinating insects, which have a specialized sucking and licking mouthparts which enables them to suck up nectar. Insects – plant pests have specialized mouthparts of the chewing type (capable of crushing hard terrestrial vegetation), piercing-sucking type for piercing plants and sucking up saps and other modifications.

About 50 % of insect species are phytophages. Accompanying the humans from time immemorial they provide serious food competition and make people seek various meth-

[1] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrmjawor@cyf-kr.edu.pl

ods of protection. These were different, starting from mechanical measures through biological ones (*eg* the use of ants in ancient China), the use of plant extracts to prayers and spells, and even court sentences. These endeavours usually proved little effective, which was also due to limited technical abilities and isolation between human communities.

Pesticides were first used successfully on a wide scale only in the second half of the 19^{th} century. At first they were inorganic compounds with a strong effect, such as lead arsenate, calcium arsenate and fluorine compounds, which at present have only historic importance as insecticides of the first generation. This group also comprised preparations of plant origin with contact activity such as pyretrum and nicotine used as a tobacco concoction.

A rapid development of pesticides started with production of synthetic preparations. In 1942 insect killing properties of DDT were discovered and soon after this compound initiated a new era in plant protection. In 1946 mass application of organic phosphorus insecticides started, which still make up a considerable share, and then the next II generation preparations were introduced, such as carbamates and pyrethroids. III generation insecticides (antifeedants, hormones and feromones) and IV generation insecticides (antihormones) appeared in the seventies.

Insecticides currently in use make up the smallest group of plant protection means applied in Poland; however, their role is still very important (eg in fighting potato beetle, rapeseed pests or orchard pests).

They are also present on farms as household chemicals used for sanitary and veterinary hygiene.

Organic compounds of anthropogenic origin, including also crop protection chemicals pose the main threat not only to human health but also to flora and fauna. The important role of plant protection chemicals results mainly from the wide scale of their application, their persistence in the natural environment and toxic properties. Diminishing the environmental impact of plant protection chemicals is one of the objectives assumed by the European Union in the Sixth Environment Action Programme of the European Community 2002–2012 (EAP6) (Decision No. 1600/2002/WE of the European Parliament and the Council adopted on 22nd July 2002). Based on four priorities, the programme made the European Commission responsible for the preparation of seven Thematic Strategies, including sustainable application of pesticides. The subject belongs to priority 3 EAP6 ("Environment and Health and Life Quality"), which sets the following goals:

– minimizing hazards and threats to human health and the environment resulting from pesticide application;

– decreasing levels of active harmful substances, particularly through replacement of the most dangerous substances by safer alternatives

– promoting farming systems using small amounts of pesticides or pesticide-free plantations.

Achieving these goals is possible only by introducing new active substances or developing new pesticide preparations (functional forms, formulations).

The number of biologically active substances applied has been decreasing systematically, which is connected with very high costs of research, especially concerning the assessment of the impact of these compounds on the environment and human health. Currently, the total costs of developing a new, original active substance which requires between 30 and 50 thousand synthesis reactions is 120 million dollars.

Pesticide biologically active chemicals are generally organic compounds revealing considerable biological activity and relatively small water solubility. Moreover, they are able to penetrate insect or plant cuticule to some degree. For these reasons they are used for plant protection only as the right functional forms called pesticide preparations. The time which elapses from synthesis of a biologically active substance to marketing its commercial preparation is ca 10 years. It is so because the manufacturer must properly select the components to ensure the stability of biologically active pesticide substances during storage and to enable obtaining stabile dispersions in the fluid phase during spraying. According to the law in force, the manufacturer of a plant protection chemical is obliged to submit to the Plant Protection Institute detailed documentation of the chemical he manufactures, comprising among others results of tests on behaviour and decomposition of the biologically active substance in soil, water and air together with the results of an ecotoxicological test, comprising data on acute toxicity for fish, daphnias and algae.

Measures undertaken to reduce the environment pollution, mainly of surface and groundwaters, should start at the stage of developing proper functional forms of the preparation. Scientific research on both the stability of a biologically active substance and its toxicity but also on paths of degradation, sorption properties and identification of potential ecological side effects should concern preparations including the effect on the above-mentioned agents applied in the process of adiuvant formulation.

Therefore, efficient plant protection does not only involve the application of a proper biologically active substance but its functional form is also important. Under conditions of increasing requirements for modern plant protection a necessity arises to create new improved variants of well-known types of formulations and new concepts of the effect which the preparation components have on active substance behaviour in various compartments of the environment. It is anticipated that the obtained insecticide preparations will reduce their dose and widen the spectrum of the optimal temperature of the preparation application. Results of testing the effect of various surficially active compounds on the processes of mass exchange in modified dispersive systems of modified forms of EC and WP insecticides from pyrethroids group will be a basis to obtain new formulations of these compounds as microcapsules, agrogels and oil dispersions[1, 2].

Preparation of appropriate formulations as products ready for sale is mainly the task of relevant departments of large chemical plants. Therefore, a great amount of data concerning technologies of formulation exists as patents [3].

The available literature contains a number of papers confirming greater persistence and toxicity of active substances used as preparations in comparison with the same properties determined for a pure active substance. It is also known that application of various types of oil adiuvants causes intensification of active substance durability and considerably contributes to decreasing the environmental pollution level.

So, scientific research aims at developing functional forms of plant protection chemicals which would be less harmful for the natural environment and human health. At this point a reference should be made to more efficient pest control in agriculture and diminishing the amount of applied active substances, which is an important factor in multifunctional development of agriculture, especially organic farming.

Because of an increasing quantity of chemical insecticides accumulating in the environment and growing pest resistance to some of them, particularly to pyrethroids, new substances which would support their effect are sought intensively. Such synergistic substances include, among others, capsaicine, a natural component present in pepper. It affects insect thermoreceptors diminishing the efficiency of their work, meaning that insects do not feel threatened by temperature. Therefore it may be assumed that capsaicine and its chemical analogues disturbing the insects natural physiological mechanism leading to an increase in the insecticide toxicity, as was demonstrated on an example of potato beetle larvae [4]. It has been common knowledge for over 40 years that the model of ionic balance, described in the physiology handbooks, causing depolarization in neuron and formation of action potential, is not the only model occurring in the whole nature. Because it has been shown that in different insect taxonomic groups, genesis of both rest and action potential is different and associated with the presence of some determined channels in the membrane, a xenobiotic affecting a determined kind of ionic channels will differently affect an insect in which the importance of these ionic channels is crucial and the one where these membrane channels are of lesser importance. This fact provides a basis for a selective, for some insect groups, insecticidal effect of pyrethroids, particularly indenooxadiazines [5].

Nowadays, plant protection abandons purely chemical pest control and more often uses biological methods – Ecologically Based Pest Management (EBPM) and Integrated Pest Management (IPM) [6]. Recommendations and requirements of Good Agricultural Practice concerning application of plant protection chemicals are legally based on the Act on Crop Protection. Meeting these requirements is most important not only from the perspective of efficacy and efficiency of plant protection measures, but even more important from the environment protection and human health viewpoint (Ministry of Agriculture and Rural Development, 2005). However, while introducing biological preparations containing live entomopathogenoc nematodes to crops, one should consider their impact on biodiversity. One of the main hazards to biodiversity is penetration of alien species (including their accidental or planned introduction), which often causes the competitionally inferior native species to drop off from the contest. Therefore, biological plant protection should necessarily apply the preparations manufactured on the basis of local species. The nematodes most frequently isolated from soils in Poland include: *Steinernema feltiae*, *S. affinis* and *Heterorhabditis megidis* [7]. Introduction of nematodes as biopreparations to crops may efficiently protect crop production reducing economic losses with a simultaneous decrease in applied chemical pesticides.

Biological methods may not only reduce a large pest population, but also maintain the pest population on a low level, safe for the crops. It is most important, since small numbers of insects settling individual niches prevent a much more dangerous invasion of other agrophagous species [6].

Entomopathogenic nematodes and their symbiotic bacteria are safe for the environment, mammals and other organisms which are not their aim [8–10].

Another crucial asset of these organisms is the morphological and physiological ability of infective juveniles (L3) to adapt to stressful conditions in soil: low temperatures, low moisture, pH, pollutants [11–16] but also long survival period of larvae without the host-insect [17]. In overdried soil an infective juvenile may survive in moisture below the level causing plant withering [8]. *H. bacteriophora* larvae resistance to pressure of 2000 kPa (290 psi) or *S. carpocapsae* and *H. megidis* resistance to the pressure of 1380 kPa (200 psi) is another asset of these organisms [18]. Owing to these properties they may be applied using the same equipment as for chemical measures: sprayers with mesh diameter bigger than 50 μm, helicopters or irrigation systems. Nematodes can also be applied using injection nozzles and pumps, which are the standard equipment for pressure application.

Moreover, nematode potential is not usually weakened when chemicals are used at the same time [19].

Nematodes have been cultured for over 70 years [8] and currently they are cultured on a large scale using three methods: *in vivo*, *in vitro* on solid media and in *vitro* in liquid media. Each of these methods has its advantages and disadvantages influenced by production costs, required technical skills or product quality. Moreover, each of them reveals potential for development. However, reduction of production costs through optimization of parameters and factors affecting final efficiency of nematodes are the most important.

Present biological substances containing *Steinernema* and *Heterorhabditis* nematodes are manufactured on a large scale by numerous firms in the world [17, 20, 21]. From among many species belonging to these two families, only 6 of them are used for commercial production.

Primary nematode lines are kept in liquid nitrogen until the start of production. The next stage is activation of these organisms when the nematodes in cuvettes are placed for 18 days in incubators where the temperature required for individual species is maintained. Initiating of the nematode reproduction process lasts for the subsequent 18 days and for this reason they are placed in cuvettes containing plant fermentation products (the composition is secret). The subsequent 18 days are devoted to final production in two containers of 6 thousand liter volume and two 40 thousand liter ones. Proper medium pH values, pressure and temperatures are maintained. Because of growing demand for that product BU, an additional container, 40 thousand liter volume, is planned for the next year. The whole production process is conducted in sterile conditions and the process is fully computerized. Proper functioning of the apparatus is monitored by four persons. The next stage involves filtering and washing the whole mass filling the containers in order to obtain a pure product containing 100 % of nematodes, whose vitality and infectivity are controlled in a laboratory. If the product meets all required quality standards it is placed together with the substance protecting these organisms against moisture loss, in unit packagings. The packed nematodes may survive at 2 °C for about two months. No more than 24 hours elapse from the moment of placing an order (by a farmer) for a nematode batch to its realization (supply to the farmer) [22]. Production of biopreparations with live nematodes is conducted also in Poland by "Owiplant" Horticultural enterprise on the basis of native nematode species. The only Polish preparation Owinema containing *Steinernema feltiae* nematodes was obtained on solid medium.

Entomopathogenic nematodes (EN) are alternatives for chemical control of sciarid flies, especially on mushroom farms. There are in Poland 8–9 week cycles of mushroom production and over such a long period chemical pesticides are ineffective. In 1998 on two Polish mushroom farms in Krakow and Rzeszow production was protected from sciarid flies using EN: commercial biopesticides "Entonem" (Dutch), "Nemasys" (English) and "Owinema" (Polish selected strain of *Steinernema feltiae*) [23]. Chemical standard pesticides: teflubenzuron, Nomolt, and diflubenzuron, Dimilin, were used for comparison. On both mushroom farms sciarid flies, *Lycoriella solani*, were the main trapped insect pests. Biological protection of mushroom against sciarids was improved. Mean yield was increased from 1.10, when chemical protection was applied, to 2.20 kg/m^2. The index of treatment profitability, the index of cost coverage and the index of percentage refund of expenditures of treatment, were the best on treatment with Polish "Owinema" biopesticide. The cost of sciarid`s control by "Owinema" was the lowest (0.48 PLN/m^2 3.9 PLN = 1US$). The data confirmed the suitability of EN commercial products as on alternative for chemical mushroom protection against sciarid flies. Biological control with Polish bio-pesticides "Owinema" was superior.

As was demonstrated in research by of Jaworska et al [24, 25], nematodes are resistant to heavy metals, whose ions contaminate soil increasingly [26]. In laboratory conditions most metal ions, except lead (Pb(II)), copper (Cu(II)) and zinc (Zn(II)) in medium concentrations do not reveal a toxic effect on entomopathogenic nematodes. However, invasive abilities of nematodes in water ion solutions weaken already after 96 hours, particularly in case of lead (Pb(II)), chromium (Cr(VI)), vanadium (V(V)), cadmium (Cd(II)) and nickel (Ni(II)) ions. Further research [27] revealed that this negative effect may be mitigated by an addition of Mn(II) (400 mg · dm^{-3}/L) or Mg (160 mg · dm^{-3}/L) or lithium to the solution.

A new biopreparation has been planned on the basis of *Heterorhabditis megidis*. These nematodes are the most frequently isolated from the soils of Poland. Nematodes of this species efficiently control not only *Lepidoptera* caterpillars, *Hymenoptera* larvae or *Diptera*, but also beetles. Preparations obtained from the liquid *in vitro* culture guarantee better productivity so they may provide competition for foreign preparations, registered in Poland (Table 1) [24].

Table 1

List of plant protection means containing entomopathogenoc nematodes licensed for sale and use in Poland

No.	Name of plant protection mean	Manufacturer of plant protection mean	Name and characteristics of living organism	Licence valid until
1.	Entonem	KOPPERT Biological Systems B.V. – The Netherlands	*Steinernema feltiae*	11.05.2010
2.	Larvanem	KOPPERT Biological Systems B.V. – The Netherlands	*Heterorhabdilis bacteriophora*	11.05.2010
3.	Owinema	"OWIPLANT" Ltd. Horticultural Enterprise – Owińska	*Steinernema feltiae*	26.11.2009
4.	Steinernema System	BIOBEST N.V. Biological Systems – Belgium	*Steinernema feltiae*	15.03.2010

Assumptions for the technological process of manufacturing a modern, environment--friendly biopreparation based on chitosan destruction products have been developed. It will be useful for plant protection against fungal, bacterial and virus diseases [25].The biopreparation will be particularly serviceable for organic crops for pro-health food production. Synthetic plant protection means currently available on the market reveal toxicity both for humans and animals posing a hazard to the environment. They are unable to simultaneously combat various fungi, bacteria and virus strains which makes necessary the application of various pesticides or their combinations depending on present threat. The use of synthetic pesticides leads to pathogen immunization to their activity. So far, no biocides which would efficiently control fungal, bacterial or virus disease at a wide spectrum of their effect have been known.

The faults of commercial pesticides, leading to environment degradation and causing, among others, genetic changes in living organisms, including the human organism, arouses growing interest in natural plant protection means, including biopreparations. The preparation based on products of chitosan destruction (derivatives of chitin, the natural polymer) should meet these requirements.

Chitosan, due to its unique properties, such as biodegradability, bioactivity, biocompatibility, fiber forming and film forming abilities and good blending ability with other polymers, finds many applications in various fields, including medicine, agriculture, environment protection and food industry.

The purposefulness of developing a plant protection biopreparation with chitosan oligomers results also from ecological reasons, since such a preparation does not cause environmental pollution. Natural origin, no toxicity for humans, animals or plants, no phytotoxic activity provides a safe alternative for toxic pesticides. It may be expected that the new preparation may reveal activity comparable with vaccines in people and animals, improving their immunity to fungi and bacteria activity. The optimal method of chitosan destruction allowing for highly productive formation of water soluble oligomers will probably be an enzymatic degradation or a combination of various methods. A new, easily applicable mean with a wide activity spectrum for cultivational applications, will be developed soon. It will be serviceable for protection against diseases caused by fungi, bacteria and viruses.

References

[1] Bondada B.R., Sams C.E., Deyton D.E. and Cummins J.C.: *Oil emulsions enhance transcuticular movement of captan in apple leaves*. Crop Protect., 2007, **26**, 691–696.

[2] Pey C.M., Maestro A., Sole I., Gonzalez C., Solans C. and Gutierrez J.M.: *Optimization of nano-emulsions prepared by low-energy emulsification methods at constant temperature using a factorial desidn study*. Colloids and Surfaces A: Physicochem. Eng. Aspects, 2006, **288**, 144–150.

[3] Green J.M. and Beestman G.B.: Recently patented and commercialized formulation and adjuvant technology. Crop Protect., 2007, **26**, 320–327.

[4] Tęgowska. E. Grajpel B. and Piechowicz B.: *Does red pepper contain insecticidal compound for Colorado beetle?* IOBC wprs Bulletin, 2005, **28**(10), 121–127.

[5] Grajpel B., Tęgowska E. and Stankiewicz M.: *Effect of pyrethroid and a new oxadiazine insecticide on bioelectrical activity and thermal behaviour in insects*. Acta Biol. Cracov. Ser. Zoologia, 2005, **47**, 43–46.

[6] Bauman D.E.: Ecologically Based Pest Management. National Academy Press, Washington, 1996.

[7] Tomalak M.: *Infectivity of entomopathogenic nematodes to soil-dwelling developmental stages of the tree leaf beetles Altica quercetorum and Agelastica alni*. Entomol. Experiment. Applic., 2004, **110**, 125–133.
[8] Brzeski N. and Sandner H.: Zarys nematologii, Warszawa, PWRiL, 1974, 400 ss.
[9] Jaworska M.: *Biological control of Haplocampa testudinea*, Klug. Acta Phytopatol. Entomol. Hungarica, 1992, **27**(1–4), 311–315.
[10] Jaworska M.: *Wpływ owadobójczych nicieni z rodzin Heterorhabditidae i Steinernematidae na śmietkę kapuścianą Delia brassicae i jej wrogów naturalnych*. Pol. Pismo Entomol., 1993, **62**, 254.
[11] Jaworska M. and Dudek B.: *Występowanie owadobójczych nicieni w glebach wybranych upraw*. Zesz. Nauk. AR Kraków, 1992, Nr 267, Ogrod. z. 20, 131–135.
[12] Jaworska M.: *Effect of the soil moisture and acidity on the activity of nematodes Steinernema feltiae and Heterorhabditis bacteriophora-parasites of the insect pest*. Entomonematologia, 1992, **1**(3), 15–21.
[13] Jaworska M., Jasiewicz Cz. and Gorczyca A.: *Wpływ zanieczyszczenia metalami ciężkimi gleb ogrodów działkowych Śląska na aktywność mikroorganizmów owadobójczych*. Prog. Plant Protect., 1997, **37**(2), 276–279.
[14] Jaworska M. and Gorczyca A.: *The effect of metal ions on mortality, pathogenicity and reproduction of entomopathogenic nematodes Steinernerma feltiae*. Polish J. Environ. Stud., 2002, **11**, 517–519.
[15] Jaworska M., Gorczyca A., Sepioł J. and Tomasik P.: *Metal-metal interactions in biological system. Sergism and antagonism of metal ion triads in Steinernema carpocapsae entomopathogenic nematodes*. Chem. Inż. Ekol. 2000, **7**(**4**), 313–326.
[16] Jaworska M.: *Wpływ niektórych czynników abiotycznych na patogeniczność nicieni owadobójczych umieszczonych na powierzchni gleby łącznie z żywicielem*. Zesz. Nauk. AR Kraków, 1992, Nr 267, Ogrod. z. 20, 113–129.
[17] Ehlers R.U. and Hokkanen H.M.T.: *Insect biocontrol with non-endemic entomopathogenic nematodes (Steinernema and Heterorhabditis spp.): Conclussion aud recommendations of a combined OECD and COST*, Sci. Technol., 1996, **6**, 403–411.
[18] Hynes R. and Boyetchko S.M.: *Research initiatives in the art and science of biopesticide formulations*. Soil Biol. Biochem., 2006, **38**, 845–849.
[19] Kamionek M.: Wpływ pestycydów na nicienie entomofilne. Rozprawa habilit. SGGW-AR, Warszawa 1992.
[20] Bedding R.A.,: *Low cost in vitro mass production of Neoplectana and Heterorhabditis species (Nematoda) for field control of insect pests*. Nematologica, 1981, **27**, 109–114.
[21] Wouts W.M.: *Mass production of entomogenous nematode Heterorhabditis heliothidis (Nematoda: Heterorhabditidae) on artificial media*. J. Nematol., 1981, **13**(4), 467–469.
[22] Kupczak K.: *Nicienie w walce z niektórymi szkodnikami roślin*. Ochr. Rośl., 2006, **12**.
[23] Jaworska M.: *Nematodes as bio-pesticides in two polish mushroom farms*. XIVth International Plant Protection Congress (IPPC). Plant Protection Towards the Third Millenium – Where Chemistry Meets Ecology Jerusalem, Israel, July 25–30, 1999.
[24] Jaworska M., Gorczyca A., Sepioł J. and Tomasik P.: *Effect of metal ions on the entomopathogenic nematode Heterorhabditis bacteriophora Poinar (Nematoda: Heterohabditidae) under laboratory conditions*. Water, Air, Soil Pollut., 1997, **93**, 157–166.
[25] Jaworska M., Gorczyca A., Sepioł J., Szeliga E. and Tomasik P.: *Metal-metal interactions in biological Systems. Part V. Steinernema carpocapsae (Steinernema) and Heterorhabditis bacteriophora (Heterorhabditidae) entomopathogenic nematodes*. Water, Air, Soil Pollut., 1997, **93**, 213–223.
[26] Jaworska M. and Tomasik P.: *Metal-metal interactions in biological Systems. Part VI. Effect of some metal ions on mortality, pathogemicity and reproductivity of Steinernema carpocapsae and Heterorhabditis bacteriophora entomopathogenic nematodes under laboratory conditions*. Water, Air, Soil Pollut., 1999, **110**, 181–194.
[27] Jaworska M. and Ropek D,: *Możliwość podwyższania patogeniczności i reprodukcji owadobójczych nicieni przez jony metali*. Mat. II konf. „Oddziaływanie jonów metali na mikroorganizmy", AR, Kraków 1997.
[28] (http://www.nettax.com.pl/serwis/publikatory/mp/2003/Nr 38/poz.562/zall.0.htm).
[29] Orlikowski L.B., Skrzypczak Cz. and Wojdyła A.: *Mikrokrystaliczny chitozan – mechanizm oddziaływania na grzyby chorobotwórcze oraz skuteczność w ochronie roślin ozdobnych*. Zesz. Nauk. AR w Krakowie, 1998, **57**(2), 729–733, 1998.

NIEKTÓRE PROBLEMY BADAWCZE W PRODUKCJI NOWOCZESNYCH PESTYCYDÓW

Katedra Ochrony Środowiska Rolniczego
Uniwersytet Rolniczy w Krakowie

Abstrakt: Skuteczna ochrona roślin polega na zastosowaniu wszystkich dostępnych metod w programie IPM (Integrated Pest Manegement). Metoda chemicznej ochrony ulega znacznym przekształceniom, poszukuje się nie tylko nowych skutecznych substancji biologicznie czynnych, ale również wzrosły wymagania co do ich bezpieczeństwa biologicznego dla organizmów niedocelowych i całego środowiska. Bez właściwej formy użytkowej nie można liczyć na sukces. Nowe formulacje i mikrokapsułki, dyspersje olejowe czy agrożele mają być biodegradalne i niezanieczyszczające środowisko. Badania naukowe idą również w kierunku poszukiwania substancji wspomagających działanie pestycydów, jak np. kapsaicyna, naturalny składnik papryki, zwiększająca toksyczność niektórych insektycydów. Preparaty przyjazne dla środowiska to produkty na bazie degradacji chitozanu, przydatne szczególnie w uprawach ekologicznych i ochronie cennych roślin ozdobnych przed chorobami. Biopestycydy, jak np. preparaty zawierające żywe nicienie owadobójcze do ochrony roślin przed szkodnikami, tworzone są w Polsce i na świecie z wykorzystaniem nowych gatunków i szczepów, przy udziale metod biotechnologicznych.

Słowa kluczowe: pestycydy chemiczne, biopestycydy, ochrona środowiska

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Teresa BANASZKIEWICZ[1]

EFFECT OF REDUCTION PHOSPHORUS CONTENT IN DIETS AND ADDITION OF PHYTASE PREPARATION ON PHOSPHORUS AND NITROGEN EXCRETION IN BROILER CHICKENS

WPŁYW OBNIŻENIA ZAWARTOŚCI FOSFORU W MIESZANKACH ORAZ DODANIA PREPARATU FITAZY NA WYDALANIE FOSFORU I AZOTU PRZEZ KURCZĘTA BROJLERY

Abstract: The objective of the study was to determine whether reduction in phosphorus content in wheat-soybean-rapeseed-based diets and an addition of a phytase preparation to a low phosphorus mixture will influence phosphorus and nitrogen excretion in broiler chickens. The experiment was carried out on 60 one-day-old broiler chickens divided into three groups at 20 birds each (4 replications, 5 birds each), and were conduced over the period from the 1st to the 21st day of age. Group I (control) – chickens were fed a starter wheat-soybean-rapeseed-based diet containing 7.26 g/kg total phosphorus. Group II diet had total phosphorus content reduced to 5.91 g/kg. Group III was offered the same mixture as group II but supplemented with an enzymatic preparation containing phytase in an amount of 0.35 g/kg. Nitrogen and phosphorus balances were determined in the last week of the experiment. Chicken body weight at 21 days of age was similar in all the groups. Reduction in the total phosphorus content of the mixture offered to group II resulted in lower phosphorus content in feces. The phosphorus amount excreted by one bird over three days of feces collection and per one day was significantly lower for the experimental groups than the control group. Reduced phosphorus content in the mixture as well as phytase addition to the low phosphorus diet did not significantly influence nitrogen excretion. Despite lack of differences in the amount of nitrogen excreted, reduced phosphorus content in the diets was followed by lower phosphorus excretion in feces.

Keywords: phosphorus, nitrogen, extraction balance, broiler chickens

Animal production is to a large extent responsible for nitrogen- and phosphorus-related pollution of the environment. It follows from the fact that nutrients are excreted to the environment, also in poultry feces. One of important ways of limiting nutrient excretion is their rational use in animal diets. By limiting the amount of phosphorus in rations the excretion of this element with poultry feces can be reduced by about 1/3 [1]. Plant feeds (mainly grains, seeds and extracted meals), which are rich in phosphorus,

[1] Departament of Animal Nutrition, University of Podlasie, ul. B. Prusa 14, 08-110 Siedlce, Poland, email: banaszt@ap.siedlce.pl, tel 25 6431342

constitute basic ingredients of feed mixtures for poultry. However, about 2/3 of phosphorus in plant feeds is phytate phosphorus which is unavailable to chickens and, as a result, excreted to the environment. Phytates can also negatively influence the availability of other minerals as well as protein [2]. Protein, phosphorus and other minerals from phytate compounds can become available only after enzymatic hydrolysis. Some feeds, like wheat bran for example, contain considerable amounts of plant phytase (1200 to 5000 U/kg) which improves phosphorus utilization. Other feeds either do not contain phytase or contain marginal amounts of it. The phytase content in rapeseed-based feeds is minimal and ranges from 15 to 50 U/kg [3]. An application of enzymatic preparations containing microbial phytase in feeds and mixtures improves phosphorus release from 5 to 25 % [4]. Żyła and Koreleski [5] found a positive effect of phytase obtained from *Aspergillus niger* on utilization by broiler chickens of phytate phosphorus from rapeseed extracted meal. In contrast, Rutherfurd et al [6] found no significant effect of an addition of phytase on ileal digestion of total and phytate phosphorus in broiler chickens fed a diet including rapeseed meal. Inorganic phosphorus in the form of feed phosphates is added to poultry mixtures in order to supplement phosphorus deficiency. In practice they are quite often applied in excess. Microbial phytase production may limit the amount of phosphates included in mixtures and phosphorus excretion to the environment by 4–9 to as much as 15 %. Appropriate levels of ingredients in mixtures can be used to influence excretions of unused compounds of nitrogen, phosphorus and microelements in particular [7, 8].

Studies were undertaken to determine whether reduction in phosphorus content in wheat-soybean-rapeseed-based mixtures and an addition of a phytase preparation to a low phosphorus mixture will influence phosphorus and nitrogen excretion in broiler chickens.

Materials and methods

The investigations were carried out on 60 one-day-old broiler chickens Ross 308 which were divided into three homogeneous experimental groups, 20 birds in a group (4 x 5 birds) in period from the 1st to the 21st day of age. Chickens were housed in metabolic cages and fed isocaloric and isoprotein experimental starter diets. Diets (in a mashed form) and water were given *ad libitum*. All diets were formulated according to the Nutritional Requirements for Poultry [9]. The chickens from control group were given experimental starter diet based on wheat (61 %), soybean meal (20.75 %), rapeseed cake of Lirajet cv. (15 %) with higher concentration of phosphorus (mixture H – 7.26 g of phosphorus/kg). In the mixture for group II the phosphorus content was lower due to the dicalcium phosphate reduction (mixture L – about 5.91 g of phosphorus/kg), mixture for group III was supplemented through the enzymatic preparation of phytase, at quantity of – 0.35 g/kg (mixture L + phytase). The enzymatic preparation – is preparation of phytase from *Peniophora lycii (Pers.) Hohn. & Litsch., 1907* derived from the fermentation of fungus *Aspergillus oryzae (Ahlb.) Cohn* (min. 2500 FYT/g). In the 1st and 21 day of life, chickens were weighed and the amount of feed consumed was determined. During the last week of experiment the balance of crude nitrogen and phosphorus was carried out. During the following 3 days, all excreta were collected and feed consumption was determined. Prior to analysis the excreta were dried at 60 °C and ground. In the feeds, diets and excreta the crude nitrogen was determined by the AOAC procedure [10]. The crude phosphorus was determined by colorimetric method (PN-76/R-64781) with eicogenic. The excretion of nitrogen, phosphorus and balance of these elements was assessed. The data obtained were statistically analyzed by analysis of variance and the Duncan's multiple range test was used to separate means (at significance $p \leq 0.05$).

Results and discussion

Table 1 presents nitrogen and total phosphorus contents in both feed materials and experimental mixtures. Crude protein (N) content in the wheat meal was similar to the values specified in the Poultry Nutrition Recommendations [9]. In contrast, soybean extracted meal contained more and rapeseed cake less crude protein than it is specified in the above-mentioned recommendations. The nitrogen content determined in the mixtures was similar whereas total phosphorus content varied (H – 7.26 g/kg vs L – 5.91 g/kg). The body weight of 21-day-old chickens in the groups was similar and ranged from 453 to 493 g. Table 2 shows percentage nitrogen content in feces, the amount of nitrogen excreted by one bird during the balance experiment, as well as an approximate amount of nitrogen excreted by the 15-thousand chicken flock during one day. Percentage nitrogen content in feces ranged from 3.15 %, for the group offered the low phosphorus mixture (L), to 3.45 % for the group fed the high phosphorus mixture (H). No significant differences were noted between the groups as to both the amount of nitrogen excreted by one bird during the balance assay, and the approximate amount of nitrogen excreted during 24 hours by the 15-thousand flock of chickens.

Table 1

Nitrogen and total phosphorus content in feeds and experimental mixtures [g/kg]

Specification	Nitrogen	Total phosphorus
Wheat meal	18.8	3.47
Soybean meal	68.5	6.71
Rape cake of Lirajet cv.	42.9	8.16
Starter mixture (H)	32.5	7.26
Starter mixture (L)	33.0	5.91

Table 2

Amount of nitrogen excreted in broiler chicken feces

Exper. group	Nitrogen content in excreta [%]	Amount of nitrogen excreted during the assay [g/bird]	Amount of nitrogen excreted [g/bird/day]	Approximate amount of nitrogen excreted by 15-thou flock of chickens [kg/day]
I	3.45	2.0 ± 0.17	0.67 ± 0.05	9.99 ± 0.83
II	3.15	1.91 ± 0.27	0.64 ± 0.09	9.57 ± 1.37
III	3.32	1.86 ± 0.24	0.62 ± 0.08	9.31 ± 1.19

Despite lack of significant differences in the amount of nitrogen excreted in the chicken feces in the experiment, decreased phosphorus content in the diet was followed by lower nitrogen excretion in the feces of the birds. An addition of phytase to the low phosphorus mixture resulted in an even lower nitrogen excretion in the feces. The results obtained indicate that, as a result of decreased amount of phosphorus in the wheat-soybean-rapeseed-based mixture, it is possible to reduce the daily amount of nitrogen excreted by the 15-thousand chicken flock by 0.42 kg. An addition of a phytase enzymatic preparation to the above mixture may additionally reduce this amount by 0.26 kg, the sum of the two being 0.68 kg nitrogen per 24 hours.

The lowest phosphorus content (Table 3) was found in the feces of group II chickens fed the low phosphorus mixture (L). Reduction in an amount of total phosphorus in the experimental mixture fed to group II was followed by lower total phosphorus content in the excreta. Supplementation with a phytase enzymatic preparation also reduced this content. The results indicate that, as a result of reducing the phosphorus content in the wheat-soybean-rapeseed-based mixture and its supplementation with the phytase enzymatic preparation, it is possible to reduce the daily amount of phosphorus excreted by a 15-thousand chicken flock by 0.44 kg. Rutkowski et al [11] mention that an addition of phytase to the low phosphorus diet improved phosphorus utilization by 24 % in soybean-based diets, and by 29 % in rapeseed-based diets. A positive effect of microbial phytase on reduction of phosphorus excretion by layer hens was also observed by Kaminska [12].

Table 3

Amount of total phosphorus excreted in broiler chicken feces

Exper. group	Total phosphorus content in excreta [%]	Amount of phosphorus excreted during the assay [g/bird]	Amount of phosphorus excreted [g/bird/day]	Approximate amount of phosphorus excreted by 15-thou flock of chickens [kg/day]
I	6.65	0.39 ± 0.06a	0.13 ± 0.02a	1.95 ± 0.32a
II	4.71	0.29 ± 0.05b	0.096 ± 0.02b	1.44 ± 0.55b
III	5.42	0.30 ± 0.04b	0.101 ± 0.01b	1.51 ± 0.18b

The amount of phosphorus excreted by one bird during the experiment and per 24 hours in the experimental groups was significantly lower compared with the control. Zanini and Sazzad [13] found that an addition of 500 U/kg phytase to the diet containing 40 % phytate phosphorus improved phosphorus, calcium, zinc and nitrogen utilization, and reduced phosphorus excretion. Table 4 presents 24-hour balances of nitrogen and phosphorus as well as retention coefficients for these elements. The amount of nitrogen consumed by one bird ranged from 1587 to 1632 mg per 24 hours whereas for phosphorus the values ranged from 270 mg for group II to 370 mg for the control group. No significant differences were found between groups in the amount of nitrogen consumed and excreted. However, there were detected significant differences in the amount of phosphorus excreted. Significantly less phosphorus was excreted by chickens fed mixtures with decreased phosphorus content compared with the control group. Smulikowska et al [14] report that phosphorus retention from rapeseed cake ranged from 23 to 27 % and did not increase as a result of phytase addition. In another experiment by Smulikowska et al [15], the authors found that retention of phosphorus from diets containing rapeseed cake amounted to about 38 % and did not increase as a result of supplementation of the mixtures with phytase. Nitrogen content in the feces of chickens fed the low phosphorus mixture decreased by 8.69 % and by 3.77 % after an addition of phytase, compared with the feces of chickens offered a diet higher in total phosphorus. The respective values of phosphorus content were 29.17 and 18.49 %.

Table 4

Nitrogen and phosphorus daily balances in broiler chickens

Experim. group	Phosphorus balance [mg/bird/day]		P retention [%]	Nitrogen balance [mg/bird/d]		N retention [%]
	Consumed	Excreted		Consumed	Excreted	
I	370 ± 88	130 ± 22a	63.15 ± 9.07	1632 ± 396	665 ± 57	57.04 ± 12.44
II	270 ± 113	96 ± 36b	61.36 ± 17.8	1587 ± 705	637.5 ± 271	55.65 ± 18.83
III	280 ± 83	101 ± 31b	63.52 ± 8.17	1640 ± 466	620 ± 170	61.72 ± 6.85

References

[1] Kirchgessner M., Windsich W. and Roth F.X.: Możliwości żywienia zwierząt w zmniejszaniu obciążenia środowiska przez rolnictwo. Wyd. Akad. Roln., Wrocław1994, 3–42.
[2] Kratzer F.H. and Vohra P.: Biochem. Biophys. Acta, 1986, **302**, 316–328.
[3] Eeckhout W. and de Paepe M.: Anim. Feed Sci. Technol., 1994, **47**,19–29.
[4] Jamroz D.: Konf. Nauk.-Techn. nt. Pasze przemysłowe a ochrona środowiska, Lublin 1997, 19–33.
[5] Żyła K. and Koreleski J.: J. Sci. Food Agric., 1993, **61**, 1–6.
[6] Rutherfurd S.M., Chung T.K. and Moughan P.J.: Brit. Poult. Sci., 2002, **43**, 598–606.
[7] Van der Hock K.W.: Environ. Pollut., 1998, **102**(suppl. 1), 127–132.
[8] Kohn R.A., Dou Z., Ferguson J.D. and Boston R.C.: J. Environ. Manage., 1997, **50**(**4**), 417–428.
[9] Normy Żywienia Drobiu: Praca zbiorowa pod red. Smulikowskiej, IFiŻZ PAN, Jabłonna 2005.
[10] AOAC: Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington DC, 1990.
[11] Rutkowski A., Śliwiński B. and Wiąz M.: J. Anim. Feed Sci. 1997, **6**, 533–540.
[12] Kamińska B.Z.: Konf. Nauk.-Techn. nt. Pasze przemysłowe a ochrona środowiska, Lublin 1997, 85–93.
[13] Zanini S.F. and Sazzad M.H.: Brit. Poult. Sci. 1999, **40**, 348–352.
[14] Smulikowska S., Mieczkowska A., Czerwiński J., Weremko D. and Nguyen C.V.: J. Anim. Feed Sci. 2006, **15**, 237–252.
[15] Smulikowska S., Czerwiński J., Mieczkowska A. and Czauderna M.: XVIII International Poultry Symposium PB WPSA, 2006, 122–126.

WPŁYW OBNIŻENIA ZAWARTOŚCI FOSFORU W MIESZANKACH ORAZ DODANIA PREPARATU FITAZY NA WYDALANIE FOSFORU I AZOTU PRZEZ KURCZĘTA BROJLERY

Katedra Żywienia Zwierząt i Gospodarki Paszowej
Akademia Podlaska

Abstrakt: Celem podjętych badań było stwierdzenie czy obniżenie zawartości fosforu w mieszankach pszenno-sojowo-rzepakowych oraz dodanie preparatu fitazy do niskofosforowej mieszanki wpłynie na wydalanie fosforu i azotu przez kurczęta brojlery. Badania przeprowadzono na sześćdziesięciu jednodniowych kurczętach brojlerach podzielonych na 3 grupy po 20 ptaków (cztery powtórzenia po 5 sztuk) w okresie od 1. do 21. dnia życia. Kurczęta z grupy I (kontrolnej) były żywione mieszanką typu starter pszenno-sojowo-rzepakową zawierającą 7,26 g/kg fosforu ogólnego. W diecie skarmianej w grupie II obniżono zawartość fosforu ogólnego do 5,91 g/kg, a grupa III otrzymywała taką mieszankę jak grupa II ale dodatkowo uzupełnioną preparatem enzymatycznym fitazy w ilości 0,35 g/kg. W ostatnim tygodniu doświadczenia określono bilans azotu i fosforu. W tym celu przez trzy kolejne dni zbierano odchody. Masa ciała kurcząt w wieku 21 dni była zbliżona we wszystkich grupach. Obniżenie zawartości fosforu w mieszance dla grupy II spowodowało niższą zawartość fosforu w odchodach. Ilość fosforu wydalanego przez jednego ptaka w okresie trzech dni kolekcji wydalin oraz przez jednego ptaka w ciągu dnia w grupach doświadczalnych była statystycznie istotnie mniejsza niż w grupie kontrolnej. Zmniejszenie zawartości fosforu w mieszance, jak również dodatek preparatu fitazy do niskofosforowej diety nie wpłynął statystycznie istotnie na wydalanie azotu. Mimo braku statystycznie istotnej różnicy w ilości wydalanego azotu, zmniejszenie zawartości fosforu w dietach spowodowało mniejsze jego wydalanie w odchodach.

Słowa kluczowe: fosfor, azot, bilans wydalania, kurczęta, brojlery

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Jerzy CABALA[1], Małgorzata PACHOLEWSKA[2] and Maria DZIUROWICZ[1]

INFLUENCE OF METALLIFEROUS MINERALS ON BIOTIC COMPONENTS OF TOPSOIL IN ZINC-LEAD FLOTATION TAILINGS PONDS

WPŁYW MINERAŁÓW METALONOŚNYCH NA BIOTYCZNE SKŁADNIKI WIERZCHNICH WARSTW SKŁADOWISK ODPADÓW Z FLOTACJI RUD CYNKU I OŁOWIU

Abstract: The mineral components of topsoil in the zinc and lead flotation tailings ponds were analyzed. To examine such mineral components the authors used methods such as scanning electron microscopy (SEM), energy dispersive spectrometry (EDS) and atomic absorption spectroscopy (AAS). The goal of this study was to pay particular attention to the secondary metalliferous mineral phases formed in rhizosphere zones mainly on plant roots and the influence of autochthonous sulphur bacteria and ferric bacteria on the chemical mechanism of waste components. This study aims both to recognize the (bio)chemical change of flotation tailings which is of great significance for the vegetation of plants, fungi and microorganisms and to properly plan the treatment connected with reclamation and phytoremediation of the area affected by waste disposal.

Keywords: Zn-Pb flotation tailings, secondary mineral transformations, autochthonous bacteria.

Mining activity and exploitation of sulphidic metal ores can produce large amounts of waste particularly during the Zn–Pb processing. One of the areas where the exploitation of Zn-Pb ores occurs on a large scale is the Olkusz region. Over 1.3 million Mg of flotation tailings are deposited in the pond per year. Nowadays this settling pond covers more than 130 hectares and it is 37 m above surface level. The waste produced includes considerable quantities of fine-grained metalliferous minerals (Zn, Pb, Fe, Cd, Cu, As, Sb and Tl) with a fraction of up to 0.04 mm (55–81 % of wastes) and a contribution of grains over 0.2 mm amounting to 3.9 % [1]. Because of easy transport by the wind the fine-grained structure allows the waste to become a source of heavy metal pollution for the soil, water and atmosphere within several kilometres of the tailings ponds [2].

[1] Faculty of Earth Sciences, University of Silesia, ul. Będzińska 60, 41-200 Sosnowiec, Poland, email: jerzy.cabala@us.edu.pl

[2] Faculty of Materials Engineering and Metallurgy, Silesian University of Technology, ul. Krasińskiego 8, 40-019 Katowice, Poland, email: malgorzata.pacholewska@polsl.pl

The vegetation in waste disposal sites becomes sparse and it is particularly difficult to cultivate with respect to a deficiency of water as well as the loss of nutrients (K, N and P). However, the negative impact of sulphidic mine waste and tailings on the environment can be significantly reduced in the cases where appropriate reclamation and phytoremediation is applied. Solutions infiltrating through the topsoils of tailings are enriched with chemically active ions such as SO_4^{2-}, Zn^{2+}, Pb^{2+}, Fe^{2+}, Cd^{2+}, Tl^{+} and Cu^{2+} which affect plant roots, symbiotic fungi and microorganisms. In conditions of high stress on metals the plants [3] and symbiotic mycorrhizal colonization [4] initiate their biological immune defences which can reduce the transfer of toxic heavy metals into the root system of plants. The reaction between biotic excretion of roots and fungi and a solution rich with metal ions results in the crystallization of the secondary metalliferous phases [5, 6]. Up to now these phases have been slightly recognized because of difficulties in their identification. As it turns out the best possibilities for their study appear on flotation tailings sites settled by plants and other living organisms. Scanning methods such as BSE and EDS can be used for an examination of the secondary phases formed on a root's rhizoderm [7] to identify the metalliferous phases.

The authors' achievements to date show that bacterial strains of species such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* isolated from tailings sites can have an influence on the ionic concentration level of heavy metals (Fe, Zn, Pb and Cd) in eluate solutions [8, 9]. Simultaneously these bacteria show a considerably low resistance both to metal ions considered to be toxic, Cu^{2+}, Cd^{2+} and Ag^{+} [10] and to residues of flotation reagents on tailings sites [11]. That is why their metabolic activity is reduced.

The chemical change, mainly Acid Waste Drainage (AWD) [5, 6], limits the plant vegetation and the development of biotic components. Submicroscopic mineral phases can be formed as a result of the interaction of metalliferous solutions with excretion of roots and fungi or bacteria metabolism products. Products of the chemical change of unstable metalliferous mineral components taken from tailings can be recognized in the BSE images [7].Their identification can prove that the organic ligands participate actively in the migration of metal ions. The primary aim of this study was to analyse the impact of metalliferous minerals occurring on Zn-Pb ore waste sites on the biotic components of rhizosphere zones and on microorganisms settled on the surface of flotation tailings ponds.

Material and methods

Samples used for study were taken from the scarp and top soils of flotation tailings ponds of ZGH Boleslaw SA located 2 km to the west of Olkusz in May, 2006. The mineralogical composition of tailings was determined using the X-ray diffraction method (XRD) with a 3710 PW Philips X-ray-diffractometer with gas monochromator. Scanning research (SEM) was done using a 30 XL Philips microscope with EDAX analyser and also an S-3400N Hitachi microscope. Accelerating voltage of 15 or 25 kV and the low vacuous mode were applied. XRD and SEM analyses were done in the laboratory at the Faculty of Earth Sciences (University of Silesia) and in the laboratory at Faculty of Materials Engineering and Metallurgy (Silesian University of Technology).

Chemical analyses of heavy metal contents were determined using the atomic absorption spektrometry (AAS) with an M6 SOLAAR spectrometer in the BOL-THERM SA laboratory in Bukowno.

Results and discussion

Mineralogical and chemical composition of flotation tailings

The mineral composition of tailings formed in zinc-lead ore processing is similar to primary ores. X-ray identification (XRD) of the mineral components in the investigated tailings reveals the presence of minerals such as dolomite $CaMg(CO_3)_2$, ankerite $Ca(Fe,Mg,Mn)(CO_3)_2$, calcite $CaCO_3$, quartz SiO_2, illite and montmorillonite $(Na,Ca)_{0,3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot n(H_2O)$, sphalerite α(Zn,Fe)S, galena PbS, marcasite FeS_2, pyrite FeS_2, smithsonite $ZnCO_3$, cerussite $PbCO_3$, monheimite $(Zn,Fe)CO_3$, gypsum $CaSO_4 \cdot 2H_2O$, goethite αFeO(OH), barite $BaSO_4$ and Fe sulphates eg, $FeSO_4 \cdot nH_2O$.

Table 1 shows the chemical analyses. It can be seen that the topsoils of tailings which can be found in the parts of the pond with the earliest deposits are characterised by high contents of Fe, Zn, Pb in comparison with those where deposition occurred later which showed considerably lower contents of the metals mentioned above. The predominant parts of Fe and Zn are bound in unstable sulphide phases, whereas Pb occurs in the secondary carbonate phases, which is proved by the great participation of Pb_{PbO} (Table 1) – all this confirms the results of previous studies [2, 7]. The large concentration of alkaline components in the investigated tailings results in the stabilisation of metals in slightly insoluble carbonate phases. A high content of sulphur bound mainly in marcasite and pyrite can have a significant impact on the chemical mechanism of flotation tailings. The contents of sulphate compounds in tailings occurring in the oldest part of the waste dump is significantly higher and this can result in the chemical or biochemical weathering of minerals. Within an early period of waste disposal the flotation reagents, supporting selective flotation, have a certain influence on the chemical mechanism of tailings.

Table 1

Chemical composition [%] of waste samples taken from topsoils of tailings ponds

Symbol of sample	Zn	Zn_{ZnO}	Pb	Pb_{PbO}	Fe	Fe_{FeO}	FeS_2	S
A-1*	2.98	0.83	1.32	0.94	16.10	2.35	29.60	14.70
A-2**	1.63	0.40	0.64	0.35	13.10	1.48	25.10	13.50
A-11***	0.95	0.20	0.48	0.26	8.35	1.26	15.20	7.39
	S_s	S_{SO_4}	As	Tl	Sb	Cu	CaO	MgO
A-1*	14.40	0.30	0.098	0.0083	0.0063	0.0040	18.20	8.80
A-2**	13.30	0.23	0.085	0.0059	0.0052	0.0044	21.20	9.94
A-11***	7.21	0.18	0.062	0.0021	0.0047	0.0021	24.50	12.30

* – the part of the tailings pond with the earliest waste deposits; ** – the part of the tailings pond with the latest waste deposits; *** – the part of the tailings pond with the mixed waste deposits.

Transformation of mineral composition in rhizosphere zones

Fine-grained mineral components of tailings characterised by the great participation of metalliferous minerals occur in the rhizosphere of root plants (Fig. 1a–f). The biological activity of roots favours the formation of polymineral aggregates on their surface. The aggregates consist of carbonates, clay minerals, Fe oxides, sulphides, Zn, Pb and Fe carbonates and sulphates (Fig. 1a). Zn and Pb, Fe sulphides undergo a secondary chemical change in subsurface layers, and as a result of oxidation SO_4^{2-} ions are released and then they are stabilised in calcium sulphate. Gypsum $CaSO_4 \cdot 2H_2O$ and bassanite $CaSO_4 \cdot 0.5\ H_2O$ crystallize in masses on polymineral aggregations (Fig. 1b) and Fe sulphides. Fe, Pb and Mg sulphates can also be identified. The oxidation and dissolution processes have an influence on the morphology of metalliferous mineral grains which show traces of erosion, leaching and recrystallization (Fig. 1b, d, f). These features imply that they become the source of heavy metal ions and sulphate ions. In the waste sites where tailings are enriched with Fe sulphides AWD can be developed. As a result of this, the secondary sulphates, seldom Fe oxides and hydroxides, crystallize on plant roots, the hyphae of fungi and other organic elements. The development of these processes on a mass scale reduces the ability of plant vegetation to occur.

Effect of metalliferous sulphide minerals on microorganisms

Metalliferous sulphide minerals become the natural habitat for chemolithoautotrophic bacteria which are characterised by the ability to oxidize iron (II) compounds such as *Acidithiobacillus ferrooxidans* and other genera eg, *Leptospirillum* or *Sulfolobus* and also oxidized sulphur compounds eg, *Acidithiobacillus thiooxidans* or *Acidithiobacillus caldus* [12]. That is why the presence of Fe, Zn, Pb and Cd sulphides occurring in flotation tailings can stimulate their metabolic activity through oxidation and solubilization of sulphides in the oxygen-rich top layer of the tailings pond. Bioleaching processes take place where there is a contact between microorganisms adhesion and the surface of the mineral in the exopolimer layer. In Figure 1c the bacterial active cells of B1 = WB1 strain belonging to the *Acidithiobacillus thiooxidans* species are presented. These cells adhere to the sulphur-rich grain. The bacteria were isolated from flotation tailings (A1).

Minerals including iron and sulphur compounds are mainly subjected to bacterial solubilization whose final products can be solutions of Fe(III) compounds and sulphuric acid as well as sulphur compounds such as jarosite as well as elementary sulphur. The oxygen-rich environment favours these processes. Figure 1 d, e, f present BSE images of Fe sulphides (marcasite and pyrite) taken from different layers of flotation tailings ponds (A1) characterised by the intermittant access to oxygen. In the top layer the most intensive chemical and biological weathering of pyrite took place (Fig. 1d). In the study area in a subsurface layer (0.3 m and 3.0 m) of the tailings pond, the biological and chemical leaching of pyrite is reduced (Fig. 1e, f).

Conclusions

Scanning methods used for research into the mineral components of waste deposited in ponds can prove that they undergo essential chemical changes when subjected to oxidation, dissolution and interaction with aggressive solutions which are generated by the biotic components of the flotation tailings.

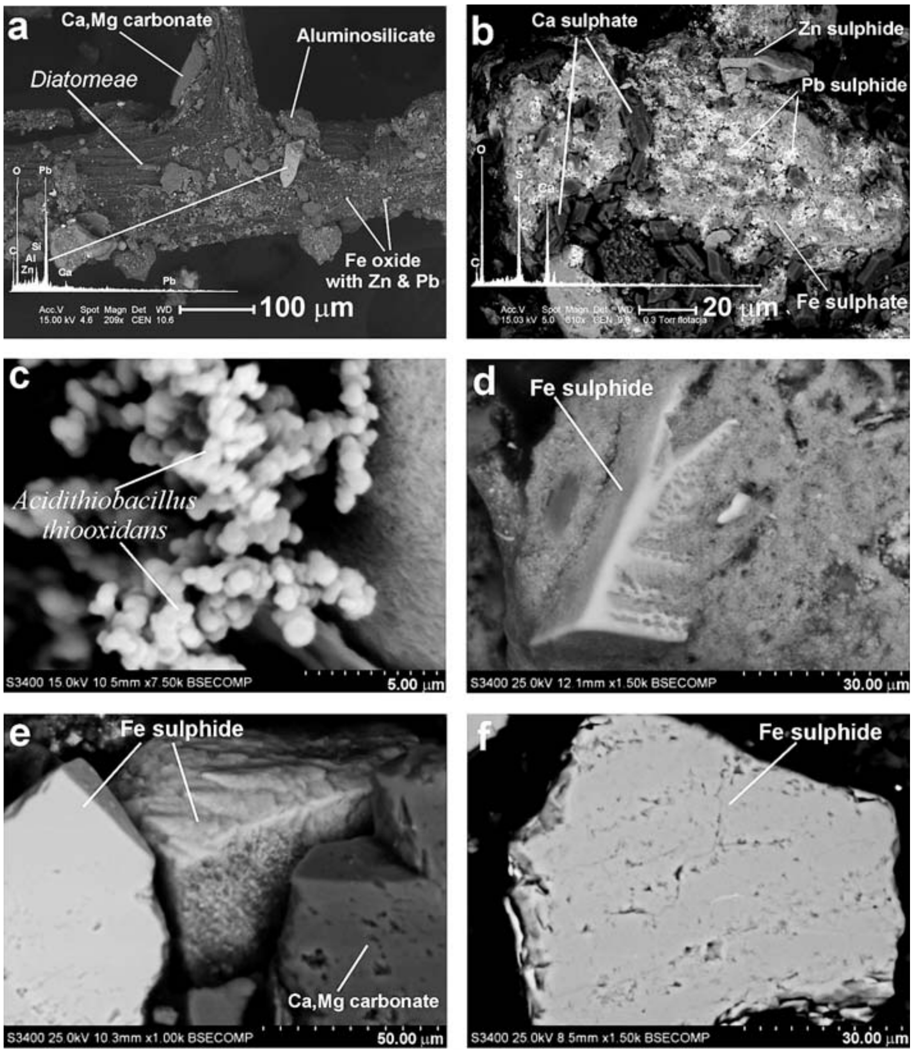

Fig. 1 BSE images of Zn-Pb flotation waste. a – Roots vegetated in the top layer of tailings ponds, b – sulphide aggregates from the top layers of tailings ponds, c – bacterial cells, B1 = WB1 strain *Acidithiobacillus thiooxidans* isolated from flotation tailings – A1 surface layer, d – corroded Fe sulphide crystal, – the place of isolated active sulphide and ferric bacteria, top layers of tailings ponds A1, e – Fe sulphide crystal from 0–0.3 m layers A1, f – surface of Fe sulphide subjected to bacterial medium stimulating metabolic activity of *Acidithiobacillus ferrooxidans* bacteria.

The morphological features of marcasite, pyrite, sphalerite and galena show that in topsoils of a tailings pond the development of the leaching process enriching the solutions with active ions of metals and sulphates occurs.

The development of the secondary mineralization that occurred on plant roots has an influence on the conditions of plant vegetation, microorganisms and fungi. Moreover, it reduces the spontaneous succession of plants and can hinder phytoremediation in sulphide-rich tailings ponds.

The ability to develop autochthonous sulphur bacteria and ferric bacteria diminishes as the quantity of oxide in flotation tailings ponds decreases. The intensive biological weathering processes of sulphide minerals take place mainly in the topsoils of tailings ponds.

Acknowledgements

This work was supported by Commissioned Research Project PBZ-KBN-111/T09/2004 from the Polish funds for science, 2005–2008.

References

[1] Górecka E., Bellok A., Socha J., Wnuk R. and Kibitlewski S.: Przegl. Geol., 1994, **10**, 834–841.
[2] Cabala J., Teper E. and Teper L.: Mine Planning and Equipment Selection 2004. Balkema Publ.: 755–760.
[3] Peer W.A, Baxter I.R, Richards E.L, Freeman J.L and Murphy A.S.: Molecular Biology of Metal Homeostasis and Detoxification. Topics in Current Genetics 2005, vol. 14, Springer, pp. 299–340.
[4] Leyval. C., Turnau K. and Haselwandter K.: Mycorrhiza 1997, **7**, 139–153.
[5] Cabała J.: *Zesz. Nauk. Pol. Śl.* 2005, **1690**(267), 63–70.
[6] Wong J.W.C., Ip C.M. and Wong M.H.: Environ. Geochem. Health 1998, **20**, 149–155.
[7] Cabala J. and Teper L.: Water, Air, Soil Pollut. 2006, **178**(1–4), 351–362.
[8] Pacholewska M., Cabała J., Cwalina B. and Sozańska M.: Rudy Metale 2007, **6**(22), 337–342.
[9] Pacholewska M. and Cabała J.: Ecol. Chem. Eng. A 2008, **15**(1–2), 103–108.
[10] Cwalina B. and Pacholewska M.: Ecol. Chem. Eng. 2009, **15**(9), 901–905.
[11] Pacholewska M., Cwalina B. and Steindor K.: The influence of flotation reagents on sulfur-oxidizing bacteria Acidithiobacillus thiooxidans. Physicochem. Probl. Min. Proc. 2008, **42**, 37–46.
[12] Rawlings D.E.: Microbial Cell Factories 2005, **4**(13), 1–15.

WPŁYW MINERAŁÓW METALONOŚNYCH NA BIOTYCZNE SKŁADNIKI WIERZCHNICH WARSTW SKŁADOWISK ODPADÓW Z FLOTACJI RUD ZYNKU I OŁOWIU

Wydział Nauk o Ziemi, Uniwersytet Śląski
Wydział Inżynierii Materiałowej i Metalurgii, Politechnika Śląska

Abstrakt: Autorzy za pomocą metod elektronowej mikroskopii skaningowej (SEM), mikroanaliz (EDS) oraz analiz chemicznych (AAS) badali składniki mineralne wierzchnich warstw odpadów zdeponowanych na składowiskach poflotacyjnych rud Zn-Pb. Szczególną uwagę zwrócono na wtórne, metalonośne fazy mineralne powstałe w strefach ryzosferowych, na powierzchni korzeni roślin oraz na wpływ autochtonicznych bakterii siarkowych i żelazowych na chemizm odpadów. Przeprowadzone badania są przydatne dla poznania (bio)chemicznych przemian odpadów poflotacyjnych, które mają duże znaczenie dla wegetacji roślin, grzybów i mikroorganizmów. Są one ważne dla prawidłowego projektowania zabiegów rekultywacyjnych i fitostabilizacji składowisk.

Słowa kluczowe: Odpady z flotacji rud Zn-Pb, składowiska odpadów, wtórne przemiany składu mineralnego, autochtoniczne bakterie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Anna CHRZAN[1], Maria MARKO-WORŁOWSKA[1]
and Tomasz ŁACIAK[2]

INFLUENCE OF SELECTED METALS ON SOIL MESOFAUNA OF GRASS HABITATS SITUATED IN DIFFERENT PLACES IN KRAKOW

WPŁYW WYBRANYCH METALI NA MEZOFAUNĘ GLEBOWĄ SIEDLISK TRAWIASTYCH W RÓŻNYCH MIEJSCACH NA TERENIE KRAKOWA

Abstrac: The researches of the four chosen grass habitats influenced by the anthropopressure of different levels were carried out in autumn (three in the center and one on the west outskirts of Krakow). The goal was to define the accumulation of Cd, Pb, Ni, Cu, Zn, Fe, and Mg in the soils of chosen areas as well as moisture, pH and temperature of the soil. The results were analyzed with regards to density and diversity of mesofauna with particular regard to *Diptera* larvae. The soils differentiate clearly in terms of the concentration of the analyzed metals. In one of the habitats in the center of Krakow where the lowest density and differentiation of both mesofauna and *Diptera* larvae were recorded, the highest concentration values of Cd, Pb, Ni, Zn, Fe and Cu were found. The research show that Cd in concentration more than three times higher on this habitat than on the others, has the biggest influence on the mesofauna number. Whereas the influence of the fact that the temperature of the soils, pH and moisture differ slightly was not detected on the density and the diversity of the mesofauna.

Keywords: soil mesofauna, soil *Diptera* larvae, abundance, diversity, metals

The anthropogenic processes such as: different branches of industry, transport, public utilities, fertilization and the use of pesticides are the main cause of the growth of toxic influence of many metals on the environment. The heavy metals deriving from these processes disperse in the environment and pollute air, water, soil and organisms. The soil pollution is considered to be the indicator of environment quality, besides, the ecological effects caused by heavy metals that pollute the soil depend not only on their quantity but also on their chemical forms, their solubility, the content of organic C and

[1] Department of Ecology, Wildlife Research and Ecotourism, Institute of Biology, Pedagogical University of Krakow, ul. Podbrzezie 3, 31-084 Kraków, Poland, email: chrzan@ap.krakow.pl, mmw@ap.krakow.pl

[2] Department of Zoology, Institute of Biology, Pedagogical University of Krakow, ul. Podbrzezie 3, 31-084 Kraków, Poland, email: tlaciak@ap.krakow.pl

sorption qualities of the soil [1]. In the soils with a big content of organic matter and reaction close to neutral the metals are effectively bind and their concentration even with relatively small inflow can rise quickly. Although, when bound, heavy metals are relatively not very harmful and reveal their toxicity at the moment they become soluble forms. At low pH heavy metals are readily soluble and that are easily freed from sorption complex [2, 3]. The presence of above standard concentrations of heavy metals in organisms cause negative mutagenic, carcinogenic, teratogenic effects [3, 4] that prevent them from normal functioning. Cd, Pb, Cr, Zn, Fe and Ni are the elements with high degree of potential danger to the environment [5].

The aim of the research was detection of relations between the number of metal forms (Cd, Pb, Ni, Cu, Zn, Fe and Mg) and quantity and quality of mesofauna with particular regard to *Diptera* larvae in the soils of grass habitats in different places around Krakow.

Materials and methods

For the purpose of the evaluation of the soil contamination with heavy metals and its influence on pedofauna of four sites (three areas situated in Krakow – Srodmiescie and one on the west outskirts) were chosen:

1. the lawn situated near the Vistula river at Podgórska Street;
2. the lawn near route next to Piłsudski bridge;
3. the tree-surrounded lawn in Bednarski Park;
4. the lawn next to the forest in Krakow-Tyniec at Jurandówny Street.

The set of samples was taken on the selected localities in autumn 2007 with the use of the soil cylinder with 10 cm in diameter. The soil cylinder was thrust into the soil to the depth of 10 cm. Each series consisted of 25 samples in the area of around 1 m^2.

Mesofauna was caught by employing the dynamic method width the modified Tullgren apparatus. After marking the selected mesofauna its density and diversity were analysed. Soil moisture and its pH, its temperature as well as the content of Cd, Pb, Ni, Cu, Zn, Fe and Mg were determined.

Dry samples of the soil (2.5 g) were mineralized. For this purpose 20 ml of 65 % HNO_3 was poured over heated to the temperature of 120 °C 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 the solutions prepared in this way the content of heavy metals was determined by atomic absorption spectrometer (AAS - Cole-Parmer, BUCK 200A).

The correlation coefficient between heavy metal contents in soil and number of *Diptera* larvae were calculated with linear type of regression.

Results and discussion

Despite of some differences in pH, the analyzed soils had slightly alkaline reaction [6]. The small differences in moisture and temperature of the soils had not actual influence on the density and diversity of the mesofauna (Table 1, 3).

Table 1

Comparison of selected parameters of the soils in the selected localities in Kraków

Selected parameters	Locality 1	Locality 2	Locality 3	Locality 4
Soil moisture [%]	14.06	17.26	14.28	13.99
Soil pH [–]	6.59	7.07	7.44	7.23
Area temperature [°C]	22.3	19.9	18.6	14.0
Soil temperature [°C]	17.7	18.0	13.7	11.0

The interpretation of the results in terms of the soil pollution degree might be difficult, because there are no norms concerning pollution of the soils of grass habitats by heavy metals. The norms used for cultivated soils are based on the total content of metals [2, 7]. As the research shows, the total quantity of Cd, Pb, Ni, Zn and Fe on position no. 2 was characteristic for polluted cultivated soils (Table 2). This result indicates that there is an influence of distance from industry, routes, forested or tree-covered areas on location of the places with high heavy metals concentration in the soils.

Table 2

Contents of heavy metals in the soils of the selected localities in Krakow [mg/kg]

Metal	Locality 1	Locality 2	Locality 3	Locality 4
Cd	2.051	7.254	2.147	0.944
Pb	197.26	215.923	54.13	46.881
Ni	20.549	25.515	16.18	8.214
Cu	34.08	83.442	15.5	3.418
Zn	10.118	24.192	8.13	3.335
Fe	506.284	747.836	427.73	207.407
Mg	4212.276	3053.807	1359.70	502.575

Locality 1 – the lawn situated near the Vistula river at Podgorska street; locality 2 – the lawn near route next to Pilsudski bridge; locality 3 – the tree surrounded lawn in Bednarski Park; locality 4 – the lawn next to the forest in Krakow-Tyniec at Jurandowny Street.

As the results concerning mesofauna and *Diptera* larvae indicate, quantity of metals analyzed influences concentration of this pedofauna group. On no. 2 site, where the highest concentration of all analyzed metals, apart from Mg, was found, the lowest density of mesofauna and *Diptera* larvae was determined (Table 3).

Table 3

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

Selected parameters	Locality 1	Locality 2	Locality 3	Locality 4
Abundance of pedofauna [sp.no.per m^2]	8600	5100	6235	6389
Abundance of larvae *Diptera* [sp.no.per m^2]	725	275	1100	1125
Diversity (number of taxonomic groups)	17	13	15	18

The analysis of the results show that the influence of the analyzed elements presented in the soil on *Diptera* larvae density is significant (Table 4).

Table 4

The correlation coefficient between heavy metals contents in soil and number of *Diptera* larvae R^2

Metal	Cd	Pb	Ni	Zn	Fe	Mg	Cu
R^2	0.8368	0.8513	0.8012	0.9097	0.8553	0.9066	0.9686

References

[1] Skwaryło-Bednarz B.: *Ogólna zawartość wybranych metali ciężkich w glebach leśnych Roztoczańskiego Parku Narodowego (RPN)*. Acta Agrophys., 2006, **8**(3), 727–733.

[2] Uziak S., Klimowicz Z., Chodorowski J. and Melke J.: *Badania zanieczyszczeń gleb Parku Krajobrazowego Lasy Janowskie*. Ann. Univ. MC-S., Lublin 1999, S.B, vol. LIV, 11.

[3] Waśniewski S.: *Zawartość i fitoprzyswajalność wybranych metali ciężkich w glebach regionu Płaskowyżu Kolbuszowskiego i Podgórza Rzeszowskiego*. Zesz. Probl. Post. Nauk Roln. 2004, **501**, 158–161.

[4] Gruca-Królikowska S. and Wacławek W.: *Metale w środowisku cz. II. Wpływ metali ciężkich na rośliny*. Chem., Dydakt., Ekol. Metrol., 2006, **11**(1–2), 41–54.

[5] Gambuś F. and Gorlach E.: *Ocena i stan zanieczyszczenia gleb w Polsce*. Aura, 2001, (7), 10–11.

[6] Ostrowska A., Gawliński S. and Szczubiałka Z.: Metody analizy i oceny gleb i roślin. 1991. Inst. Ochr. Środ. Warszawa, 47–50.

[7] Kabata-Pendias A., Motowicka-Terlak T., Piotrowska M., Terelak H. and Witek T.: Ramowe wytyczne dla rolnictwa. Wyd. IUNG, Seria P[53], Puławy 1993.

WPŁYW WYBRANYCH METALI NA MEZOFAUNĘ GLEBOWĄ SIEDLISK TRAWIASTYCH W RÓŻNYCH MIEJSCACH NA TERENIE KRAKOWA

Zakład Ekologii, Badań Łowieckich i Ekoturystyuki, Instytut Biologii,
Uniwersytet Pedagogiczny w Krakowie
Zakład Zoologii Kręgowców, Instytut Biologii, Uniwersytet Pedagogiczny w Krakowie

Abstrakt: Badania przeprowadzono w okresie jesiennym na czterech wybranych stanowiskach trawiastych (trzy w centrum i jedno na zachodnich obrzeżach Krakowa) będących pod wpływem różnego stopnia antropopresji.

Określono akumulację pierwiastków: Cd, Pb, Ni, Cu, Zn, Fe i Mg w glebach stanowisk, a także ich wilgotność, odczyn oraz temperaturę. Otrzymane wyniki opracowano w odniesieniu do zagęszczenia i zróżnicowania mezofauny, ze szczególnym uwzględnieniem larw muchówek *Diptera*. Gleby stanowisk zasadniczo różniły się koncentracją badanych metali. Na jednym ze stanowisk w centrum Krakowa, gdzie stwierdzono najmniejsze zagęszczenie i zróżnicowanie mezofauny, w tym również najmniejsze zagęszczenie i różnorodność larw Diptera odnotowano największą zawartość Cd, Pb, Ni, Zn, Fe i Cu. Z badań wynika, że największy wpływ na liczebność mezofauny ma kadm, którego koncentracja jest na ww. stanowisku ponad trzykrotnie większa niż na pozostałych. Nie stwierdzono natomiast wpływu niewielkich różnic w temperaturze gleb, wartości pH, a także wilgotności na zagęszczenie i zróżnicowanie mezofauny.

Słowa kluczowe: mezofauna glebowa, larwy *Diptera*, zagęszczenie, różnorodność, metale

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Joanna DŁUŻNIEWSKA[1] and Maria NADOLNIK

OCCURRENCE AND HARMFULNESS OF FUNGAL DISEASES ON ROSE BUSHES CULTIVATED IN KRAKOW PART II. BLACK SPOT (*Diplocarpon rosae*) INFECTION

WYSTĘPOWANIE I SZKODLIWOŚĆ CHORÓB GRZYBOWYCH NA KRZEWACH RÓŻ UPRAWIANYCH NA TERENIE KRAKOWA CZ. II PORAŻENIE PRZEZ CZARNĄ PLAMISTOŚĆ (*Diplocarpon rosae*)

Abstract: The aim of the paper was to determine the occurrence of black spot on rose bushes growing in convent gardens and in a Krakow park. The research was conducted in 2002–2004. In the selected points black spot posed the gravest hazard in 2002. In all years of the research rose in the Carmelite convent garden and in Polish Aviators' Park were most strongly infected by *Diplocarpon rosae*. The diseases were the least intensified in the St. Bernard monastery garden and in Cistercians' garden, except the year 2002.

Keywords: roses, rose black spot, city green areas, air pollution

Krakow is one of large European cities with the worst air quality [1]. The main element of the urban landscape, which also alleviates the effects of pollution are urban green areas. Green areas are capable of capturing a considerable part of gaseous and dust pollutants from the air [2]. Plants respond to air pollution by their shoot injuries and growth inhibition and even by die-back [3]. Pollutants may also affect the interactions between host plants and pathogens. These substances may directly influence the growth and development of mycelium, and spore germination, but also fungal pathogenicity. Pollutant compounds originating from the atmosphere may affect pathogens directly or indirectly through a change of host plant susceptibility [4].

In compliance with the act of 16th April 2004 [5] the aim of environmental protection is among others protection of city green areas involving maintenance, sustainable use and restoration of green resources and elements. Green areas in cities fulfil aesthetic, recreational, health and protective functions. Green areas on the estates included in the historic monument register are particularly important. In Krakow such places include *eg* convent gardens.

[1] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrdluzni@cyf-kr.edu.pl

Rose black spot caused by *Diplocarpon rosae* Wolf fungus is a common and dangerous rose disease, which generally causes considerable losses on plantations. Recommendations on its control prove little efficacious in practice. As a result numerous rose bushes lose leaves already in midsummer. Infection by *D. rosae* greatly diminishes decorative qualities of the plants [6–9].

The present study aimed to determine the occurrence of black spot on rose beds situated in convent gardens and a park in Krakow.

Material and methods

The research was conducted in 2002-2004 on rose beds in four convent gardens of the following orders: Carmelite Sisters (40, Łobzowska str.), Felician Sisters (6, Smoleńsk str.), St. Bernard's order (2, Bernardyńska str.), Cistercians' order (11, Klasztorna str.) and in Polish Aviators' Park (John Paul II Av.). Only the first three gardens are located in the very centre of Krakow, whereas Cistercians' garden and Polish Aviators' Park are locates in the eastern part of the city in a sparsely build-up area.

The research methods were presented in the paper by Dłużniewska and Nadolnik [9].

Results

The weather conditions during the period when the research was conducted were presented in the paper by Dłużniewska and Nadolnik [9]. The lowest average temperature in the period from May to August was registered in 2004. Also considerable differences in precipitation amounts were observed in the respective years of the research. In 2002 the highest rainfall was noted in July when also excessive humidity occurred. Superfluous rainfall was also noticed in October of the same year. The year 2003 was characterized by the most unfavourable precipitation distribution. Excessive humidity was noted in May and July, whereas in June, August and September too little humidity was registered. In the third year of the research the dependencies between the precipitation amount and the temperature were favourable and the humidity was optimal for the entire vegetation season, except September.

Data on air pollution were provided by the Department of Environment Monitoring and Automatic Air Analyses Laboratory in Krakow (Table 1) [10]. The results from the Main Square station referred to the convent gardens of Carmelite Sisters, Felician Sisters and St. Bernard's order. Polish Aviators' Park and Cistercians' garden are located within the Nowa Huta station range. The measuring stations did not register all selected pollutants over the investigated period. No data were available from August to October 2004 because of modernization of monitoring network, communication and data processing systems. SO_2 concentration was similar at both measuring points. In the years 2002 and 2003 the suspended particulate matter, NO and NO_2 concentrations were higher in Nowa Huta. The admissible level for airborne substance – 40 $\mu g \cdot m^{-3}$ was exceeded. In 2002 the norm was exceeded four times, in 2003 three times and once in 2004. At the Main Square the exceeded values for suspended particulate matter were observed in April 2002 and 2003.

Table 1.

Air pollution during the research period in 2002–2004 annual average [11]

Month	SO_2 [µg/m^3]			PM [µg/m^3]			NO [µg/m^3]			NO_2 [µg/m^3]		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Main Square measuring station												
April	13	14	11	48	45	–	–	4	–	–	28	–
May	9	10	6	37	36	–	–	4	–	–	23	–
June	8	8	6	32	32	–	–	4	–	–	24	–
July	9	7	6	32	28	–	–	4	–	–	22	–
August	7	7	–	39	32	–	–	5	–	–	26	–
Sept.	7	9	–	36	39	–	8	14	–	22	32	–
Oct.	11	14	–	38	38	–	22	17	–	30	32	–
Nowa Huta measuring station												
April	11	14	–	55	51	55	10	8	11	32	30	30
May	8	7	–	37	40	28	8	8	8	25	28	24
June	7	9	–	28	34	32	9	9	9	24	30	29
July	10	7	–	39	30	33	7	11	12	24	27	28
August	9	9	–	59	39	–	10	11	–	25	31	–
Sept.	7	9	–	63	54	–	12	25	–	26	34	–
Oct.	10	11	–	55	42	–	34	24	–	29	29	–

In 2002 black spot symptoms were noticed by the end of May on all investigated sites except the Carmelite Sisters' garden (Table 2). The disease was exacerbating significantly at subsequent dates. In mid-August the infection index by *D. rosae* increased rapidly. In Polish Aviators' Park this index reached 100 %. By the end of August 100 % of infected plants were observed in Carmelite Sisters' and Cistercians' gardens and in Polish Aviators' Park. The plants were rapidly loosing leaves. Such fast development of the disease might have been caused by too much precipitation in July and resulting excessive humidity. Significantly smallest infection was observed in St. Bernard's monastery garden.

Table 2

Dynamics of rose infection by *Diplocarpon rosae* in 2002

Date of observation 2002	Infection index [%] in respective observation points				
	Carmelite Sisters	Felicjan Sisters	St. Bernard's monastery	Cistercian monastery	Polish Aviators' Park
31.05	0.0 a	11.7 a–e	7.7 a–c	13.3 a–f	8.3 a–d
14.06	5.7 b	20.0 b–g	21.0 c–g	24.3 e–i	15.0 b–f
30.06	10.0 a–e	35.7 h–k	21.0 c–g	39.7 j–m	35.7 n–k
15.07	24.3 e–i	43.0 k–o	22.7 d–h	55.7 n–g	50.0 l–p
30.07	37.7 i–l	52.0 m–p	27.7 f–j	55.7 n–g	56.7 o–q
14.08	71.3 r	66.3 q–r	32.7 g–k	63.3 p–r	100.0 s
28.08	100.0 s	72.3 r	42.3 k–h	100.0 s	100.0 s

In 2003 the disease symptoms occurred later than in the first year of the research (Table 3). In Carmelite Sisters' garden and in Polish Aviators' Park rose leaves with black spots were noticed at the beginning of June. On the other hand in the gardens of St. Bernard's and Cistercians' monastery the disease symptoms appeared only in the first decade of July. Later in the vegetation season black spot infection of roses was increasing significantly. By the end of September bushes growing in the Carmelite Sisters' garden were apparently the most heavily infected by the pathogen. The plant infection index exceeded 83 %. Very strong infection was also noted in Polish Aviators' Park, where the index reached the level of about 77 %. Notably healthiest roses were cultivated in St. Bernard's and Cistercians' monastery gardens.

Table 3

Dynamics of rose infection by *Diplocarpon rosae* in 2003

Date of observation 2003	Infection index [%] in respective observation points				
	Carmelite Sisters	Felicjan Sisters	St. Bernard's monastery	Cistercian monastery	Polish Aviators' Park
6.06	21.7 ef	3.3 ab	0.0 a	0.0 a	11.7 c
20.06	29.2 gh	20.0 de	0.0 a	0.0a	36.7 ik
9.07	43.3 l	25.4 fg	5.8 b	2.8 ab	42.2 l
20.07	54.2 m	30.0 h	24.6 f	16.9 d	51.7 m
15.08	60.4 n	36.2 ij	32.4 hi	30.0 h	55.3 m
30.08	68.3 op	43.3 l	31.2 h	33.9 hj	66.9 o
15.09	79.6 q	50.9 m	36.2 ij	37.5 jk	71.7 p
30.09	83.3 r	55.4 m	40.8 kl	42.0 l	77.3 q

In 2004 black spot symptoms were visible at the beginning of June (Table 4). The disease was developing gradually in subsequent months. At the beginning of October significantly most serious infection by black spots was observed in the Carmelite Sisters' garden and in Polish Aviator's Park.

Table 4

Dynamics of rose infection by *Diplocarpon rosae* in 2004

Date of observation 2004	Infection index [%] in respective observation points				
	Carmelite Sisters	Felicjan Sisters	St. Bernard's monastery	Cistercian monastery	Polish Aviators' Park
11.06	22.5 c–g	13.3 a–d	4.2 ab	0.9 a	4.2 ab
27.06	17.5 b–e	22.5 c–g	23.3 c–f	10.9 a–c	28.3 c–g
14.07	29.2 d–j	30.9 e–j	25.9 c–f	22.5 c–g	30.0 c–h
4.08	35.0 f–j	42.5 i–k	29.2 d–h	27.5 c–f	30.5 d–j
28.08	35.5 f–j	43.0 i–k	30.5 d–j	27.0 c–f	35.6 f–j
18.09	37.5 g–k	45.0 jk	37.5 f–i	27.5 d–i	40.5 g–k
1.10	62.5 l	52.5 k–l	42.5 i–k	30.0 e–j	64.5 l

Discussion

The research confirmed that black spots belong to the most serious rose diseases. The pathogen considerably diminishes the plant decorative qualities and causes premature defoliation of bushes [7, 12]. On the analyzed sites, black spots posed the gravest hazard in 2002. In all investigated years roses were the most seriously infected by *D. rosae* in Carmelite Sisters' convent garden and in Polish Aviators' Park. On the contrary, the disease was the least exacerbated in St. Bernard's monastery garden and in Cistercians' garden, except the year 2002.

D. rosae infestation is visible as black round or irregular spots with frayed edges. The tissue around the spots is yellowing and the leaves fall prematurely. The leaves from the lower part of bushes fall first, then those from the upper part. Bushes are less frost resistant, have less vitality and die faster. Weakened plants are easily infested by other fungi pathogens and pests [7, 8, 11].

Development of the disease is connected with atmospheric conditions. Wet weather during summer favours infection. Germination of *D. rosae* spores, therefore rose infection by black spots occurs when the leaves are moistened for 9–18 hours [3, 13–14].

Infection may be also affected by pollutants originating from the atmosphere, which in host plants lead to changes, such as a change of wettability and chemical composition of the surface and a change of leaf secretion products [4].

Air pollution may also influence plant-pathogen-endophyte interactions through a change of the species composition of endophyte communities, which may be antagonistic to pathogenic fungi [15]. Yeast, which is an antagonist of leaf pathogens is particularly sensitive to SO_2. It was noticed that at low concentrations of SO_2 (< 50 ppb) yeast *Sporobolomyces roseus* and *Cryptococcus laurentii* reduced *Alternaria brassicae* infection in the cruciferous. On the other hand at SO_2 concentration over 100 ppb an apparently increased plant infection was registered. The results of the experiment show that high concentrations of SO_2 reduce the activity of saprophytic yeast, which favours plant pathogen infections [4].

Nadolnik [2] observed that lesions on rose leaves caused by black spot were less intensified on bushes growing on the site with high traffic density. *D. rosae* fungus is counted among species sensitive to high pollutant concentrations [16].

The effect of air pollution on plant pathogenic fungi depends on many factors, such as: concentration, amplitude of daily seasonal concentrations, time of activity composition of vegetation cover and degree of its injury by phytotoxic components of pollution, land topography, climatic conditions, soil and habitat quality and sensitivity of the pathogens themselves [16].

Monitoring of the healthiness of plants cultivated in urban green areas is useful for making decisions concerning cultivation measures to maintain plants in good condition. Damage due to improper care of green areas may result in a penalty imposed by the president of the city [17].

References

[1] Bach A. and Pawłowska B.: Ecol. Chem. Eng., 2007, **14**(9), 911–917.
[2] Nadolnik M.: Chem. Inż. Ekol., 1998, **5**(8–9), 743–749.

[3] Bell J.N.B. and Treshow M.: Zanieczyszczenie powietrza a życie roślin. WNT, Warszawa 2004, 526 pp.
[4] Flückiger W., Braun S. and Hiltbrunner E.: [in:] Zanieczyszczenie powietrza a życie roślin. Bell J.N.B. and Treshow M. (eds.), WNT, Warszawa 2004, 425–456.
[5] Wojdyła A., Kamińska M., Łapanowski G. and Orlikowski L.: Ochrona róż, Plantpress, Kraków 2002.
[6] Czyżewski J.A.: Choroby i szkodniki roślin ozdobnych, PWRiL, Warszawa 1975.
[7] Kowalik M., Pokora B. and Chimowska B.: Róże w chorzowskim rosarium, ich patogeny a człowiek, Ochrona Środowiska Naturalnego w XXI w. – nowe wyzwania i zagrożenia, Wiech K., Kołoczek H. and Kaszycki P. (eds.), Fundacja na rzecz wspierania badań naukowych Wydz. Ogr. AR, Kraków 2005.
[8] Czekalski M. and Żyła M.: Hasło Ogrodn., 1992, 10, 21–22.
[9] Dłużniewska J. and Nadolnik M.: Ecol. Chem. Eng. A, (in press).
[10] Informacja o zanieczyszczeniu powietrza w województwie Małopolskim w okresie IV–X 2002, 2003, 2004 roku (system automatycznego monitoringu powietrza). Oprac. Wydział Monitoringu Środowiska i Pracownia Badań Automatycznych Powietrza, Kraków.
[11] Cardillo R.: Horticulture, 2002, (5/6), 18 p.
[12] Carlson-Nilsson B.U. and Davidson C.G.: Acta Horticult., 2000, **508**, 141–147.
[13] Wojdyła A.: Hasło Ogrodn., 1997, (6), 47–48.
[14] Wiśniewska-Grzeszkiewicz H., Wojdyła A. and Rejman S.: Zesz. Nauk. Inst. Sadow. Kwiac., 1999, (6), 189–199.
[15] Magan N. and Smith M.K.: Phyton – Ann. Rei Bot., 1996, **36**(3), 103–110.
[16] Grzywacz A.: Ochr. Rośl., 1973, **XVII**(7), 22–23.
[17] Ustawa z dnia 16 kwietnia 2004 r. o ochronie przyrody, DzU z 2004 r. Nr 92, poz. 880.

WYSTĘPOWANIE I SZKODLIWOŚĆ CHORÓB GRZYBOWYCH NA KRZEWACH RÓŻ UPRAWIANYCH NA TERENIE KRAKOWA CZ. II PORAŻENIE PRZEZ CZARNĄ PLAMISTOŚĆ (*Diplocarpon rosae*)

Katedra Ochrony Środowiska Rolniczego, Uniwersytet Rolniczy w Krakowie

Abstrakt: Celem pracy było określenie występowania czarnej plamistości na skwerach różanych położonych w trzech ogrodach przyklasztornych i jednym parku Krakowa. Badania prowadzono w latach 2002–2004. W wybranych punktach największe zagrożenie czarna plamistość stanowiła w 2002 r. We wszystkich latach badań najbardziej opanowane przez *D. rosae* były róże w ogrodzie Karmelitanek i Parku Lotników Polskich. Natomiast w najmniejszym nasileniu choroby obserwowano w ogrodach Bernardynów i z wyjątkiem 2002 r. u Cystersów.

Słowa kluczowe: róże, czarna plamistość, zieleń miejska, zanieczyszczenie powietrza

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Urszula DOPIERAŁA[1]

EFFECT OF COPPER AND TEMPERATURE ON THE GROWTH AND CHLOROPHYLL CONTENT OF SCENTLESS MAYWEED (*Tripleurospermum indorum* (L.) SCHULTZ-BIP.) ORIGINATED FROM VICINITY OF GLOGOW COPPER SMELTER

WPŁYW MIEDZI I TEMPERATURY NA WZROST I ZAWARTOŚĆ CHLOROFILU U MARUNY BEZWONNEJ (*Tripleurospermum indorum* (L.) SCHULTZ-BIP.) POCHODZĄCEJ Z OKOLICY HUTY MIEDZI „GŁOGÓW”

Abstract: Scentless mayweed (*T. indorum* (L.) Schultz-Bip., *Asteraceae*), as the dominant weed in winter cereals, on account of producing big biomass makes dangerous concreation for the crops cultivated in the vicinity of GLOGOW Copper Foundry (GCF) situated in the Silesia region. The aim of this study was to compare the influence of increasing doses of copper in two temperature ranges on the growth and chlorophyll content among biotypes of scentless mayweed coming from the vicinty of GCF and Wielkopolska province. The experiments were conducted in greenhouse conditions in two temperature ranges: 40/10 °C and 25/5 °C (day/night). The seedlings of *T. indorum* were grown in pots filled with the soil including different doses of copper ($CuSO_4 \cdot 5H_2O$): 0, 50, 100, 200, 400, 600 and 800 mg Cu/kg of dry soil. The higher doses of copper inhibited the shoot growth and decreased leaf chlorophyll content. The reduction rate of both parameters was considerably slower in lower temperatures.

Keywords: Chlorophyll, copper, plant growth, industrial pollution, *Tripleurospermum indorum*

In the Lower Silesia province of Poland, agricultural areas situated in the vicinity of GLOGOW Copper Foundry (GCF) have been polluted by heavy metals included in the dust emitted into the atmosphere. Within the last ten years, thanks to intensive modernizing activities by GCF, the condition of agricultural environment got radically better. At present, increased contents – mainly of copper and lead – are detected in cultivated fields situated in the nearest vicinity of the copper foundry [1]. The results of the latest

[1] Department of Ecology and Protection of Agricultural Environment, Institute of Plant Protection-National Research Institute, ul. Władysława Węgorka 20, 60-318 Poznań, Poland, email: u.dopierala@ior.poznan.pl

investigations showed that over 80 % of total concentration of Cu in a soil arable layer is bound in the fraction hardly available to plants [2].

In the region of GCF 60–70 % of cultivated crops are cereals. Scentless mayweed, as the dominant dicotyledonous weed in winter cereals, on account of producing big biomass, makes dangerous competition for these crops. In agricultural environment weeds and crops are often exposed to the co-occurrence of different abiotic stresses such as heat, drought, cold, salt, high light, mechanical injuries or abundance of heavy metals [3].

The aim of this study was to compare the influence of increasing doses of copper in two temperature ranges on the growth and chlorophyll content among biotypes of scentless mayweed coming from the vicinity of GCF and the Wielkopolska province.

Material and methods

Seeds of *T. indorum* (L.) Schultz-Bip., *Asteraceae* were collected from two cultivated fields (marked as G-1 and G-2) in the vicinity of GCF in the Lower Silesia province. Comparative seeds were collected from the fields in Skoków (Sw) in Wielkopolska province (about 120 km far from Glogow). The level of copper content in soils from which seeds were collected amounted to 223, 88 and 28 mg/kg of dry matter of soil respectively for localizations G-1, G-2 and Sw. Polish standard concentration of copper in soil – according to the law: Journal of Laws, No. 165 Item 1359 – amount to 150 mg/kg of dry matter of soil.

Seedlings obtained from seeds collected near Glogow (G-1, G-2) and near Poznan (Sw) were grown in greenhouse conditions. Plants were grown in pots filled with mix of compost soil and sand (2 : 1) including different doses of copper (as $CuSO_4 \cdot 5H_2O$): 0, 50, 100, 200, 400, 600 and 800 mg Cu/kg of dry soil. In such prepared soils pH in 1M KCl ranged from 7.02 to 6.71 according to the dose of copper. Four pots per treatment were used, with 5 seedlings in each pot. Each experimental combination was made in two replications. After four weeks, the aboveground parts (shoots) of plants were cut and the fresh weight [in g] of 5 shoots per pot was estimated. Presented results are the average of eight measurements for each combination of copper level.

Experiments were conducted in two terms: in summer – with the temperature ranges of 40/10 °C (day/night) and in autumn with temperature 25/5 °C (day/night).

Chlorophyll content was determined in 80 % acetone extract, using spectrophotometer measuring the absorbeance at λ = 645 and 663 nm (UV/VIS Helios alfa, UNICAM, Great Britain). Chlorophyll $a + b$ were calculated according to Arnon method [4]. Results are presented as arithmetical average from two series in which two parallel analyses were made. Statistical evaluation has been made using the analysis of variance, ANOVA test.

Results and discussion

Both shoot growth and chlorophyll content in leaves of the plants from Glogow and Skokow locations changed according to the amount of Cu content in the soil and temperature. In all experiments copper at sublethal concentrations (600 and 800 mg Cu^{2+}/kg of dry soil) reduced shoot growth as well as chlorophyll content, but these reduction rates were considerably slower at lower temperatures (Table 1 and 2). At temperatures 40/10 °C and 25/5 °C, the fresh biomass of three weed populations increased in the range of supplied copper 0–200 mg Cu^{2+}/kg of dry soil. Additionally for population G-2 at low temperatures fresh biomass still increased at the level of 400 mg Cu^{2+}/kg of dry soil. In high temperatures, reduction of chlorophyll content was observed already at the lowest level of copper dose applied. However in low temperatures the reduction of chlorophyll content was still observed at 600 and 800 mg Cu^{2+}/kg of dry soil, but there was not significant decrease.

Table1

Fresh weight of shoots [g] of *T. indorum* populations in respect of copper dose and temperature range

Population	Dose of copper [mg Cu/kg dry matter of soil]						
	0	50	100	200	400	600	800
Temperature 40/10 °C (day/night)							
Sw	*5.51 a	5.54 a	5.75 a	5.81 a	5.40 a	4.25 b	2.04 c
G-1	5.65 a	5.89 a	5.85 a	5.99 a	5.49 a	4.49 b	2.94 c
G-2	5.65 a	5.80 a	5.97 a	5.83 a	5.54 a	4.61 b	2.73 c
Temperature 25/5 °C (day/night)							
Sw	2.25 a	2.27 a	2.42 a	2.31 a	2.07 ab	1.80 bc	1.42 c
G-1	2.57 a	2.58 a	2.62 a	2.64 a	2.50 ab	2.43 ab	2.07 b
G-2	2.44 ab	2.55 ab	2.75 a	2.62 ab	2.51 ab	2.28 bc	1.98 c

* Values in lines marked by the same letter are not significantly different at $p<0.05$

Table 2

Chlorophyll *a + b* content [mg/g fresh mass] in leaves of *T. indorum* populations in respect of copper dose and temperature range

Population	Dose of copper [mg Cu/kg dry matter of soil]						
	0	50	100	200	400	600	800
Temperature 40/10 °C (day/night)							
Sw	*1.88 a	1.80 ab	1.72 b	1.72 b	1.42 c	1.16 d	0.68 e
G-1	1.89 a	1.84 ab	1.76 bc	1.69 c	1.45 d	1.20 e	0.87 f
G-2	1.89 a	1.84 a	1.77 ac	1.70 bc	1.48 d	1.18 e	0.87 f
Temperature 25/5 °C (day/night)							
Sw	1.63 a	1.66 a	1.71 a	1.67 a	1.64 a	1.58 a	1.53 a
G-1	1.77 a	1.82 a	1.83 a	1.81 a	1.79 a	1.73 a	1.69 a
G-2	1.78 a	1.80 a	1.82 a	1.81 a	1.79 a	1.74 a	1.68 a

* Values in lines marked by the same letter are not significantly different at $p < 0.05$

Although the influence of copper on the growth and the metabolism of several photosynthetic organisms has been extensively studied, little information is still available concerning the relationship between the growth and metabolism of plant for different levels of copper concentrations. Many authors reported that, photosynthesis and growth respond to the presence of different Cu^{2+} levels separately. Perales-Vela at al, found that the growth is more sensitive to Cu^{2+} than metabolism [5].

In agricultural environment, often several abiotic stress factors appear simultaneously rather than a particular stress factor. Plants' response to a combination of two different abiotic stresses may be unique and cannot be directly inferred from the response of plants to each of the different stresses applied individually. In addition, combined different stresses might evoke conflicting or antagonistic responses. Heavy metal stress might pose that problem to plants when combined with heat stress because enhanced transpiration could result in enhanced uptake of heavy metals [3, 6].

There are some reports about weed species growing on naturally metalliferous sites, near copper mines or on piles with metallic ores which developed different tolerance strategies that protected them against copper toxicity [7–9]. Near the copper smelter LEGNICA situated in the Lower Silesia province, among many weed species spontaneously migrating to the area around the emitter, copper tolerant *Agropyron repens* and *Convolvulus arvensis* were found. Activity of this oldest smelter in Poland (with constantly high emissions of fly-ash, reduced distinctly only in the nineties) theoretically gave some weed species a sufficient period to develop tolerance [10]. The results of presented studies *in vivo* showed small differences in the reaction of plant growth and its chlorophyll content to Cu stress between populations of *T. indorum* originated from the vicinity of GCF and from the site studied in Wielkopolska province.

References

[1] Rosada J. and Urbańczyk J.: *Ocena stopnia zanieczyszczenia gleb i roślin w rejonie Huty Miedzi „Głogów" w świetle nowych ustaw o dopuszczalnym poziomie metali ciężkich*, [in] Kwas Siarkowy – Nowe Wyzwania, (eds.) Grzesiak P., Schroeder G., Pruszyński S., IOR Poznań 2003, 245–252.

[2] Rosada J., Grzesiak J., Grzesiak P., Schroeder G., Orlicka A., Ratajczak J. and Rissmann I.: *The application of AAS and ICP techniques for the speciation of trace metals insulated by sequential chemical extraction*, [in:] Development in Production and Use of New Agrochemicals, (eds.) Górecki H., Dobrzański Z., Kafarski P.: Chemistry for Agriculture, 2005(6), 464–477.

[3] Mittler R.: *Abiotic stress, the field environment and stress combination*, Trends in Plant Sci. 2006, **11**(1), 15–19.

[4] Arnon D.J.: *Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris*, Plant Physiol. 1949, **24**, 1–15.

[5] Perales-Vela H.V., González-Moreno S., Montes-Horcasitas C. and Cañizares-Villanueva R.O.: *Growth, photosynthetic and respiratory responses to sub-lethal copper concentrations in Scenedesmus incrassatulus (Chlorophyceae)*, Chemosphere, 2007, **67**(11), 2274–2281.

[6] Anand P., Isar J., Saran S. and Saxena R.K.: *Bioaccumulation of copper by Trichoderma viride*, Biores. Tech. 2006, **97**(8), 1018–1025.

[7] Boojar M.M.A. and Goodarzi F.: *The copper tolerance strategies and role of antioxidative enzymes in three plant species grown on copper mine*, Chemosphere, 2007, **67**(11), 2138–2147.

[8] Liu J., Xiong Z., Li T. and Huang H.: *Bioaccumulation and ecophysiological responses to copper stress in two populations of Rumex dentatus L. from Cu contaminated and non-contaminated sites*, Environ. Exp. Bot. 2004, **52**(1), 43–51.

[9] Masarovičová E. and Holubová M.: *Effect of copper on growth and chlorophyll content of some herbs*, Rostlinná Výroba, 1998, **44**(6), 261–265.

[10] Brej T.: *Heavy metal tolerance in Agropyron regens (L.) P. Bauv. populations from the Legnica copper smelter area, Lower Silesia*, Acta Soc. Bot. Pol. 1998, **67**(3–4), 325–333.

WPŁYW MIEDZI I TEMPERATURY NA WZROST I ZAWARTOŚĆ CHLOROFILU U MARUNY BEZWONNEJ (*Tripleurospermum indorum* (L.) SCHULTZ-BIP.) POCHODZĄCEJ Z OKOLICY HUTY MIEDZI „GŁOGÓW"

Zakład Ekologii i Ochrony Środowiska, Instytut Ochrony Roślin – Państwowy Instytut Badawczy

Abstrakt: Maruna bezwonna to chwast dwuliścienny, dominujący w zbożach ozimych uprawianych na terenie Śląska, gdzie usytuowana jest Huty Miedzi „GŁOGÓW" (HMG). Ze względu na wytwarzanie dużej biomasy stanowi ona silną konkurencję dla rośliny uprawnej. Celem badań było porównanie wpływu wzrastających dawek miedzi w dwóch zakresach temperatur na wzrost i zawartość chlorofilu między biotypami maruny bezwonnej pochodzącymi z rejonu oddziaływania emisji przemysłowych HMG a biotypem pochodzącym z Wielkopolski. Doświadczenia prowadzono w warunkach szklarniowych w przedziale temperatur: 40/10 °C i 25/5 °C (dzień/noc). Siewki maruny rosły w doniczkach wypełnionych ziemią zawierającą wzrastające dawki miedzi ($CuSO_4 \cdot 5H_2O$): 0, 50, 100, 200, 400, 600 i 800 mg Cu/kg suchej gleby. Większe dawki miedzi hamowały wzrost pędów oraz zmniejszały zawartość chlorofilu w liściach. Tempo zmniejszania się obu badanych parametrów ulegało znacznemu spowolnieniu w niższych temperaturach.

Słowa kluczowe: chlorofil, huta miedzi, miedź, temperatura, *Tripleurospermum indorum*, wzrost roślin

Vol. 16, No. 7 2009

Żaneta FIEDLER and Danuta SOSNOWSKA[1]

INFLUENCE OF TEMPERATURE ON EFFECTIVENESS OF PATHOGENIC FUNGI FOR CONTROL OF WESTERN FLOWER THRIPS *Frankliniella occidentalis* (PERGANDE) (*THYSANOPTERA: THRIPIDAE*)

WPŁYW TEMPERATURY NA SKUTECZNOŚĆ GRZYBÓW PASOŻYTNICZYCH W ZWALCZANIU WCIORNASTKA ZACHODNIEGO *Frankliniella occidentalis* (PERGANDE) (*THYSANOPTERA: THRIPIDAE*)

Abstract: *Frankliniella occidentalis* is the most damaging pest of protected cucumbers in Poland and reliable control measures are required that are compatible with other components in the Integrated Pest Management programme. Successful control of this pest is very difficult to achieve due to life cycle where some stages are not available to the insecticides. In practice, entomopathogenic fungi are mostly used in control of insect pest of greenhouse crops because environmental factors such as temperature and humidity are optimal for their development and efficacy. Entomopathogenic fungi are able to have a major role in the regulation of insect populations in greenhouse conditions. The study shows efficacy of following pathogenic fungi: *Paecilomyces lilacinus*, *Beauveria bassiana* and *Acremonium* sp depending on temperatures. The fungus *Beauveria bassiana* was effective against larvae and adults of *F. occidentalis* when applied at the temperature of 25 °C. *Acremonium* sp. was more effective against western flower thrips at higher temperatures.

Keywords: pathogenic fungi, temperature, *Frankliniella occidentalis*

For many years entomopathogenic fungi have been applied in the biological control of the most severe greenhouse pests, such as aphids, mites, greenhouse whiteflies and western flower thrips. However, their efficacy depends upon several abiotic factors and therefore, results are not always satisfactory [1, 2]. In Poland only one bioinsecticide is available based on fungus *Isaria fumosorosea* (old name – *Paecilomyces fumosoroseus)*. When applied together with the parasitoid *Encarsia formosa* it can sufficiently decrease populations of the whitefly [3, 4].

The main objective of the research was to evaluate the influence of temperature on pathogenicity of the Polish strain of three species of pathogenic fungi such as: *Paecilomyces lilacinus*, *Beauveria bassiana*, *Acremonium* sp. at different stages of *Frankliniella occidentalis*.

[1] Department of Biological Control and Quarantine, Institute of Plant Protection – National Research Institute, ul. Władysława Węgorka 20, 60-318 Poznań, Poland, email: z.fiedler@ior.poznan.pl, d.sosnowska@ior.poznan.pl

Materials and methods

Experiments were carried out in laboratory conditions. *P. lilacinus*, *Acremonium* sp. and *B. bassiana* were applied as a suspension of conidia. Spore suspensions were prepared by harvesting spores grown on PDA by washing the cultures with tap water plus Triton X-100 (0.05 %). The spores were collected from a 12-day-old fungus culture grown on potato-dextrose-agar medium (PDA). Spores were harvested by adding 20 cm^3 of sterile distilled water and scraped off with a sterile cell scraper and then homogenized in a glass homogenizer. The concentration of conidial suspension was subsequently adjusted to 108 conidia cm^{-3} using a Goriaev haemocytometer. The isolates were maintained in a collection at the Department of Biological Control and Quarantine, Institute of Plant Protection – PIB, Poznan, Poland.

In laboratory tests, 10 individuals of different stages of western flower thrips were placed on bean leaves covered with moistened filter paper in Petri dishes (9 cm diam.). These leaves were sprayed with a spore suspension in sterile water at a concentration of $10^8/cm^3$ with Triton X-100. The dishes were preserved during tests at 20 °C, 25 °C and 30 °C temperatures. Observations were conducted 2, 5 and 7 days after a treatment and each time the number of live and dead pest individuals were recorded. The degree of fungal infection was evaluated under a microscope.

There was one Petri dish with bean leaves with 10 individual stages of thrips per treatment, with 5 replications.

Data were analyzed by ANOVA after angle transformation. The differences were examined with the Tukey's test at $p < 0.05$.

Results and discussion

P. lilacinus was most successful when applied against soil stages of *Frankliniella occidentalis*, this fungus caused about 70 percentage of mortality. No effect of tested temperatures was observed on effectiveness *P. lilacinus* for control soil stages of western flower thrips. This fungus is effective against different species of plant pathogenic nematodes, and mainly infects eggs and females [5, 6]. In Poland, a domestic strain of this fungus species was investigated as a potential biological agent against root-knot nematodes in greenhouses [7].There is no information in the literature about using *P. lilacinus* against *F. occidenatlis.* Our results showed that *P. lilacinus* could also be recommended for control of thrips.

Table 1

Mean percentage mortality (± sd) of soil stages of *Frankliniella occidentalis*, on *P. lilacinus* treated and control in different temperatures

Treatment	Mortality [%]		
	20 °C	25 °C	30 °C
1. *P. lilacinus*	68 ± 4.8 b	72 ± 8.2 b	70 ± 6.4 b
2. Control	0 ± 0.0 a	0 ± 0.0 a	0 ± 0.0 a

Means marked by the same letter in each column are not significantly different, $p < 0.05$, Tukey's test)

Table 2

Mean percentage mortality (± sd) of larvae (L1) of *Frankliniella occidentalis*, on *Acremonium* sp. and *B. bassiana* treated and control in different temperatures (7 days observation).

Treatment	Mortality [%]		
	20 °C	25 °C	30 °C
1. *B. bassiana*	38 ± 4.6 b	84 ± 8.8 c	40 ± 4.6 b
2. *Acremonium* sp.	48 ± 4.4 b	52 ± 10.2 b	84 ± 2.8 c
3. Control	4 ± 0.8 a	4 ± 0.2 a	2 ± 0.8 a

Means marked by the same letter in each column are not significantly different, $p < 0.05$, Tukey's test)

Table 3

Mean percentage mortality (± sd) of larvae (L2) of *Frankliniella occidentalis*, on *Acremonium* sp. and *B. bassiana* treated and control in different temperatures (7 days observation).

Treatment	Mortality [%]		
	20 °C	25 °C	30 °C
1. *B. bassiana*	52 ± 4.2 c	80 ± 6.2 c	60 ± 2.2 b
2. *Acremonium* sp.	32 ± 2.0 b	42 ± 2.4 b	76 ± 4.4 c
3. Control	2 ± 0.4 a	4 ± 1.0 a	2 ± 0.2 a

Means marked by the same letter in each column are not significantly different, $p < 0.05$, Tukey's test)

Generalny, the greatest mortality of larvae *F. occidentalis* was obtained with *Acremonium* sp. (84 % and 76 %) at the temperature of 30 °C. If the temperature increases, the mortality of different stages will also increase. This isolate was isolated from *Trialeurodes vaporariorum* in Palm House in Poznan. The effect of temperature was recorded for fungus *B. bassiana*, where larvae and adults of *F. occidentalis* mortality was significantly greater at 25 °C than at 20 °C and 30 °C. The fungus caused 92 % mortality of adults 7 days after application (Fig. 1). Other authors also examined the efficacy of *B. bassiana* strain. They obtained similar results [8, 9].

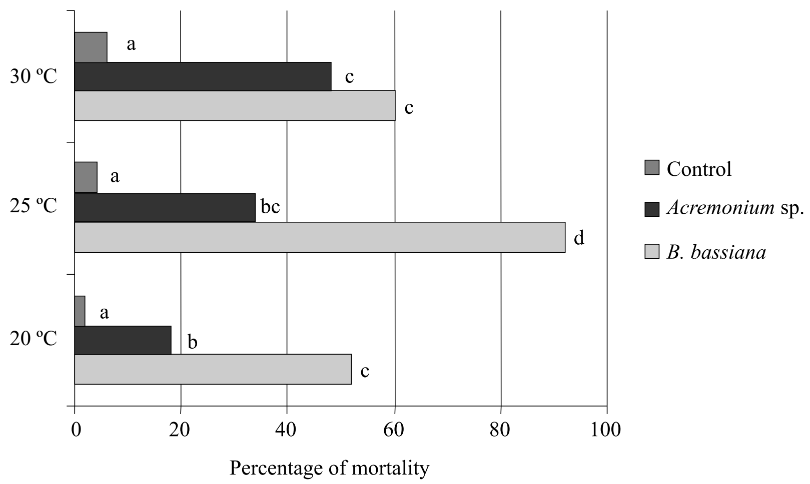

Fig. 1. Mean percentage mortality of *Frankliniella occidentalis* adults on *Acremonium* sp. and *B. bassiana* treated and control (7 days observation).

Microbiological control of arthropod pests using entomopathogenic fungi is not a new idea. In practice, entomopathogenic fungi are mostly used in control of insect pests of greenhouse crops because environmental factors such as temperature and humidity are optimal for their development and efficacy. However, application of natural enemies and entomopathogenic nematodes is still the most common biological control method. Chemical products are applied in cases of failure of the biological methods or their insufficient effect. According to the Polish Act on Plant Protection issued on December 18th 2003, biological methods should be used prior to any application of chemical products. Thus, biological control is a priority in plant protection, and *P. lilacinus*, *B. bassiana*, *Acremonium* sp. seem to be excellent candidates to be used in greenhouse biocontrol programs. The pathogenic fungi are good alternative for control pests in greenhouse crops.

Conclusions

1. The fungus *P. lilacinus* was effective against prepupal and pupal stages of *Frankliniella occidentalis* when applied at different temperatures, from 20 °C to 30 °C. This is the first report on efficacy of this fungus in western flower thrips control in Poland or anywhere else.

2. *B. bassiana* was the greatest pathogenic to L1-L2 larvae and adults of western flower thrips when fungus was applied at the temperature of 25 °C and *Acremonium* sp. was more effective at temperature 30 °C.

References

[1] Vestergaard S., Gillespie A.T. Butt T.M., Schreiter G. and Eilonberg J.: *Pathogenicity of the hyphomycetes fungi Verticillium lecanii and Metarhizium anisopliae to the western flower thrips Frankliniella occidentalis*. Biocontrol Sci. Technol., 1995, **5**, 185–192.

[2] Murphy B.C., Morisawa T.A., Newman J.P., Tiosvold S.A. and Parrella M.P.: *Fungal pathogen controls thrips in greenhouse flowers*. California Agricult., 1998, **52**(3), 32–36.

[3] Sosnowska D. and Piątkowski J.: *Nowy preparat biologiczny do zwalczania mączlika szklarniowego*. Ochr. Rośl., 1995, **11**, 7–9.

[4] Sosnowska D. and Piątkowski J.: *Efficacy of entomopathogenic fungus Paecilomyces fumosoroseus against whitefly (Trialeurodes vaporariorum) in greenhouse tomato cultures*. Insect Pathogens and Insect Parasitic Nematodes. Ed. P.H. Smits. IOBC WPRS Bulletin, 1996, **19**(9), 179–182.

[5] Amancho A. and Sasser J.N.: *Biological control of Meloidogyne incognita with Paecilomyces lilacinus.* Biocontrol, 1995, **1**, 51–61.

[6] Borisov B.A.: *Semi-industrial cultivation of the nematoparasitic fungus Paecilomyces lilacinus (Thom) Samson (Deuteromycotina, Hyphomycetes) and its application against root-knot nematodes in greenhouses*. Russ. J. Nematol., 1998, **6**, 59–60.

[7] Sosnowska D.: *Możliwości zastosowania Pochodnia chlamydosporia (Goddard) Zare et Gams oraz Paecilomyces lilacinus (Thom) Samson w biologicznym zwalczaniu mątwika burakowego (Heterodera schachtii Schmidt) i guzaków korzeniowych (Meloidogyne spp.).* Rozprawy Nauk. Inst. Ochr. Rośl., 2003, (9), 95 pp.

[8] Shipp L., Zhang Yun., Hunt D., Ferguson G. and Enkegaard E.: *Influence of greenhouse microclimate on the efficacy of Beauveria bassiana (Balsamo) Vuillemin for control of greenhouse pests*. Bull. OILB-SROP, 2002, **25**, 237–240.

[9] Jacobson R.J., Chandler D., Fenlon J. and Russell K.M.: *Compatibility of Beauveria bassiana (Balsamo) with Amblyseius cucumeris Oudemans to control Frankliniella occidentalis Pergande on cucumber plants*. Biocontrol Sci. Technol., 2001, **11**(3), 391–400.

WPŁYW TEMPERATURY NA SKUTECZNOŚĆ GRZYBÓW PASOŻYTNICZYCH W ZWALCZANIU WCIORNASTKA ZACHODNIEGO *Frankliniella occidentalis* (PERGANDE) (*THYSANOPTERA: THRIPIDAE*)

Instytut Ochrony Roślin – Państwowy Instytut Badawczy, Poznań

Abstrakt: Wciornastek zachodni (*Frankliniella occidentalis*) należy do najgroźniejszych szkodników występujących w uprawach szklarniowych. Zwalczanie tego szkodnika za pomocą środków chemicznych i entomofagów nie rozwiązało problemów z jego występowaniem w uprawach szklarniowych. W doświadczeniach laboratoryjnych przebadano wpływ temperatury 20 °C, 25 °C i 30 °C na skuteczność trzech gatunków grzybów pasożytniczych: *Paecilomyces lilacinus*, *Beauveria bassiana* i *Acremonium* sp. w ograniczaniu liczebności różnych stadiów rozwojowych wciornastka zachodniego. Doświadczenia wykazały, że powyższe temperatury nie mają wpływu na skuteczność grzyba *P. lilacinus* w zwalczaniu szkodnika. Inna sytuacja wystąpiła w przypadku grzyba *B. bassiana,* gdzie optymalną temperaturą dla jego stosowania okazała się temperatura 25 °C, natomiast dla grzyba *Acremonium* sp. stwierdzono, że wraz ze wzrostem temperatury wzrasta skuteczność tego gatunku w zwalczaniu wciornastka zachodniego.

Słowa kluczowe: grzyby pasożytnicze, temperatura, *Frankliniella occidentalis*

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Katarzyna GLEŃ[1] and Elżbieta BOLIGŁOWA[1]

EFFECT OF SELECTED FOLIAR FERTILIZERS ON PHYTOPATHOGENIC FUNGI UNDER CONDITIONS *IN VITRO*

WPŁYW WYBRANYCH NAWOZÓW DOLISTNYCH NA GRZYBY FITOPATOGENNE W WARUNKACH *IN VITRO*

Abstract: The research aimed at an assessment of the effect of foliar fertilizers, ie Mikrovit Fe – Iron chelate, Wapnovit and Fostar recommended for agronomic and vegetable crops and in orchards on linear growth, biomass and sporulation of the following fungi: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn and *Phoma exiqua* Desm. var. *exiqua* under conditions *in vitro*.

The results obtained show a not unanimous response of the tested fungi species to applied foliar fertilizers and their various concentrations. Among the analyzed foliar fertilizers, Mikrovit Fe most strongly inhibited linear growth, biomass increment and mitigated spore production in all tested fungi. Also Fostar revealed a strong fungistatic effect on *Phoma exiqua* and *Rhizoctonia solani*. At the highest applied concentration (1.0 mm^3/cm^3) growth inhibition coefficients for these species were respectively 67.54 % and 46.45 %. On the other hand, Wapnovit revealed very weak fungistatic properties because it inhibited the linear growth of the analyzed fungal organisms only between 0.02 and 8.24 %. At the same time this fertilizer stimulated growth of the aerial mycelium and sporulation process in the tested fungi. Moreover, *Sclerotinia sclerotiorum* was the species which most weakly responded to fertilizer presence in the medium.

Keywords: foliar fertilizers, phytopathogenic fungi, growth, biomass, sporulation

The growing assortment of foliar fertilizers available on the market answers plant producers' requirements and provides a challenge to undertake new research on their effect on the amount of yield and the environment. The main objective of the introduction of foliar fertilizer to the agrocenosis is increasing the quantity of obtained yields [1-7]. While improving plant nutrition through foliar fertilization, one may also strengthen their resistance to pathogen infestation [8–14]. Moreover, the protective effect of foliar fertilizers is connected with their direct effect upon pathogens. Several studies in this area show that foliar fertilizers may inhibit development of plant pathogen under conditions *in vitro* [15–21].

[1] Departament of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrglen@cyf-kr.edu.pl

The paper shows the influence of various concentrations of foliar fertilizers, ie Mikrovit Fe, Fostar and Wapnovit on linear growth, biomass increment and sporulation of phytopathogenic fungi: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn and *Phoma exiqua* Desm. var. *exiqua* under conditions *in vitro*.

Material and methods

Foliar fertilizers: Mikrovit Fe, Fostar and Wapnovit manufactured by Intermag Enterprise in Olkusz were selected for a laboratory experiment. Mikrovit Fe – Iron chelate contained 3.0 % Fe, 32 g Fe/dm^3 of the fertilizer, 4.5 % N, pH – 32; Wapnovit had 12.16 % CaO, (256 g CaO/dm^3 of fertilizer), 10 % of nitrogen (N-NO_3), 0.48 % – MgO, 0.05 % – B, 0.02 % – Cu, 0.02 % – Zn, pH – 2.7,whereas Fostar; contained: 14.8 % – P, 34.1 % – P_2O_5 (500.3 g P_2O_5/dm^3 of fertilizer), pH – 1.4. Fungi species: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn and *Phoma exiqua* Desm. var. *exiqua* originated from the cultures of the Agricultural Environment Protection Department. The fungi were cultured under conditions *in vitro,* in five replications, on the PDA medium (glucose-potato) and at the temperature of 23 °C. The PDA medium with added Mikrovit Fe, Wapnovit and Fostar were prepared to obtain their concentrations of : 0.01, 0.1 and 1 mm^3 per 1 cm^3 of the medium. Prior to the experiment, the outset pH was measured in the media. The media were inoculated with an agar ring, 5 mm in diameter, overgrown with two-week old mycelium. The control was provided by Petri dishes with the medium without the fertilizer supplement. The effect of individual foliar fertilizers and their concentrations on linear growth of the analyzed fungal organisms was presented as a difference between the mean fungus colony diameter on the control dishes and the diameter of the mycelium colony on dishes with individual concentrations of foliar fertilizers. Coefficients of linear growth rate and inhibition/stimulation coefficients were computed following the formula presented by Gleń [13]. After three weeks of the fungi culture growing on the PDA media with the foliar fertilizers and control the numbers of spores were assessed in Thom haemocytometer.

Fungi biomass growth was maintained in 300 cm^3 Erlenmayer flasks on 100 cm^3 of the modified PDA medium (without agar) with the supplements of foliar fertilizers in the same concentrations as in the above mentioned experiment. The culture was maintained for 21 days at the temperature of ca 23 °C. After this period the post-culture liquid with mycelium was filtered through filter paper. Then the mycelium was dried on a sterile glass at 80 °C to constant weight and weighed. The results were verified statistically using ANOVA and the significance of differences was assessed on the basis of the t-Student test.

Results and discussion

The laboratory experiments allowed to assess the direct effect of foliar fertilizers, ie Mikrovit Fe, Fostar and Wapnovit on linear growth, biomass and sporulation of *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn and *Phoma exiqua* Desm. var. *exiqua* fungi. However, the obtained results do not provide grounds for unanimous determination of the fertilizer effect on the fungal organisms, since each investigated fungus species responded differently to individual fertilizer preparations and their concentrations in the medium. This fact was confirmed by other authors [15–22].

Among the analyzed foliar fertilizers Mikrovit Fe, irrespective of the concentration applied, revealed strong fungistatic properties towards *Phoma exiqua* and *Rhizoctonia solani.* It has been visible as a significantly slower rate of the linear growth of these species in comparison with the growth on Petri dishes, inhibition of surface growth of these species colonies by respectively 49.28 % and 44.76 %, biomass increments on average by 41.36 % and 64. 36% and sporulation (Fig. 1, Table 1–3).

Table 1

Coefficients of rate [T] and inhibition of the linear growth of the tested fungi [%]

Foliar fertilizers	Concentration [mm^3/cm^3]	*Rhizoctonia solani*		*Sclerotinia sclerotiorum*		*Phoma exiqua*	
		[T]	[%]	[T]	[%]	[T]	[%]
Mikrovit Fe – Iron chelate	1	33.38 a*	59.97	76.94 b	4.82	20.83 a	74.99
	0.1	40.75 b	51.14	68.14 a	15.71	37.45 c	55.05
	0.01	64.12 d	23.18	80.56 c	0.35	68.48 f	17.8
Fostar	1	44.66 c	46.45	80.44 c	0.49	27.04 b	67.54
	0.1	64.03 d	23.22	79.92 c	1.14	55.17 d	33.77
	0.01	65.30 e	21.70	83.55 d	+3.35	56.09 e	32.67
Wapnovit	1	76.53 f	8.24	80.42 c	0.52	79.00 g	5.17
	0.1	81.94 g	1.75	79.66 bc	1.46	80.23 e	3.70
	0.01	83.96 i	+0.67	79.66 bc	1.46	83.29 i	0.02
Control		83.40 h		80.84 cd		83.31 i	

* Values in columns marked by the same letter are not significantly different,
\+ means stimulation of biomass increment.

At the same time it was found that the inhibitory effect of Mikrovit Fe on *P. exiqua* and *R. solani* was diminishing with their decreasing concentrations in the media (Table 1–4). A similar relationship was found in the research on the influence on Mikrovit Fe on *Botrytis cinerea* [21]. Moreover, Mikrovit Fe used in 0.1 mm^3/cm^3 concentration very strongly (91.46 %) inhibited *S. sclerotiorum* biomass growth, but it limited its linear growth only in 15.40 % (Table 1–2). However, in the same object a stimulation of *S. sclerotiorum* sporulation was detected (Table 3). In the opinion of Hodges [23] such a result may be considered as a defensive response of the fungus to unfavourable conditions and protection of the colony durability.

The activity of foliar fertilizers on fungal organisms under *in vitro* conditions greatly depends not only on the kind and dose of the fertilizer preparation but, as reported by Weber and Wyrwa [16], also on the medium reaction. On the other hand, in common opinion, microscopic fungi may develop within a wide range of environment reaction values, but for the majority the optimum pH ranges from 4 to 7 [24–26]. In the Authors' studies the share of individual concentrations of Mikrovit Fe changed the medium pH to a slight extent (4.34–5.20). Moreover, a characteristic feature of Mikrovit Fe used in the experiment was the presence of chelated Fe cations. Gumiński [27] reports that Fe^{3+} is the most strongly bound at pH of 3 and the bond strength decreases with growing pH. Therefore, the role of the medium reaction is connected rather with the availability of individual nutrients to fungi. In their own studies the Authors observed a significant and at the same time very strong inhibition of biomass increment, particularly in *S. sclerotiorum* and *Rhizoctonia solani* at 1.0 and 0.1 mm^3/cm^3 of Mikrovit Fe supplement (Table 4). On the other hand, it may be supposed that at a lower concentration of this preparation in the medium and pH 5.20, Fe ions might have been better utilized by the analyzed fungal organisms to build fungal structures. On the other hand, irrespectively on the applied concentration, Mikrovit Fe strongly reduced sporulation process in the tested fungi. This fact was also corroborated by the studies of Glen and Boliglowa [19].

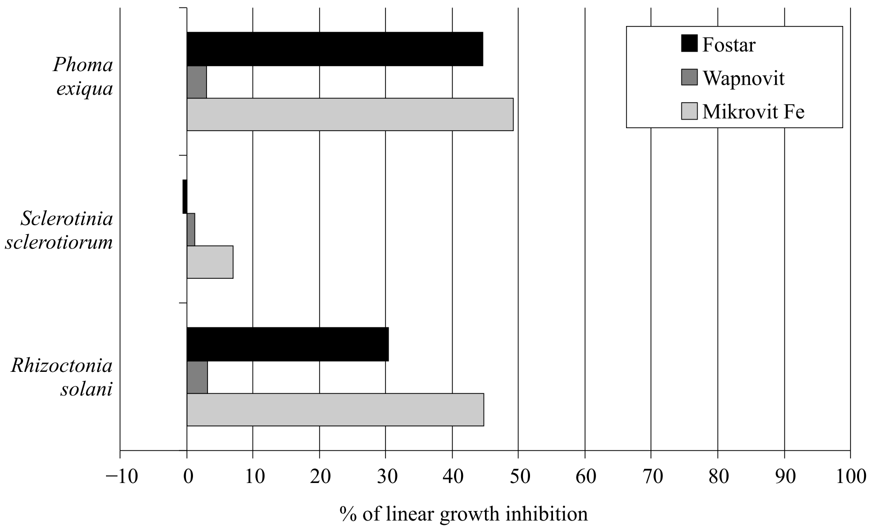

Fig. 1. Effect of foliar fertilizers on tested fungi growth inhibition

The response of the tested fungal organisms to Fostar was rather diversified. Generally the fertilizer revealed weaker fungistatic properties. In its highest concentrations (1.0 and 0.1 mm^3/cm^3) it notably reduced the development of all tested fungi colonies, but as in the case of Mikrovit Fe, *P. exiqua* and *R. solani* proved to be the most sensitive species (Table 1). An apparent inhibition of their linear growth (ca 34 and 23 %) was observed also on media containing 0.1 mm^3/cm^3 of this fertilizer preparation (Table 1). However, on liquid media Fostar, especially in the lowest concentration it stimulated biomass increments in the tested organisms (Table 2). *R. solani* biomass increment reached even 68.34 %. In the Authors' former studies [22] a strong inhibition of linear growth reaching ca 80 % was noted as well as very intensive biomass increments (54.62–106.17 %) of *Fusarium* fungi in the presence of 0.01 mm^3/cm^3 of Fostar. ANOVA conducted in the Authors' own research did not reveal any notable effect of individual Fostar concentrations on *S. sclerotiorum* growth rate (Table 1). However, in each of the analyzed concentrations of this fertilizer a four time smaller number of *S. sclerotiorum* spores was registered (Table 3). On the other hand at 1.0 mm^3/cm^3 of Fostar content in the medium the number of *P. exiqua* spores declined even ten times. Also Gleń [22] reported that irrespective of the applied concentration, this fertilizer totally blocked the macroconidia forming the process in *F. coeruleum*, *F. culmorum* and *F. graminearum*. In the Authors' own research, pH of media containing 1.0, 0.1 and 0.01 mm^3/cm^3 of Fostar was respectively: 3.61, 3.95 and 4.32. A strongly acid medium pH assuredly did not favour the growth of the tested phytopathogenic fungi, particularly during the short experiment on Petri dishes. It was confirmed by the research by Gleń and Boligłowa [19]. Foliar fertilizers characterized by a low pH applied in 1.0 mm^3/cm^3 concentration led to a complete inhibition of *F. avenaceum, F. coeruleum, F. graminearum.*

The Wapnovit foliar fertilizer revealed a lack of fungistatic activity. Slight inhibition of *R. solani*, *P. exiqua* and *S. sclerotiorum* colony growth was registered only when the highest concentrations were used (Table 1). Irrespective of its concentration Wapnovit had a significant influence on stimulation *R. solani* biomass increments (Table 3). The 0.01 mm^3/cm^3 concentration also greatly affected intensive growth of *P. exiqua* biomass. On the other hand, although higher concentrations of Wapnovit revealed a tendency to stimulate this species, the biomass increments did not differ notably from the control. Moreover, a considerable (nine-fold) smaller *P. exiqua* spore number was observed (Table 3). The analyzed foliar fertilizer did not have any marked influence either on linear growth or biomass of *S. sclerotiorum* (Table 1, 2). Very weak fungistatic properties of Wapnovit applied in 1.0 mm^3/cm^3 concentration (field dose) towards *Fusarium* fungi were also observed by Gleń [22]. Unlike Fostar, Wapnovit is a mutlicomponent fertilizer which apart from calcium contains also nitrogen, magnesium, boron, copper and zinc. Moreover the pH of the media with added Wapnovit ranged from 4.51 to 5.75, remaining within the range optimal for the fungi tested in the experiment. The tested fungi species revealed a great tolerance to Wapnovit presence in the medium.

Table 2

Effect of foliar fertilizers on biomass increment of tested fungi

Foliar fertilizers	Concentration [mm^3/cm^3]	*Rhizoctonia solani*		*Sclerotinia sclerotiorum*		*Phoma exiqua*	
		Biomas [g]	T [%]	Biomas [g]	T [%]	Biomas [g]	T [%]
Mikrovit Fe – Iron chelate	1	0.046 a*	92.29	0.181 a	80.43	0.259 a	53.75
	0.1	0.164 a	72.53	0.079 de	91.46	0.311 ab	44.46
	0.01	0.424 b	28.98	0.442 b	52.21	0.415 c	25.89
Fostar	1	0.525 bc	12.06	0.755 c	18.38	0.394 bc	29.64
	0.1	0.533 bc	10.72	0.893 cde	3.46	0.569 de	+1.61
	0.01	1.005 d	+68.34	0.994 e	+7.46	0.664 ef	+18.57
Wapnovit	1	1.009 d	+69.01	0.953 de	+3.03	0.563 d	+0.53
	0.1	0.977 d	+63.65	0.814 cd	12.00	0.608 de	+8.57
	0.01	0.970 d	+62.48	0.863 cde	6.70	0.714 f	+27.50
Control		0.597 c		0.925 de		0.560 d	

* values in columns marked with the same letter do not differ significantly,
\+ means stimulation of biomass increment.

Table 3

Sporulation of tested fungi

Fungus species	Spore number per 1 cm^3 x $[10^8]$									
Fungus species	Control	Mikrovit Fe			Fostar			Wapnovit		
		concentration $[mm^3/cm^3]$								
		1.0	0.1	0.01	1.0	0.1	0.01	1.0	0.1	0.01
Sclerotinia sclerotiorum	12.48	8.36	7.40	6.79	4.06	4.21	4.93	13.61	8.59	8.39
Phoma exiqua	72.24	0.23	5.47	7.38	7.18	8.10	11.69	7.23	8.02	8.14

Conclusions

1. Among the tested fungal organisms, *Phoma exiqua* and *Rhizoctonia solani* revealed high sensitivity to the applied fertilizer preparations, particularly to Mikrovit Fe and Fostar.

2. Mikrovit Fe revealed the strongest fungistatic properties, which became apparent as strong inhibition of biomass growth of all tested fungi species, a considerable limitation of *P. exiqua* and *R. solani* linear growth, and inhibition of sporulation of *P. exigua* and *S. sclerotiorum*.

3. Fostar foliar fertilizer applied in 1.0 mm^3/cm^3 concentration strongly inhibited linear growth of *R.solani* and *P. exiqua*, reduced their biomass increment by 12.06–29.64 %. Irrespective of its concentration, it inhibited sporulation in *P. exiqua* and *S. sclerotiorum.*

4. The share of 1.0 mm^3/cm^3 of Wapnovit in the medium stimulates biomass increment in all tested fungi species and favours intensification of the sporulation process in *S. sclerotiorum.*

References

[1] Czuba R.: *Efekty dolistnego dokarmiania roślin uprawnych. Cz. II. Reakcja roślin na dolistne stosowanie mikroelementów i azotu łącznie z mikroelementami*. Rocz. Glebozn. 1993, **XLIV**(3/4), 79–87.

[2] Boligłowa E.: *Wpływ dolistnego dokarmiania na plonowanie i jakość bulw ziemniaka*. Rozp. Habilit., 41. Wyd. WSR-P, Siedlce 1995.

[3] Jabłoński K. and Bernat E.: *Wpływ dolistnego nawożenia Mikrosolem Zn na kształtowanie się plonu ziemniaka i jego jakość oraz możliwość ograniczenia stosowania fungicydów do zwalczania zarazy ziemniaka*. Progr. Plant Protect., 2001, **41**(1), 299–305.

[4] Boligłowa E.: *Wpływ dolistnego dokarmiania ziemniaka na plon jego strukturę, zdrowotność i trwałość przechowalniczą bulw*. Acta Agrophys., 2003, **85**, 99–106.

[5] Tobiasz-Salach R. and Borecka-Jamro D.: *Wpływ wieloskładnikowych nawozów dolistnych na plonowanie i skład chemiczny owsa*. Acta Agrophys., 2003, **85**, 89–98.

[6] Chwil S. and Szewczuk C.: *Wpływ dolistnego dokarmiania buraka cukrowego na jego plon i niektóre cechy jakościowe*. Acta Agrophys., 2003, **85**, 117–124.

[7] Sady W.: Nawożenie warzyw polowych. Plantpress, Kraków 2000.

[8] Nowosielski O.: *FloroGama (FGO) – Pierwszy nawóz nalistny ochroniarski*. Ochr. Rośl., 1986, **7**, 14–16.

[9] Solarska E.: *Ocena przydatności działania ochronnego nawozów dolistnych na chmielu*, Mat. 35 Sesji Naukowej IOR, cz. II Postery, Poznań 1995.

[10] Piszczek J.: *Wpływ dokarmiania dolistnego preparatami Bornit i Tytanit na zdrowotność i jakość buraka cukrowego*. Progr. Plant Protect., 2001, **41**(1), 306–311.

[11] Kapsa J.: *Możliwość ograniczania dawek fungicydów przez dodatek Insolu 7 w ochronie plantacji ziemniaka przed Phytophthora infestans (Mont.) de Bary*, Zesz. Nauk. AR Kraków, 2002, nr 387, 82, 75–79.

[12] Sawicka B.: *Wpływ łącznego stosowania agrochemikaliów na tempo szerzenia się Phytophthora infestans na roślinach ziemniaka*. Acta Agrophys., 2003, **85**, 157–167.
[13] Kruczek A.: *Wpływ dolistnego dokarmiania kukurydzy azotem i nawozem wieloskładnikowym na porażenie roślin przez choroby i szkodniki oraz wyleganie.* Acta Agrophys., 2003, **85**, 77–87.
[14] Gleń K.: *Evaluation of foliar fertilizers for horse radish Armoracia rusticana Gaertn protection against fungal diseases*. Ecol. Chem. Engin., 2007, **14**, 527–532.
[15] Przeździecki Z., Wojciechowska-Kot H., Mikołajska I. and Murawa D.: *Wpływ mikroelementów na rozwój grzybni, zarodnikowanie i zawartość kwasów tłuszczowych Fusarium i Fusarium avenaceum.* Acta Acad. Agricullt. Tech., Olsztyn 1991, **53**, 229–239.
[16] Weber Z. and Wyrwa P.: *Wpływ nawozu dolistnego Bonga na wzrost czterech gatunków grzybów in vitro*. Rocz. Akad. Roln. Poznań, Roln., 1993, **42**(247), 133–137.
[17] Skrzypczak Cz., Orlikowski L.B. and Matysiak B.: *Effect of some fertilizer chelates on the growth on pathogenic fungi and development of Phytophtora foot-rot of gerbera and population dynamics of Phytophtora cryptogen.* Phytopathol. Polon., 1996, **11**, 41–50.
[18] Boligłowa E. and Gleń K.: *Wpływ nawozów dolistnych na wzrost i zarodnikowanie grzybów patogenicznych z rodzaju Fusarium w warunkach in vitro*. Acta Agrophys., 2003, **85**, 107–116.
[19] Gleń K. and Boligłowa E.: *Effect of Mikrovit Fe and Mikrovit Mn on development of Fusarium culmorum (W.G. Smith) Sacc.* Ecol. Chem. Eng. 2006, **8**(13), 743–750.
[20] Gleń K., Boligłowa E. and Trela S.: *Assessment of Tytanit in vitro effect on selected phytopathogenic fungi.* Ecol. Chem. Eng. 2006, **7**(13), 743–750.
[21] Gleń K. and Boligłowa E.: *Response of some polyphagous fungi on microelement foliar fertilizers under conditions in vitro.* Ecol. Chem. Eng. 2007, **14**, 537–543.
[22] Gleń K.: *Comparison of Fostar and Wapnovit foliar fertilizers effect on phytopathogenic Fusarium fungi of genus*. Ecol. Chem. Eng. 2007, **14**, 529–536.
[23] Hodges C.F: *Vegetative growth and sporulation of Biopolaris sorokiniana on sequentially older infected leaves of Poa pratensis exposed to postemergence herbicides*, Mycopathologia. 1994, **128**(2), 105–109.
[24] Kirlay Z., Klement Z. and Solymosy F.: *Fitopatologia*. *Wybór metod badawczych*. PWRiL. Warszawa 1977
[25] Kochman J. and Węgorek W.: Ochrona roślin. Plantpress. Kraków 1997.
[26] Borecki Z.: *Nauka* o chorobach roślin. PWRiL, Warszawa 2001.
[27] Gumiński S.: Znaczenie chelatów i związków próchniczych w odkażaniu środowiska, Bioindykacja skażeń przemysłowych i rolniczych. PAN, Wrocław 1983, 57–66.

WPŁYW WYBRANYCH NAWOZÓW DOLISTNYCH NA GRZYBY FITOPATOGENNE W WARUNKACH *IN VITRO*

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

Abstrakt: Celem podjętych badań było określenie wpływu nawozów dolistnych, tj. Mikrovit Fe – Chelat żelaza, Wapnovit i Fostar zalecanych do stosowania w uprawach rolniczych, warzywniczych i sadach, na wzrost liniowy, biomasę i zarodnikowanie grzybów: *Sclerotinia sclerotiorum* (Lib.) de Bary*, Rhizoctonia solani* Kühn i *Phoma exiqua* Desm. v. *exiqua* w warunkach *in vitro.*

Uzyskane wyniki wskazują, że reakcja badanych gatunków grzybów na zastosowane nawozy dolistne i ich różne stężenia nie jest jednoznaczna. Spośród badanych nawozów dolistnych Mikrovit Fe niezależnie od stężenia w podłożu najsilniej ogranicza rozrost liniowy, przyrost biomasy oraz osłabia proces sporulacji wszystkich grzybów testowych. Silne właściwości fungistatyczne w odniesieniu do *Phoma exiqua* i *Rhizoctonia solani* wykazywał również Fostar. Szczególnie zaś w największym z zastosowanych stężeń (1.0 mm^3/cm^3) – współczynnik zahamowania wzrostu dla tych gatunków wynosił odpowiednio 67,54 % i 46,45 %. Natomiast Wapnovit wykazał bardzo słabe właściwości fungistatyczne, bowiem hamował on rozrost liniowy badanych organizmów grzybowych w zakresie 0,02–8,24 %. Jednocześnie nawóz ten stymulował wzrost grzybni powietrznej i proces sporulacji testowanych gatunków. Ponadto gatunkiem najsłabiej reagującym na obecność nawozów w podłożu okazał się *Sclerotinia sclerotiorum*.

Słowa kluczowe: nawozy dolistne, grzyby fitopatogenne, wzrost, biomasa, zarodnikowanie

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Katarzyna GLEŃ[1] and Krzysztof GONDEK[2]

EFFECT OF MINERAL FERTILIZATION ON THE DYNAMICS OF *Rhizoctonia solani* KÜHN GROWTH

WPŁYW NAWOŻENIA MINERALNEGO NA DYNAMIKĘ WZROSTU *Rhizoctonia solani* KÜHN

Abstract: The research focused on determining the effect of water extracts of soil fertilized with mineral fertilizers: NPK, NPK + S(ammonium sulphate), NPK + S(Potafoska) on the linear growth and biomass of *Rhizoctonia solani* under conditions *in vitro*. The research has shown that *R. solani* fungus is very sensitive to the presence in the medium of water extracts of soils fertilized with mineral fertilizers. It has been reflected both by linear growth rate coefficients, inhibition of linear growth and biomass increments. All investigated extracts and their concentrations significantly affected the dynamics of *R. solani* growth. The strongest inhibitory effect on the *R. solani* hyphae linear growth was observed in the presence of the water extract of the soil fertilized with NPK + S(Potafoska). On the other hand, when 50 mm^3/cm^3 of the extract of the soil fertilized with NPK and 25 mm^3/cm^3 of the extract of the NPK + S(ammonium sulphate) treated soil was added, the tested fungus responded by strong inhibition of biomass increment, respectively by 55.71 and 57.07 %. The experiments conducted *in vitro* may suggest that supplying mineral NPK and other fertilizers, such as ammonium phosphate or Potafoska to the soil may limit the population of *R. solani* pathogenic fungi. However, in agrocenoses one should also consider the complicated interrelations between individual elements of the environment.

Keywords: *Rhizoctonia solani*, mineral fertilization, soil extracts, linear growth, biomass

Fungi from the Rhizoctonia genus cause tiresome diseases in many plants, mainly due to injuries of their underground organs. *Rhizoctonia solani* Kühn species belongs to the *Mycelia sterilia* group and occurs in soil where it is one of the most serious polyphagous pathogens. In the opinion of many authors [1–5] *R. solani* is the cause of shoot rotting and rhizoctonosis of potato and beetroot seedling disease [6]. It causes cereal root diseases and also other diseases [7–9]. Mineral fertilization, beside soil and climatic conditions, is one of the most important factors affecting the quantity of pathogenic

[1] Departament of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrglen@cyf-kr.edu.pl

[2] Departament of Agricultural Chemistry, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland

organisms accumulated in soil [10, 11]. However, the interaction between the fertilization level and the development of pathogens infecting roots has not been fully recognized yet.

The research aimed at determining the effect of water extracts from soil fertilized with mineral fertilizers: NPK, NPK + S(ammonium sulphate) and NPK + S(Potafoska) on linear growth and biomass of *Rhizoctonia solani* under conditions *in vitro*.

Table 1

Contents of macro- and some microelements in water extracts of soils

Water extracts of soil	Macroelements [mg/dm^3]							
	$N-NO_3$	$N-NH_4$	P	K	Ca	Na	Mg	S
Non-fertilized soil	1.6	traces	0.31	1.05	2.82	2.02	1.40	0.80
NPK	0.19	traces	0.33	1.79	8.41	2.20	2.10	5.70
NPK + S(ammonium sulphate)	1.84	traces	0.27	2.28	5.91	1.99	1.63	2.39
NPK + S(Potafoska)	0.43	traces	0.40	2.29	5.52	2.31	2.32	6.75
	Microelements and trace elements [mg/dm^3]							
	Cr	Zn	Pb	Cu	Cd	Ni	Fe	Mn
Non-fertilized soil	0.005	0.079	0.004	0.012	0.000	0.008	1.010	0.029
NPK	0.005	0.037	0.001	0.008	0.000	0.004	0.890	0.020
NPK + S(ammonium sulphate)	0.004	0.067	0.000	0.008	0.000	0.003	0.690	0.030
NPK + S(Potafoska)	0.005	0.060	0.000	0.009	0.000	0.006	1.030	0.020

Subsequently individual water extracts were added to the PDA (glucose-potato) medium, so that the extract constituted 50 and 25 mm^3/cm^3 of the medium. *R. solani* was cultured in five replications for each concentration at the temperature of 23 °C. The control were Petri dishes with a medium without the water extract of soil. Before inoculation of the media, their pH was measured; it ranged between 6.31 and 6.45. The effect of individual water extracts of soil on *R. solani* linear growth was presented as a difference between the fungus colony diameter on the control dishes and the mycelium colony diameter on the dishes with individual extract concentrations. Subsequently the value was converted into the growth inhibition/stimulation coefficient according to the Abbot formula and the linear growth rate coefficient of *R. solani* was computed according to the formulas presented by Gleń [12].

R. solani fungus biomass increment was conducted in Erlenmayer flasks on 100 cm^3 of the modified PDA medium (without agar) with added water solutions of soils in the same concentrations as described above. The culture was maintained for 21 days and then the post-culture liquid with mycelium was filtered through filter paper. Dry matter was assessed in the biomass and then in dry mass of the mycelium assessed were the content of mineral components, phosphorus, potassium, sodium, calcium, magnesium and trace elements (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) using ICP-AES method on JY 238 Ultrace apparatus.

The results were elaborated statistically using ANOVA.

Results and discussion

On the basis of conducted laboratory analyses it was found that water extracts of soils and their concentrations had a significant effect on the rate of linear growth of the *R. solani* colony and biomass. The analyses also revealed that the tested fungus was a sensitive species, since it responded by an apparent inhibition of its linear growth and biomass increment to the presence of individual soil extracts. It should be said that the analyzed water extracts of soils were diversified with respect to their contents of individual macro- and microelements (Table 1). The water extract from the soil fertilized with NPK + S(Potafoska) was characterized by the highest contents of PK, Na, Mg, S and Fe and it also inhibited the *R. solani* growth rate the most. The diameter of the *R. solani* colony on the medium with added 50 mm^3/cm^3 of this extract was by 39.01 % smaller in comparison with the control (Fig. 1). Higher (50 mm^3/cm^3) concentrations of extracts of soils fertilized with NPK and NPK + S(Potafoska) revealed a markedly stronger fungistatic activity than in 25 mm^3/cm^3 (Table 2).

Table 2

Growth rate coefficients of *Rhizoctonia solani*

Water extracts of soil	Growth rate coefficients [T]			$LSD_{0.05}$
	concentration [mm^3/cm^3]		Mean	
	50	25		
Non-fertilized soil	63.08	62.91	62.99	8.98
NPK	72.69	82.96	77.83	
NPK + S(ammonium phosphate)	82.99	80.83	81.91	
NPK + S(Potafoska)	54.80	60.72	57.76	
Control (PDA medium)	89.41		89.41	
Mean	72.60	75.36		
$LSD_{0.05}$	4.21			

The *R. solani* culture on the media with added water solutions of soils fertilized with mineral materials contributed to inhibition of biomass growth within the range of 42.92–57.07 % (Table 3). Generally in combinations with 25 mm^3/cm^3 content of soil extracts and therefore more deficient in macro- and microelements ca 5–10 % lower biomass was registered. On the other hand on the medium with the water extract of NPK fertilized soil an opposite response of the *R. solani* fungus was observed to the applied concentrations. Moreover, no significant differences in the obtained *R. solani* biomass were noted between the combinations with the extract of non-fertilized soil and the soil fertilized with NPK + S(Potafoska) (Table 3). Fungal organisms reveal an ability to accumulate both macro- and microelements. They use them to build cell structure or they penetrate into cell organelle where biochemical processes take place and participate in the regulation of secondary metabolism [13, 14]. On the basis of the chemical analysis of the *R. solani* mycelium it was demonstrated that in the presence of 50 mm^3/cm^3 of water extracts of soil in the medium the content of potassium decreases in the mycelium

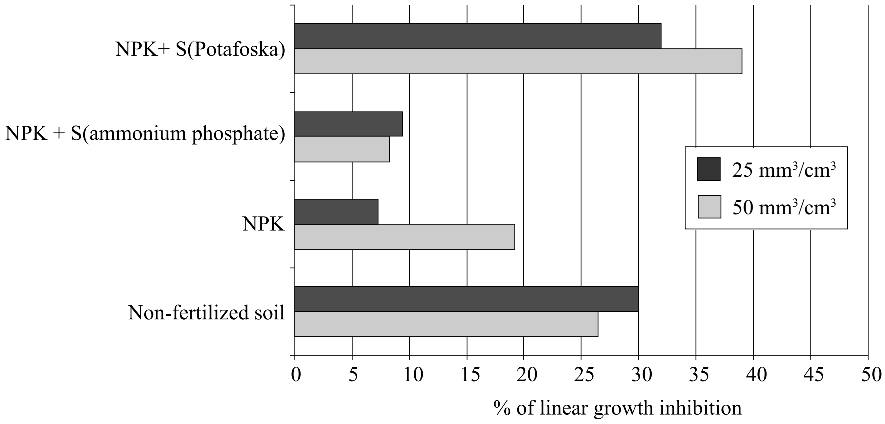

Fig. 1. Effect of water extracts of soil on linear growth of *Rhizoctonia solani*

dry mass but the contents of phosphorus and calcium increase (Table 4). Water extracts of soil fertilized with NPK + S(ammonium sulphate) and NPK + S(Potafoska) contained the greatest quantities of potassium, yet in *R. solani* mycelium cultured in the above mentioned objects the content of this element was the lowest (Tables 1 and 4). It is a common opinion that plant fertilization with potassium and phosphorus favours improvement of their resistance to infectious diseases [14, 15]. In the light of the conducted experiments it may be deduced that the greater amounts of bioavailable potassium in the environment, the lesser its utilization by *R. solani*. It shows that potassium is an element mainly utilized by plants, but it may have a direct inhibitory effect on microorganisms, eg *R. solani*, which settle plants. Soil liming is often used as a measure to disinfect the soil and get rid of phytopathogenic organisms [14]. In the presented experiments, calcium was the element taken up by the tested fungal organism in amounts proportional to its contents in the medium. The fact was corroborated by other research where calcium fertilizer stimulated linear growth and biomass increments of *Fusarium* fungi [16].

Table 3

Effect of water extracts of soils on *Rhizoctonia solani* biomass

Water extracts of soils	Biomass			
	Concentration [mm^3/cm^3]			
	50		25	
	g	[%]	g	[%]
Non-fertilized soil	1.20 cde*	45.2	1.09 bcd	50.23
NPK	0.97 a	55.71	1.25 de	42.92
NPK + S(ammonium phosphate)	1.16 cd	47,03	0.94 a	57.07
NPK + S(Potafoska)	1.19 cde	45,66	1.09 bcd	50.23
Control without extract	2.19 f			

*values in columns marked by the same letters do not differ significantly

Table 4

Contents of macro- and microelement in *Rhizoctonia solani* mycelium

Water extracts of soil	Concentration [mm^3/cm^3]	g/kg d.m.						
		P	K	Ca	Mg	Na	Fe	Cu
Non fertilized soil	50	2.42	72.24	9.66	13.26	1.63	1.40	0.043
	25	2.06	84.40	8.02	12.86	1.57	1.07	0.039
NPK	50	2.99	78.82	15.74	15.11	3.49	0.41	0.046
	25	2.50	88.71	11.40	13.15	1.29	0.30	0.039
NPK + S(ammonium sulphate)	50	2.15	44.75	10.87	16.35	3.83	1.37	0.088
	25	1.99	65.99	10.14	13.73	2.87	1.21	0.079
NPK + S(Potafoska)	50	2.55	69.65	10.16	14.18	1.49	1.32	0.040
	25	1.60	86.41	9.39	13.20	2.10	0.92	0.039
Control without extract		2.44	94.35	7.84	13.84	2.47	0.28	0.043

Water extracts of soil tested in the experiment did not favour the development of the *R. solani* fungus. Under field conditions mineral NPK fertilization also inhibited potato tuber infection by *R. solani* [17]. According to Górski and Gaj [18] high contents of Mg, Cu, and Mn in plants reduce beetroot leaf infection by *Cercospora beticola*, *Erisiphe betae* and *Uromyces betae.* Therefore it is supposed that the presence of these elements in water extracts of soils may contribute to inhibition of *Rhizoctonia solani* fungus development.

Conclusions

1. Under conditions *in vitro* the *R. solani* fungus reveals high sensitivity to the presence in the medium of water extracts of soils fertilized with mineral fertilizers. All tested soil extracts applied in concentrations of 50 and 25 mm^3/cm^3 inhibit both colony surface growth and increments of *R. solani* biomass.

2. The strongest inhibitory effect on linear growth of the *R. solani* mycelium was observed in the presence of the water extract of soil fertilized with NPK + S(Potafoska).

3. The share of 50 mm^3/cm^3 of the extract of NPK fertilized soil and 25 mm^3/cm^3 of NPK with the S(ammonium sulphate) extract contributes to inhibiting *R. solani* biomass growth to the same extent.

References

[1] Bogucka H. and Pawińska M.: *Występowanie ospowatości bulw ziemniaka w Polsce w latach 1977–1980.* Biul. Inst. Ziemn. 1983, **29**, 141–150.

[2] Deacon J.W. and Scott D.B.: *Rhizoctonia solani associated with crater disease (stunting) of wheat in South Africa*. Trans. Brit. Mycol. Soc., 1985, **85**, 319–327.

[3] Mazzola M., Wong O.T. and Cook R.J.: *Virulence of Rhizoctonia oryzae isolates on wheat and detection of the pathogen in plant tissue using PCR protocol*. Phytopathology 1995, **85**, 1133.

[4] Burgieł Z. and Gleń K.: *Wpływ herbicydu Afalon na zdrowotność bulw ziemniaka*. Pestycydy 1997, (3–4), 85–91.
[5] Błaszkowski J., Tadych M. and Madej T.: *Choroby powodowane przez grzyby z podgromady Basidiomycotina, klasy Hymenomycetes, rzędu Ceratobasidiales, rodziny Ceratobasidiaceae.* Przewodnik do zajęć z fitopatologii. Szczecin 1999, 293–295.
[6] Boligłowa E., Gleń K. and Klima K.: *Stopień porażenia bulw parchem zwykłym i ospowatością w zależności od położenia na stoku*. Folia Univer. Agricul. Stetin. 201, Agricultura 2000, **78**, 7–12.
[7] Żółtańska E.: *Zdolność grzybów z rodzaju Rhizoctonia do porażania korzeni pszenicy ozimej*. Progr. Plant Protect. 2001, **441**(2), 776–778.
[8] Szymczak-Nowak J.: *Wrażliwość wybranych odmian buraka cukrowego na Rhizoctonia solani Kühn.* Progr. Plant Protect. 2005, **45**(2), 1141–1144.
[9] Gleń K., Boligłowa E. and Trela S.: *Assessment of Tytanit in vitro effect on selected phytopatogenic fungi*. Chem. Inż. Ekol. 2006, **13**(7), 649–656.
[10] Kapsa J.: *Sucha zgnilizna bulw ziemniaka*. Ziemniak Polski, 1993, (2), 15–17.
[11] Choroszewski P.P.: *Mikroflora środowiska glebowego pól ziemniaczanych*. Zesz. Probl. Nauk Roln. 1989, **374**, 101–118.
[12] Gleń K. and Boligłowa E.: *Response of Fusarium fungi isolated from winter wheat culm base to selected foliar fertilizers*. Ecol. Chem. Eng. 2006, **13**(1–2), 29–36.
[13] Przeździecki Z., Wojciechowska-Kot H., Mikołajska I. and Murawa D.: *Wpływ mikroelementów na rozwój grzybni, zarodnikowanie i zawartość kwasów tłuszczowych Fusarium i Fusarium avenaceum.* Acta Acad. Agricult.-Tech., Olsztyn, 1991, **53**, 229–239.
[14] Borecki Z.: Nauka o chorobach roślin. PWRiL, Warszawa 2001.
[15] Kochman J. and Węgorek W.: Ochrona roślin. Plandpress. Kraków, 1997.
[16] Gleń K.: *Comparison of Fostar and Wapnovit foliar fertilizers effect phytopatogenic fungi of genus Fusarium*. Ecol. Chem. Eng. 2007, **14**, 529–536.
[17] Zimny L.Wacławowicz R. and Oliwa T.: *Porażenie bulw przez Rhizoctonia solani w zależności od systemów uprawy ziemniaka.* Progr. Plant Protect. 2006, **46**(1), 388–394.

WPŁYW NAWOŻENIA MINERALNEGO NA DYNAMIKĘ WZROSTU *Rhizoctonia solani* KÜHN

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

Abstrakt: Badania dotyczyły określenia wpływu wodnych wyciągów z gleb nawożonych nawozami mineralnymi: NPK, NPK + siarczan amonu, NPK + Potafoska na rozrost liniowy i biomasę *Rhizoctonia solani* w warunkach *in vitro*. Z badań wynika, że grzyb *R. solani* wykazuje dużą wrażliwość na obecność w podłożu hodowlanym wodnych wyciągów z gleb nawożonych nawozami mineralnymi. Odzwierciedlają to współczynniki tempa wzrostu liniowego oraz zahamowania rozrostu liniowego i przyrostów biomasy. Wszystkie badane wyciągi glebowe i ich stężenia znacznie wpływały na dynamikę wzrostu *R. solani*. Najsilniejsze oddziaływanie inhibitujące wzrost liniowy strzępek grzybni *R. solani* obserwowano w obecności wodnego wyciągu z gleby nawożonej NPK + Potafoska. Natomiast na udział w podłożu hodowlanym 50 mm^3/cm^3 wyciągu z gleby nawożonej NPK oraz 25 mm^3/cm^3 NPK z siarczanem amonu testowany gatunek grzyba reagował silnym zahamowaniem przyrostu biomasy odpowiednio o 55.71 i 57.07 %. Przeprowadzone badania *in vitro* mogą wskazywać, iż wprowadzanie do gleby nawozów mineralnych NPK oraz innych takich jak siarczan amonu czy Potafoska mogą ograniczać populację grzyba chorobotwórczego *R. solani*. Jednakże w agrocenozach należy uwzględnić wzajemny skomplikowany układ zależności pomiędzy poszczególnymi elementami środowiska.

Słowa kluczowe: *Rhizoctonia solani*, nawożenie mineralne, wyciągi glebowe, wzrost liniowy, biomasa

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Anna GORCZYCA[1], Marek J. KASPROWICZ[2], Tadeusz LEMEK[3]
and Magdalena JAWORSKA[1]

INFLUENCE OF MULTI-WALLED CARBON NANOTUBES (MWCNTs) ON VIABILITY OF *Paecilomyces fumosoroseus* (WISE) BROWN & SMITH (*Deuteromycotina*: *Hyphomycetes*) FUNGUS SPORE

WPŁYW WIELOŚCIENNYCH NANORUREK WĘGLOWYCH (MWCNTS) NA ŻYWOTNOŚĆ ZARODNIKÓW GRZYBA *Paecilomyces fumosoroseus* (WISE) BROWN & SMITH (*Deuteromycotina*: *Hyphomycetes*)

Abstract: The study aimed at testing the influence of pristine multi-walled carbon (MWCNTs) and carboxyl (MWCNT(COOH)) nanotubes on spores of entomopathogenic *Paecilomyces fumosoroseus* fungus. The effect of the nanotubes on the fungus linear growth, biomass increment and sporulation was determined. The character of linear growth of *P. fumosoroseus* mycelium obtained from the spores contacted with MWCNTs was different in comparison with the control. Pristine carbon nanotubes significantly stimulated *P. fumosoroseus* mycelium linear growth and limited its sporulation in comparison with the control. Carboxylation changed the influence of carbon nanotubes on the spores of the tested fungus. The activity of MWCNT(COOH) was definitely weaker and the obtained linear growth and sporulation did not differ significantly from the control. No apparent effect of MWCNTs on the increment of *P. fumosoroseus* biomass was found.

Keywords: multi-walled carbon nanotubes, carboxylated multi-walled carbon nanotubes, *Paecilomyces fumosoroseus*

Nanotechnology is a modern scientific discipline evoking wide interest. Nanomaterials and nanocompounds find numerous applications in various fields of economy. Carbon nanotubes (CNTs) are novel nanotechnological material researched by numerous scientists. Currently, as in the case of a majority of such materials, analyses focus primarily on their physical and chemical properties and potential practical applications [1–5].

[1] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrgorczy@cyf-kr.edu.pl;

[2] Department of Physics of the University

[3] Department of Chemistry of the University

There have been reports pointing out the necessity to test carbon nanotube toxicity, especially for humans. Some of the obtained results may cause some anxiety [6–9]. Few of the studies conducted so far aim to test carbon nanotube toxicity for lower living organisms [10]. Researchers from the Yale University pointed out to the strong antibacterial effect of single-walled carbon nanotubes (SWNTs) on *Escherichia coli* [11]. Toxicity of this kind of nanomolecules for prokaryotic bacteria cells was demonstrated in the direct contact with the cell wall, which caused the death of the bacteria.

The studies aimed at determining the influence of multi-walled carbon nanotubes (MWCNTs) and carboxylated multi-walled carbon nanotubes (MWCNTs(COOH)) on the spores of *Paecilomyces fumosoroseus* fungus.

Material and methods

Carboxylated multi-walled nanotubes MWCNTs(COOH) were obtained by oxidation of pristine MWCNTs purchased in Echo-Nanobio Trading Co. Ltd., (Taipei, Taiwan). These are carbon nanotubes with the outer diameter of 40–60 nm, specific weight between 140 and 300 g/dm^3 and carbon content over 80 %.

Commercial MWCNTs were heated in nitric(V) acid for 2 days at boiling temperature. Subsequently they were drained and washed with water to obtain a neutral filtrate. They were dried at 120 °C for two days. TG analysis and acid-base titration of the carboxylated carbon nanotubes obtained in this way revealed the presence of carboxyl groups gravimetrically 3(± 0.5), purity > 99 %, pH = 4.5, outer diameter 10–40 nm (SME). The nanotubes prepared in this way were used for further analyses.

The experiment used a Polish strain of *P. fumosoroseus* entomopathogenic fungus, from the Department's own collection and was isolated from the soil for trap insects. The strain was multiplied to obtain sporulating mycelium. Fungus spore suspensions, in concentrations calculated in Bürker haemocytometer, were prepared in sterile distilled water. 200 cm3 of spore suspension in $5.5 \cdot 10^8$ pcs per cm^3 concentration was introduced into glass flasks. The control was provided by pure suspension without added nanotubes. The experimental objects contained multi-walled carbon nanotubes and carboxylated multi-walled carbon nanotubes, which were added to the spore suspensions in the volume of 0.1 cm^3 each. The control flask and flasks with added nanotubes were placed in a shaker and shaken for 1 hour. 10 mm^3 of the solution was collected with a micropipette from each of the shaken suspensions and inoculated on dishes with a standard Sabouraud solid medium to obtain mycelium linear growth. Six replications were used. When the observation of linear growth was completed (23rd day of fungi culturing) and sporulating mycelia were obtained, spore suspensions were prepared again by means of collecting 5 mycelium rings, 1 cm in diameter, from each experimental object and counting their sporulation. Mycelium biomass increment was also examined after 1 hour contact with the nanotubes by inoculating 3 cm^3 of the suspension to the flasks containing 100 cm^3 of liquid Sabouraud medium in 6 replications and incubating them at 20 °C for 40 days. After this period the post culture liquid with the mycelium was drained through filter paper. The mycelium was dried in sterile glass at 80 °C to constant weight. Dry mycelium mass was weighed on electronic analytical balance.

Analysing the obtained results, the curves of the linear growth trend were matched with the Gompertz curve according to the following formula:

$$Y = A \cdot \exp(-\exp(-k(x-x_c)))$$

where:
A – amplitude,
k – process speed rate,
x_c – curve inflection point.

Results and discussion

Figure 1 presents linear growth of *P. fumosoroseus* obtained from the fungi spores after one-hour contact with pristine multi-walled carbon and carboxylated nanotubes inoculated on solid media.

The trend of mycelium growth after the contact with multi-walled carbon nanotubes was different in character as compared with the control (spore suspension stirred for 1 h). Following the contact with pristine carbon nanotubes, at the five hundredth hour of growth, the mycelium reached a significantly larger size in comparison with the control. It was not observed for carboxylated carbon nanotubes. The analysis of growth according to Gompertz distribution revealed differences in the rate of the process (k) and the growth saturation time, which corresponds to the curve inflection point. The results of analysis were compiled in Table 1.

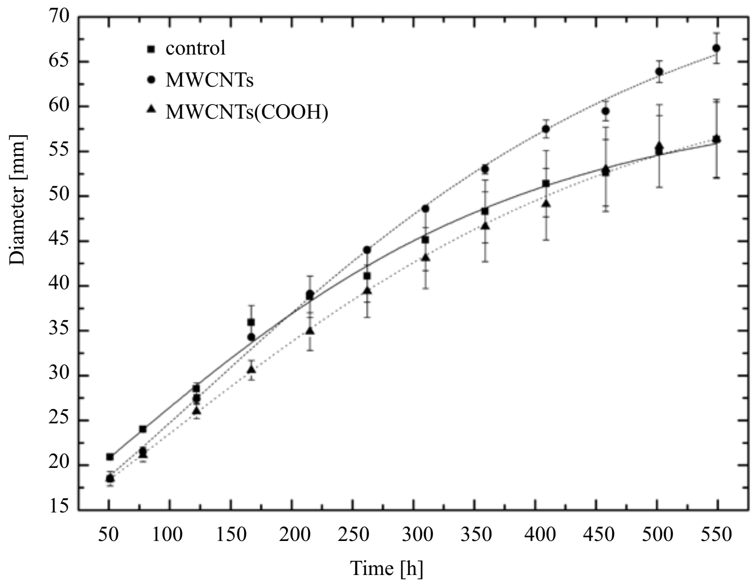

Fig. 1. Linear growth of *P. fumosoroseus* fungus obtained in culturing after 1 hour contact with carbon nanotubes

Table 1

Linear growth parameters obtained on the basis of the Gompertz distribution.

Specification	Control		MWCNTs		MWCNTs(COOH)	
	value	error	value	error	value	error
Amplitude – A	60.76	4.12	77.35	2.03	64.98	6.74
Growth rate – k	0.00510	0.00067	0.00435	0.00024	0.00439	0.00076
Time of fastest growth – x_c	63.76	13.28	130.21	6.62	103.43	24.89

In the case when both kinds of carbon nanotubes were used, the growth trend revealed a greater amplitude in comparison with the control, a lower process rate and a significantly longer time of growth saturation, which means that the control mycelium was growing faster, but also its growth rate was slowed earlier. In may be concluded that culturing the fungus from *P. fumosoroseus* spores following their contact with both kinds of MWCNTs initially only slightly limited the linear growth intensity in time, whereas its collapse was observed significantly later in comparison with the control and the mycelia reached greater sizes, particularly for non-carboxylated multi-walled carbon nanotubes.

Biomass obtained from the spores after their contact with carbon nanotubes did not change notably in comparison with the control. Differences were observed within the measuring error range, as has been shown in Figure 2.

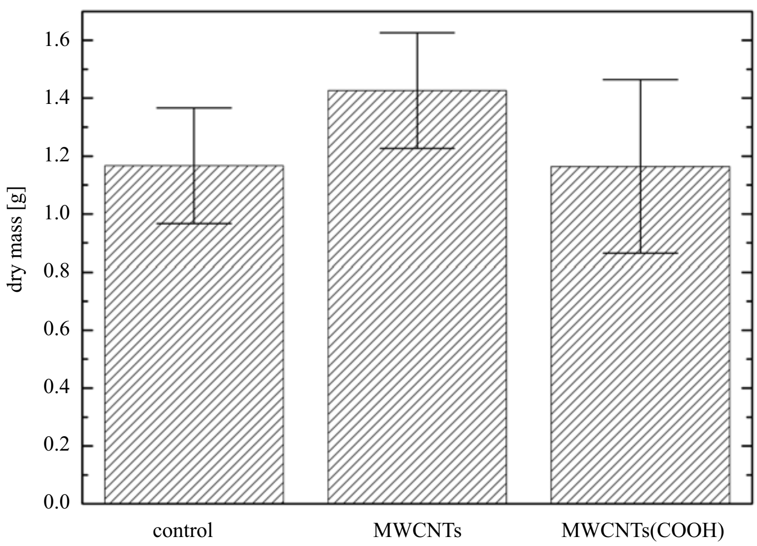

Fig. 2. Biomass of *P. fumosoroseus* obtained in the fungus culturing after 1 hour contact with carbon nanotubes

Results of fungus sporulation were diversified (Fig. 3). *P. fumosoroseus* sporulated significantly worse after the contact with non-carboxylated nanotubes, which was not noted for carboxylated nanotubes, where the results obtained were very close to the control.

Kang et al [12], who observed a strong antibacterial effect of carbon nanotubes on *E. coli*, obtained different results. However, in their studies the above-mentioned authors were using single-walled carbon nanotubes (SWNTs) at the concentration on of 5 µg/cm^3 introduced to 0.9 % NaCl. Moreover, their test organism were bacteria and not a fungus spore.

Initial observations conducted on *P. fumosoroseus* fungus spores require more detailed research including, among others, testing if carbon nanotubes may contain free radicals. At present the *P. fumosoroseus* spore response to the contact with nanotubes may be explained by the fact that non-carboxylated carbon nanotubes, although hydrophobic, provide a source of carbon necessary for the growth of living organisms. However, the applied dose of nanotubes was not a considerable source of amorphous carbon. The more probable cause of the fungus growth stimulation may be the mutagenicity of non-carboxylated carbon nanotubes expressed as a faster cell division and therefore growth at simultaneous incapacitating sporulation.

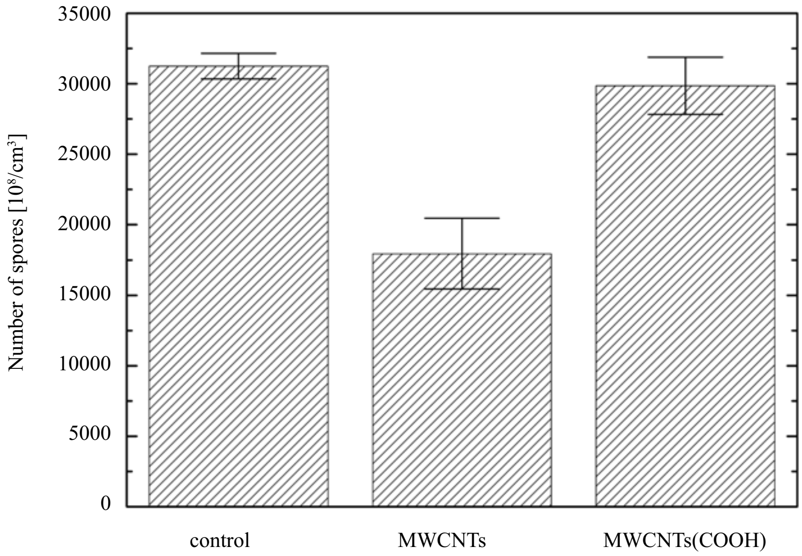

Fig. 3. Sporulation of *P. fumosoroseus* obtained in the fungus culturing after 1 hour contact with carbon nanotubes

Conclusions

1. A different character of *P. fumosoroseus* mycelium linear growth obtained from the spores in contact with multi-walled carbon nanotubes was observed in comparison with the control.

2. Pristine carbon nanotubes led to a significant stimulation of surface growth of *P. fumosoroseus* mycelium and reduction of its sporulation in comparison with the control.

3. Carboxylation changes the properties of multi-walled carbon nanotubes towards fungus spores.

4. The effect of carboxylated carbon nanotubes was definitely weaker and the obtained linear growth and sporulation did not differ significantly form the control.

5. No significant effect of multi-walled carbon nanotubes on *P. fumosoroseus* fungus biomass increment was observed.

References

[1] Iijima S.: *Helical microtubules of graphitic carbon*. Nature, 1991, **354**(6348), 56–58.
[2] Stafiej A. and Pyrzyńska K.: *Adsorption of heavy metal ions with carbon nanotubes*. Separ. Purific. Technol., 2007, **58**, 49–52.
[3] Wong D.H. and Lieber C.M.: *Probing electrical transport in nanomaterials: conductivity of individual carbon nanotubes*. Science, 1996, **272**(5261), 523–526.
[4] Ebbesen T.W., Lezec H.J., Hiura H., Bennet J.W., Ghaemi H.F. and Thio T.: *Electrical conductivity of individual carbon nanotubes*. Nature, 1996, **382**(6586), 54–55.
[5] Saito Y., Hamaguchi K., Hata K., Uchida K., Tasaka Y. and Ikazaki F.: *Conical beams from open nanotubes*. Nature, 1997, **389**(6651), 554.
[6] Hurt R.H., Monthioux M. and Kane A.: *Toxicology of carbon nanomaterials: Status, trends, and perspectives on the special issue*. Carbon 2006, **44**, 1028–1033.
[7] Lacerda L., Bianco A., Prato M. and Kostarelos K.: *Carbon nanotubes as nanomedicines: From toxicology to pharmacology*. Adv. Drug Delivery Rev. 2006, **58**, 1460–1470.
[8] Smart S.K., Cassady A.I., Lu G.Q. and Martin D.J.: *The biocompatibility of carbon nanotubes*. Carbon 2006, **44**, 1034–1047.
[9] Kalbacova M., Kalbac M., Dunsch L. and Hempel U.: *Influence of single-walled carbon nanotube films on metabolic activity and adherence of human osteoblasts*. Carbon 2007, **45**, 2266–2272.
[10] Tuzen M., Saygi K.O., Usta C. and Solak M.: *Pseudomonas aeruginosa immobilized multiwalled carbon nanotubes as biosorbent for heavy metal ions*. Bioresource Technol. 2008, **99**, 1563–1570.
[11] Reinhold C.: *Carbon nanotubes show germ-fighting promise*. Toxicol. Environ. Nanotoday., 2007, **2**(5), 10.
[12] Kang S., Pinault M., Pfefferle L.D. and Elimelech M.: *Single-Walled Carbon Nanotubes Exhibit Strong Antimicrobial Activity*. Langmuir., 2007, **23**, 8670–8673.

WPŁYW WIELOŚCIENNYCH NANORUREK WĘGLOWYCH (MWCNTS) NA ŻYWOTNOŚĆ ZARODNIKÓW GRZYBA *Paecilomyces fumosoroseus* (WISE) BROWN & SMITH (*Deuteromycotina*: *Hyphomycetes*)

Katedra Ochrony Środowiska Rolniczego; Katedra Fizyki; Katedra Chemii;
Uniwersytet Rolniczy w Krakowie

Abstrakt: Zbadano wpływ wielościennych nanorurek węglowych surowych (MWCNTs) i karboksylowanych (MWCNT(COOH)) na zarodniki owadobójczego grzyba *Paecilomyces fumosoroseus*. Określono wpływ nanorurek na wzrost liniowy grzyba, przyrost biomasy i zarodnikowanie. Charakter wzrostu liniowego grzybni *P. fumosoroseus* uzyskanego z zarodników kontaktowanych z MWCNTs w porównaniu do kontroli był inny. Nanorurki węglowe surowe powodowały znaczną stymulację wzrostu powierzchniowego grzybni *P. fumosoroseus* i ograniczenie jej zarodnikowania w porównaniu do kontroli. Karboksylacja zmieniła wpływ nanorurek węglowych na zarodniki badanego grzyba. Oddziaływanie MWCNT(COOH) było zdecydowanie słabsze, a uzyskany wzrost liniowy i zarodnikowanie nie różniły się istotnie od kontroli. Nie stwierdzono istotnego wpływu MWCNTs na przyrost biomasy grzyba *P. fumosoroseus*.

Słowa kluczowe: wielościenne nanorurki węglowe, karboksylowane wielościenne nanorurki węglowe, *Paecilomyces fumosoroseus*

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Janina GOSPODAREK[1]

EFFECT OF MAGNESIUM TREATMENT ON BEAN BEETLE (*Bruchus rufimanus* BOH.) FEEDING ON BROAD BEAN (*Vicia faba* L. ssp. *maior*) IN CONDITIONS OF SOIL CONTAMINATION WITH HEAVY METALS

WPŁYW NAWOŻENIA MAGNEZOWEGO NA ŻEROWANIE STRĄKOWCA (*Bruchus rufimanus* BOH.) NA BOBIE (*Vicia faba* L. ssp. *maior*) W WARUNKACH SKAŻENIA GLEBY METALAMI CIĘŻKIMI

Abstract: The aim of the research was to determine the effect of magnesium treatment on the harmfulness of bean beetle (*Bruchus rufimanus* Boh.) for broad bean growing in conditions of soil contaminated with single heavy metals on III level of pollution acc. to the IUNG classification. The observations were conducted on broad bean (*Vicia faba* L. ssp. *maior*), White Windsor c.v. cultivated in two series: in soil subjected to magnesium fertilization and unfertilized with it. 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 with mineral fertilization (Control + NPK); cadmium contaminated soil (4 mg $\cdot$ kg^{-1} d.m.); soil polluted with lead (530 mg $\cdot$ kg^{-1} d.m.); soil polluted with copper (85 mg $\cdot$ kg^{-1} d.m.); soil polluted with zinc (1000 mg $\cdot$ kg^{-1} d.m.) and soil contaminated with nickel (110 mg $\cdot$ kg^{-1} d.m.). Identical magnesium fertilization applied for all objects was 20.4 mg Mg $\cdot$ kg^{-1} d.m.

The level of magnesium treatment applied to the soil contaminated with single heavy metals did not significantly affect broad bean seed yield, degree of injuries caused by bean beetle or their germinating ability. Magnesium treatment may slightly improve germination energy of broad bean seeds originating from plants growing in soil polluted with cadmium.

Keywords: heavy metals, magnesium fertilization, *Bruchus rufimanus* Boh.

Soil concentrations of heavy metals, such as copper, lead or cadmium reaching the level of elevated content or medium pollution according to the IUNG classification do not affect negatively the amount or quality of broad bean seeds [1, 2]. Therefore soil polluted with these metals may be used for broad bean seed crop cultivation. Magnesium fertilization may additionally beneficially affect the growth and crop yield quality, also when the plants are growing in heavy metal polluted soil [3]. It is due to potential

[1] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrjgospo@cyf-kr.edu.pl

reduction of the heavy metal uptake by plants as a result of the application of this measure and diminishing the harmfulness of some agrophages [3–5].

The studies were undertaken to determine the effect of magnesium treatment on the harmfulness of bean beetle (*Bruchus rufimanus* Boh.) for broad bean growing in soil contaminated with single heavy metals on the III level of pollution according to the IUNG classification. Assessed were also the energy and germination ability of seeds both injured by broad bean beetle and healthy ones.

Material and methods

The experiment was conducted in 2005 on degraded chernozem developed from loess revealing acid reaction and organic carbon concentration 1.13 %. Observations were conducted on broad bean (*Vicia faba* L., ssp. *maior*), White Windsor c.v. cultivated in the following objects: unpolluted soil with natural content of heavy metals (Control); unpolluted soil with natural heavy metal content receiving mineral fertilization (Control + NPK); soil contaminated with cadmium dosed: 4 mg $\cdot$ kg^{-1} d.m.; soil contaminated with lead dosed: 530 mg $\cdot$ kg^{-1} d.m.; 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. The same magnesium fertilization, with 20.4 mg Mg $\cdot$ kg^{-1} d.m., was applied in all objects.

The analyzed level of soil heavy metal pollution corresponded to the III class of pollution according to the IUNG classification [6]. The heavy metals in the form of water solutions of the following salts: $3CdSO_4 \cdot 8H_2O$, $NiSO_4 \cdot 7H_2O$, $CuSO_4$, $ZnSO_4 \cdot 7H_2O$ and $Pb(NO_3)_2$ were supplied to the soil in the year preceding the experiment. Basic fertilization, the same on all objects (except for the non-fertilized control), dosed: 0.7 g N (in NH_4NO_3); 0.8 g P_2O_5 (in KH_2PO_4); 1.2 g K_2O (in KCl) per pot (9.8 kg of soil d.m.) was applied simultaneously with heavy metal addition to the soil. In the year of the experiment some pots containing the contaminated soil received magnesium fertilization. The dose of the magnesium fertilizer was established on the basis of the soil analysis conducted by the Agro-Chemical Station in Krakow. The content of bioavailable magnesium in the initial soil was 7.2 mg $\cdot$ 100g^{-1} of soil dry mass. Magnesium was added to the soil as water solution of $MgSO_4 \cdot 7H_2O$.

The harmfulness of bean beetle was estimated on the basis of the weight of injured seeds in relation to the total seed weight. The germination energy and ability of broad bean seeds were assessed in laboratory conditions according to the generally accepted standards. The test was performed on Petri dishes on filter paper as the substratum. Germinating energy was assessed after 4 days and germination ability after 14 days. Seeds injured by bean beetle (*Bruchus rufimanus* Boh.) and healthy seeds were assessed separately.

Results and discussion

Soil pollution with zinc and nickel inhibited broad bean plant growth so that they were unable to develop seeds. Applied magnesium fertilization did not lead to any major changes in the soil reaction [7]. No statistically significant effect of the level of magnesium treatment applied to the soil contaminated with individual heavy metals was registered with respect to the obtained broad bean seed yield and the level of their injury due to bean beetle (Table 1). The largest number of seeds was gathered from plants growing in lead and cadmium contaminated soil when magnesium treatment was applied in the object where the soil was polluted with lead and fertilized with magnesium also the highest proportion of seeds injured by bean beetle was found, significantly higher than in the control receiving mineral fertilization.

Table 1

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

Object	Average seed weight per plant [% in relation to NPK]	Weight of seeds injured by *Bruchus rufimanus* Boh. [% in relation to NPK]
Cu+Mg	88.22 ab	137.62 ab
Cu	117.13 ab	110.66 ab
Pb+Mg	173.33 b	176.86 b
Pb	139.92 ab	167.37 ab
NPK+Mg	118.12 ab	163.22 ab
NPK	100.00 ab	100.00 a
Cd+Mg	153.64 ab	130.34 ab
Cd	139.41 ab	114.55 ab
K+Mg	52.12 a	155.15 ab
K	116.69 ab	161.42 ab

A greater germination energy was observed in seeds uninjured by bean beetle from plants growing in cadmium contaminated soil and fertilized with magnesium than when this measure was not applied. The situation was similar in the control object where mineral fertilizers were used. In the other objects no significant effect of magnesium fertilization on seed germination energy was observed (Fig. 1b). Also the seeds injured by bean beetle originating from plants cultivated in cadmium polluted soil subjected to magnesium treatment were germinating faster than the seeds from the object contaminated with cadmium but not fertilized with magnesium (Fig. 2b). The cadmium content in roots decreased ca 2.5 times as a result of magnesium fertilization. On the other hand, no effect of magnesium fertilization was observed on the concentrations of this element in broad bean shoots. Magnesium fertilization had a similar effect in the case of copper and lead – diminished metal concentrations as a result of this measure was observed only in the underground parts [7].

The analysis of the germination of the seeds uninjured by bean beetle did not reveal a statistically significant effect of magnesium treatment (Fig. 2a). No major differences in seedling condition were registered, either (Table 2). Bean beetle feeding considerably inhibited seed germination ability (Fig. 2b). Also in this case no statistically significant differences were found between objects subjected to magnesium fertilization and ones non-fertilized with this element. Seeds from the control, from the object with mineral fertilization and additional magnesium treatment and from the lead contaminated object revealed the greatest germination ability.

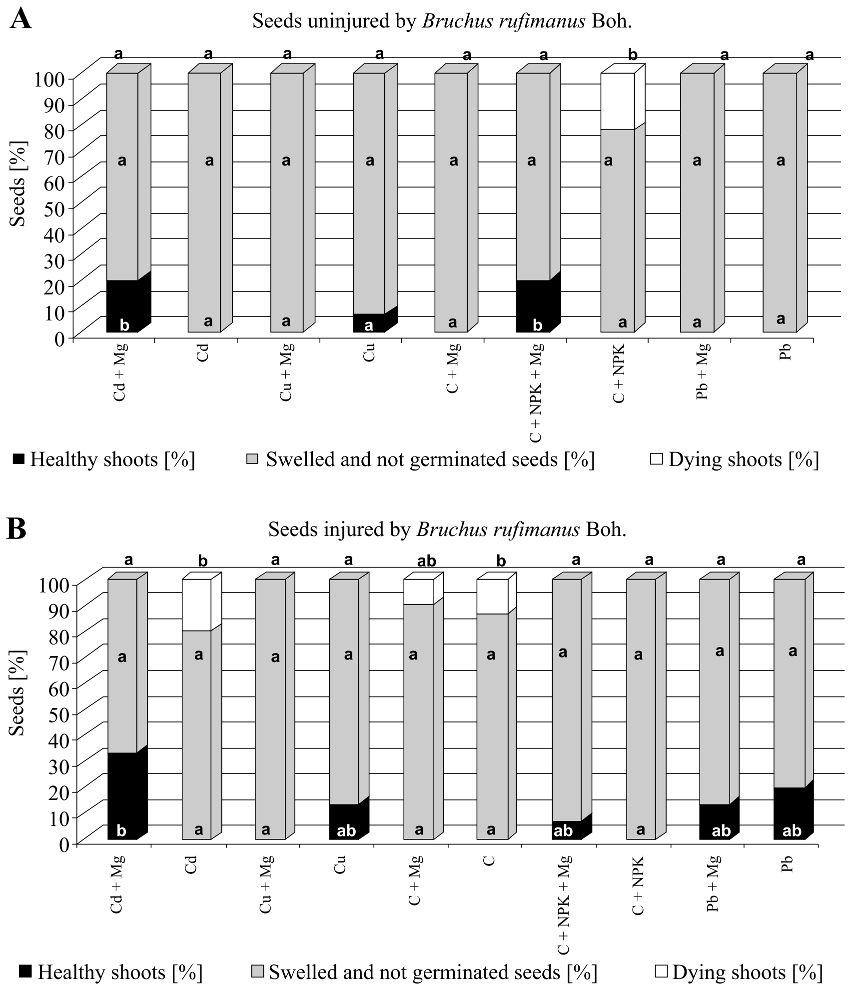

Fig. 1. Germination energy of broad bean seeds uninjured (A) and injured (B) by *Bruchus rufimanus* Boh. originating from plants growing in natural and heavy metal contaminated soil with magnesium fertilization. Values for individual metals or control and for individual features, marked by different letters in columns are statistically different (p = 0.05).

Table 2

Characteristics of germinating broad bean seeds (injured or uninjured by *Bruchus rufimanus* Boh.) from plants cultivated in natural soil and in heavy metal contaminated soil with applied magnesium treatment.

Objects	Shoot length [cm]		Underground part length [cm]		Number of lateral roots > 2 mm	
	Uninjured seeds	Injured seeds	Uninjured seeds	Injured seeds	Uninjured seeds	Injured seeds
Cu + Mg	5.99 a	2.76 a	10.60 ab	4.33 ab	6.82 a	7.92 a
Cu	7.22 a	5.19 a	8.80 ab	8.32 ab	12.88 ab	12.61 a
Pb + Mg	8.12 a	8.03 a	6.25 ab	9.59 ab	8.45 ab	11.44 a

Table 2. contd.

Objects	Shoot length		Underground part length		Number of lateral roots > 2 mm	
	Uninjured seeds	Injured seeds	Uninjured seeds	Injured seeds	Uninjured seeds	Injured seeds
Pb	9.10 a	8.80 a	9.35 ab	12.65 b	13.50 ab	10.49 a
NPK + Mg	15.62 a	4.77 a	8.50 ab	6.85 ab	17.00 ab	6.88 a
NPK	9.03 a	5.40 a	5.50 a	4.60 ab	10.05 ab	5.35 a
Cd + Mg	10.66 a	5.68 a	12.94 b	6.91 ab	15.05 ab	6.53 a
Cd	9.10 a	2.36 a	9.00 ab	2.78 a	11.00 ab	4.89 a
K + Mg	14.00 a	0.73 a	7.00 ab	2.98 a	18.00 b	7.75 a
K	–	5.98 a	–	8.92 ab	–	15.19 a

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

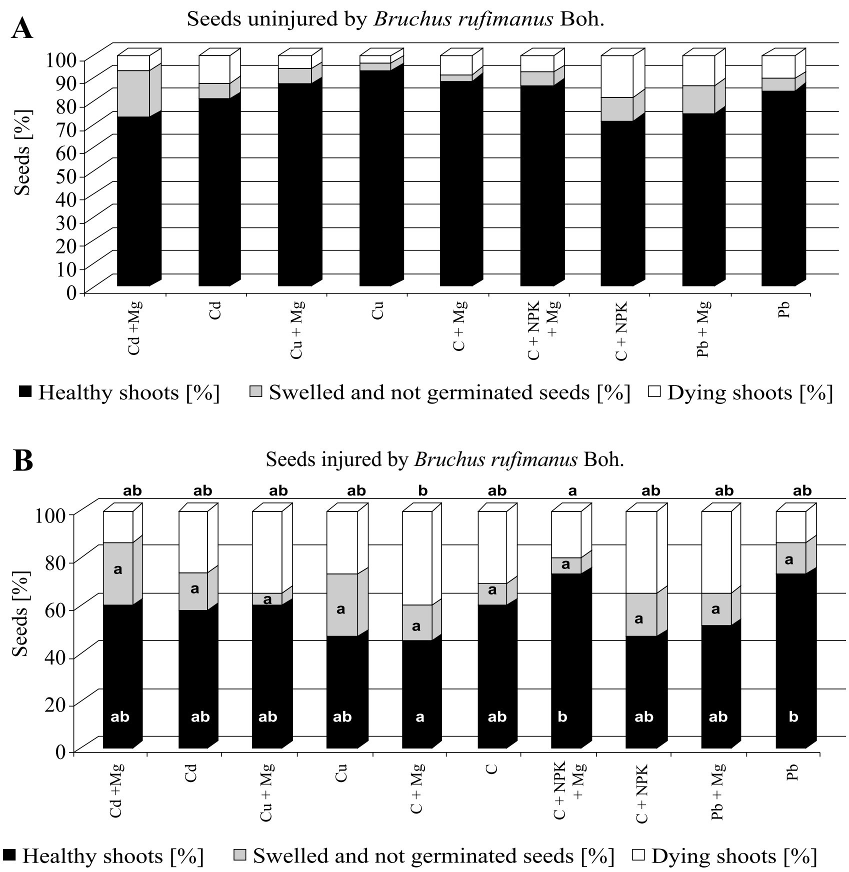

Fig. 2. Germination ability of broad bean seeds uninjured (A) and injured (B) by *Bruchus rufimanus* Boh. originating from plants growing in natural and heavy metal contaminated soil with magnesium fertilization. Values for individual metals or control and for individual features, marked by different letters in columns are statistically different (p = 0.05).

Conclusions

1. The applied level of magnesium fertilization of soil contaminated with single heavy metals does not significantly affect either broad bean seed yield, or the degree of injuries caused by bean beetle, or their germination ability.

2. Magnesium treatment may slightly improve the germination energy of broad bean seeds from plants growing in cadmium contaminated soil.

References

[1] Gospodarek J.: Ecol. Chem. Eng. 2006, **13**(6), 497–504.

[2] Gospodarek J.: Ecol. Chem. Eng. 2006, **13**(1–2), 47–54.

[3] Jaworska M. and Gospodarek J.: Acta Agrophys. 2003, **1**(4), 647–652.

[4] Slansky F. and Rodriguez J.G.: Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates, John Wiley & Sons, New York 1987, 1016 pp.

[5] Jaworska M. and Gospodarek J.: J. Elementol. 2004, **9**(3), 321–328.

[6] Kabata-Pendias A. and Piotrowska M.: *Ocena stopnia zanieczyszczenia gleb i roślin metalami ciężkimi i siarką.* 1993, Wyd. IUNG Puławy, ser. P: 53 pp.

[7] Gospodarek J. and Nadgórska-Socha A.: Ecol. Chem. Eng. 2007, **14**(9), 966–974.

WPŁYW NAWOŻENIA MAGNEZOWEGO NA ŻEROWANIE STRĄKOWCA (*Bruchus rufimanus* BOH.) NA BOBIE (*Vicia faba* L. ssp. *maior*) W WARUNKACH SKAŻENIA GLEBY METALAMI CIĘŻKIMI

Katedra Ochrony Środowiska Rolniczego, Uniwersytet Rolniczy w Krakowie

Abstrakt: Celem podjętych badań było określenie wpływu nawożenia magnezowego na szkodliwość strąkowca bobowego (*Bruchus rufimanus* Boh.) dla bobu rosnącego w warunkach gleby zanieczyszczonej pojedynczymi metalami ciężkimi na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG. Obserwacje prowadzono na bobie (*Vicia faba* L. ssp. *maior*) odm. Windsor Biały uprawianym w dwóch seriach: na glebie poddanej nawożeniu magnezowemu i 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 (Kontrola + 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. Nawożenie magnezowe zastosowano jednakowe dla wszystkich obiektów: 20,4 mg Mg · kg^{-1} s.m.

Zastosowany poziom nawożenia magnezowego gleby skażonej pojedynczymi metalami ciężkimi nie wpływa znacząco na plon nasion bobu, stopień ich uszkodzenia przez strąkowca bobowego ani też ich zdolność kiełkowania. Nawożenie magnezowe może nieco zwiększać energię kiełkowania nasion bobu pochodzących z roślin rosnących w glebie zanieczyszczonej kadmem.

Słowa kluczowe: metale ciężkie, nawożenie magnezowe, *Bruchus rufimanus* Boh.

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Janina GOSPODAREK[1] and Aleksandra NADGÓRSKA-SOCHA[2]

EFFECT OF LIMING OF HEAVY METAL POLLUTED SOIL ON THE CONTENT OF MAGNESIUM, CALCIUM AND IRON IN BROAD BEAN (*Vicia faba* L., ssp. *maior*) PLANTS

WPŁYW WAPNOWANIA GLEBY SKAŻONEJ METALAMI CIĘŻKIMI NA ZAWARTOŚĆ MAGNEZU, WAPNIA I ŻELAZA W ROŚLINACH BOBU (*Vicia faba* L., ssp. *maior*)

Abstract: The research aimed at determining the effect of diversified lime doses on the content of magnesium, calcium and iron in broad bean plants growing in conditions of soil polluted with heavy metals on the III level of pollution according to the IUNG classification. Analyses were conducted on broad beans (*Vicia faba* L. ssp. *maior*), White Windsor c.v. cultivated in two series: in limed and non-limed soil. In each series the plants were cultivated in the following objects: unpolluted soil with natural heavy metal concentrations (Control); unpolluted soil with natural heavy metal content receiving mineral fertilization (NPK); soil polluted with cadmium dosed 4 mg · kg^{-1} d.m.; soil contaminated with lead dosed 530 mg · kg^{-1} d.m.; soil contaminated with copper dosed 85 mg · kg^{-1} d.m.; soil polluted with 1000 mg · kg^{-1} d.m.of zinc and soil contaminated with a dose of 110 mg · kg^{-1} d.m. of nickel. Liming was conducted on the basis of hydrolytic acidity analysis of soil from individual objects. Two doses were applied: according to 1 and 2 Hh. The lime doses were respectively: Control – 356 and 712 mg CaO · kg^{-1} d.m.; NPK – 420 and 840 mg CaO · kg^{-1} d.m.; cadmium polluted soil – 504 and 1008 mg CaO · kg^{-1} d.m.; soil polluted with lead – 420 and 840 CaO · kg^{-1} d.m.; copper contaminated soil – 398 and 796 CaO · kg^{-1} d.m.; soil polluted with zinc – 860 and 1720 CaO · kg^{-1} d.m. and soil polluted with nickel 524 and 1048 CaO · kg^{-1} d.m.

Soil contamination with heavy metals such as Cu, Cd, Pb, Ni or Zn causes considerable changes in Ca, Mg and Fe concentrations in broad bean plants. Application of liming contributes to balancing the content of the studied elements in plants. After the measure was applied the content of Ca, Fe and Mg in the plants growing in soil contaminated with individual heavy metals in most cases was on a level similar as in the plants growing in unpolluted soil.

Keywords: heavy metals, liming, accumulation, Mg, Ca, Fe

The harmful effect of heavy metals in soil may be revealed as blocking the uptake of some macro- and microelements by plants. For instance, Zn and Cd have an inhibitory

1 Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrjgospo@cyf-kr.edu.pl

2 Departament of Ecology, University of Silesia, ul Bankowa 9, 40-007 Katowice, Poland, email: olan@hoga.pl

effect upon Fe, whereas lead on P and Mn absorption. Disturbances in the uptake of these elements may result in their deficiency in plants but may also reduce the nutritional value of plants cultivated for forage. Calcium in soil is identified as an agent neutralising the negative effect of many harmful constituents, including heavy metals [1, 2].

The investigations aimed at determining the effect of diversified calcium doses on the content of selected elements: magnesium, calcium and iron in broad bean plants growing in conditions of soil contaminated with heavy metals on the III level of pollution according to the IUNG classification [3].

Material and methods

The soil used for the experiment was a degraded chernozem developed from loess with acid reaction (pH in 1 mol · dm^{-3} KCl solution was 5.7 and in water 6.5) and organic carbon content 1.13 %. The observations were conducted on broad bean (*Vicia faba* L. ssp. *maior*), White Windsor, c.v. cultivated in two series: in limed and non-limed soil. The doses of heavy metals and calcium are given in Table 1. The way in which heavy metals were supplied to the soil, the amount of basic fertilization and methods of chemical analyses conducted on the soil were presented in other papers [4, 5]. Liming was conducted on the basis of hydrolytic acidity analyses of the soil from individual objects. Two doses were applied: according to 1 (…+Ca1) and 2 Hh (…+Ca2). The soil reaction from individual objects after the end of the growing period was presented in another paper [6].

Table 1

Experimental design

Object	Metal dose [mg/kg soil d.m.]	CaO dose [mg/kg soil d.m.] (acc. to 1 Hh) (+Ca1)	CaO dose [mg/kg soil d.m.] (acc.to 2 Hh) (+Ca2)
Non-fertilized control	–	356	712
Control receiving mineral fertilizers	–	420	840
Cadmium contaminated soil	4	504	1008
Copper contaminated soil	85	398	796
Lead contaminated soil	530	420	840
Nickel contaminated soil	110	524	1048
Zinc contaminated soil	1000	860	1720

The samples for chemical analyses were collected at the stage of seed milk maturity. Chemical analysis of the plant material consisted in determining the content of selected macroelements: iron, magnesium and calcium.

Plant material was washed in tap and in distilled water, dried at 105 °C to a constant weight and ground to fine powder, then mineralized and dissolved in 10 % HNO_3. After filtration: Mg, Ca, Fe content was measured using Flame Atomic Absorption Spectrometry (FAAS) [7, 8]. The quality of the analytical procedure was controlled by using samples of the reference material in each series of analysis (Certified Reference material CTA-OTL-1 Oriental Tobacco Leaves). The data were processed using software Statistica to compute significant statistical differences between samples ($p < 0.05$) according to Tukey's multiple range test.

Results and discussion

Soil contamination with copper, nickel and zinc caused a significant decline in magnesium concentrations in broad bean shoots in comparison with the objects with unpolluted soil (Fig. 1A). The decline was slight for copper, in the case of nickel it was almost two-fold, whereas for zinc even 5-fold. Liming of copper contaminated soil (both using a higher and a lower dose) led to a further slight decrease in the Mg content. A similar result of liming was observed when broad bean was growing in cadmium contaminated soil. In conditions of nickel polluted soil a double lime dose diminished the magnesium content in broad bean shoots by 1/3 in comparison with the object contaminated with this metal but without liming. On the other hand in the case of zinc contaminated soil, liming (both at the lower and higher CaO dose) incurred growth in the Mg content reaching almost the level similar to unpolluted plants. However, liming did not affect the content of magnesium in the shoots of broad bean cultivated in lead contaminated soil. Due to soil pollution with cadmium or lead, between 3 and 4 times larger amounts of magnesium were detected in broad bean roots growing in this soil than in the control plant roots (Fig. 1B). In conditions of copper and cadmium contaminated soils, a double dose of lime significantly lowered magnesium concentrations in broad bean roots. In broad bean grown in lead contaminated soil, a high, over two fold decline in magnesium concentration in the roots was registered when liming was done with both the lower and the higher dose.

This measure made the Mg content in roots approximate the same level as in plants unpolluted with heavy metals. Liming of zinc polluted soil caused an increase in the Mg content in broad bean underground parts, particularly at a higher lime dose. In nickel contaminated soil a lower dose of lime slightly elevated the Mg content in these plant parts, whereas a higher dose did not lead to any notable changes in this macroelement content. Similarly, a slight increase in the magnesium content under the influence of liming was noted in broad bean roots growing in the soil contaminated with a joint dose of heavy metals on the level corresponding to the I pollution degree according to the IUNG classification [9].

Soil pollution with all of the analyzed heavy metals, except cadmium, led to a decline in the calcium content in broad bean shoots (Fig. 2A). Liming of copper polluted soil apparently did not affect the concentration of this element. In the case of soil pollution with cadmium liming with both a higher and a lower dose led to a decline in the Ca content in broad bean shoots by ca 1/3. When the soil was polluted with lead the lower dose of lime elevated the Ca content (almost twice in comparison with Pb contaminated but non-limed soil), whereas the higher dose lowered this concentration (by ca 1/3 in comparison with the non-limed object). In conditions of soils contaminated with zinc

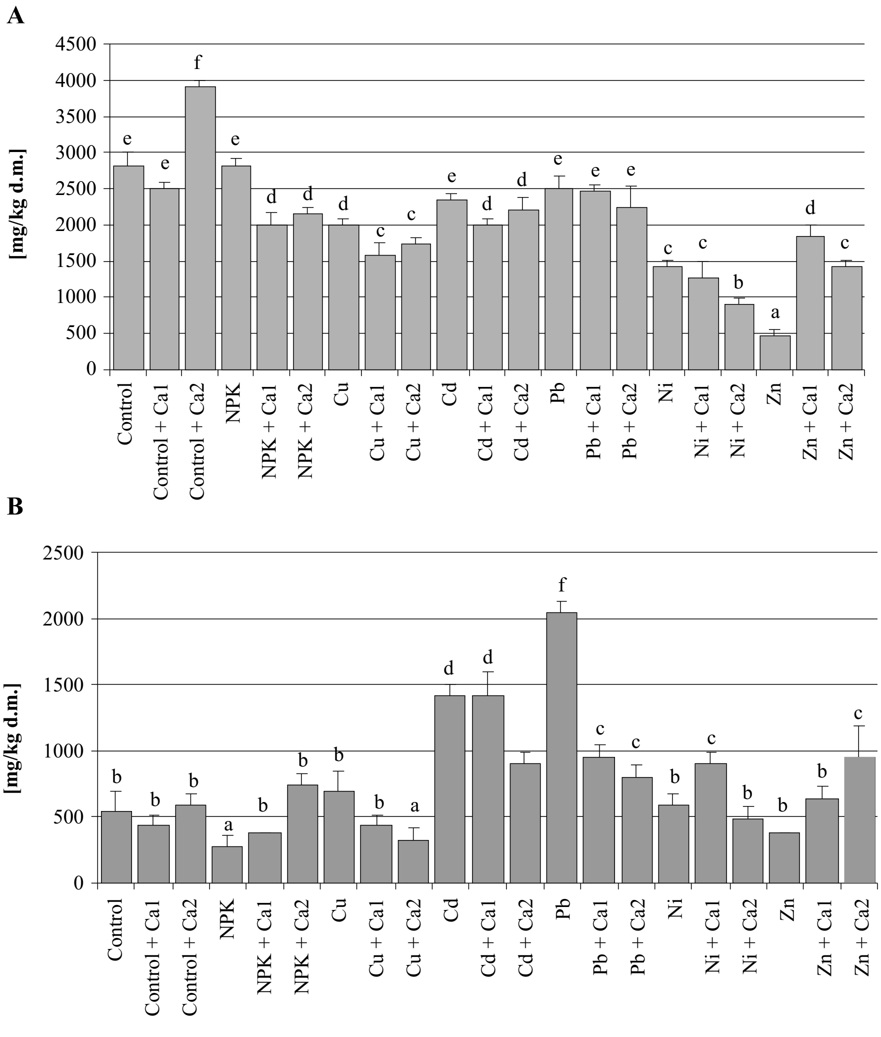

Fig. 1. Magnesium content in shoots (A) and roots (B) of broad bean (*Vicia faba* L., ssp. *maior*) cultivated in unpolluted soil (Control, NPK) and in soil contaminated with single heavy metals and limed with a dose acc. to 1 Hh (…+Ca1) and acc. to 2 Hh (…+Ca2). Values marked with different letters are statistically different at p = 0.05

and nickel, liming (both with a dose computed acc. to 1 Hh and acc. to 2 Hh) apparently raised the Ca content in broad bean shoots. In the object with nickel contaminated soil the rise was almost twofold, while on the object with zinc contaminated soil – 20-fold. In the latter case Ca concentrations in broad bean shoots after applied liming approximated the level of this element in the control plants. Similarly as in the shoots, also in roots soil contamination with a majority of the analyzed heavy metals caused a decline in Ca content (Fig. 2B).

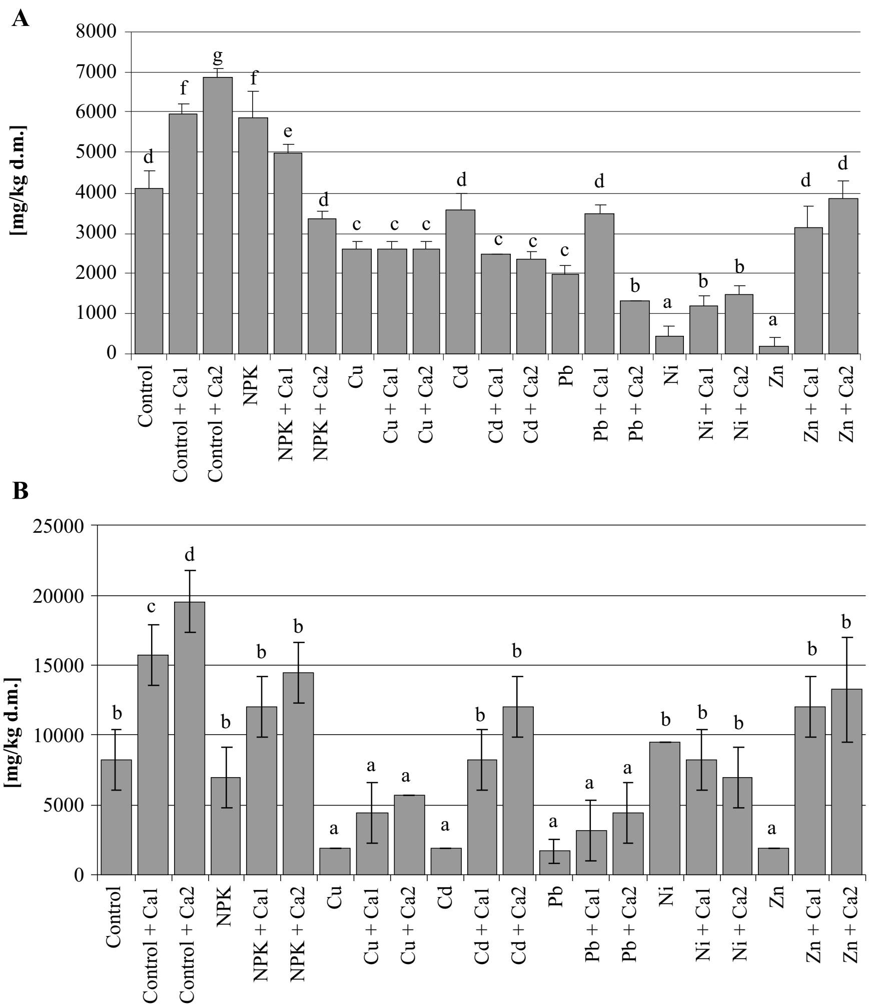

Fig. 2. Calcium content in shoots (A) and roots (B) of broad bean (*Vicia faba* L., ssp. maior) cultivated in unpolluted soil (Control, NPK) and in soil contaminated with single heavy metals and limed with a dose acc. to 1 Hh (…+Ca1) and acc. to 2 Hh (…+Ca2). Values marked with different letters are statistically different at $p = 0.05$.

On the other hand, application of liming usually caused an increase in the calcium content in these broad bean parts, which was the higher, the higher was the lime dose. In objects where the soil was contaminated with cadmium and zinc this measure contributed to increasing the Ca level in broad bean roots to the same one as in unpolluted plants. Only in the case of soil polluted with nickel no changes in the Ca content were registered in broad bean roots growing in it as an effect of liming, but the level of this element did not differ significantly either from the calcium content in plants growing in natural soil. The Author's former studies showed that at joint application of all heavy metal doses the concentrations of this element in broad bean roots growing in contaminated and limed soil were also apparently higher in comparison with the roots growing in polluted soil but without liming [9].

Soil contamination with copper, lead and cadmium did not affect significantly the iron content in broad bean shoots (Fig. 3A). However, soil contamination with nickel and zinc caused a considerable (ca 3 –fold for Ni and 4-fold for Zn) increase in iron content. It was connected with a decrease in soil pH value in the objects polluted with these elements [6]. Solubility of iron compounds increases proportionally to the soil acidification degree. The iron content in plants changes considerably during the growing period. In legumes the content on the level of between 75 and 400 mg/kg d.m. is registered [10]. In conditions of nickel contaminated soil, the lime dose calculated acc. to 1 Hh caused further development of the Fe level in broad bean shoots, whereas the dose computed acc. to 2 Hh caused that the Fe level declined to the level noted in the control plants. In the case of zinc contaminated soil, a lower CaO dose decreased the iron content in broad bean shoots by ca 1/3, while the higher one led to a further decline to the level similar to the one registered in the control plants. No apparent differences in the iron content between unpolluted plants and those growing in Pb, Cu or Cd contaminated soil were registered either in broad bean shoots or roots (Fig. 3B). In these objects liming caused only slight fluctuations of the iron content in these plant parts. On the other hand, soil contamination with zinc affected a considerable, 6-fold decrease in the iron content in broad bean roots. Liming with a dose established on the basis of double hydrolytic acidity raised the content of this element to the level similar to the control plants. Similarly, in conditions of soil contamination with nickel, already the lime dose calculated according to single hydrolytic acidity raised the iron level in broad bean roots to the same as noted in the control plants, whereas a higher CaO dose caused a further increase in the Fe content in broad bean roots. In studies on the cooperation of NPK treatment with the liming effect on bioavailability of some metals to meadow plants, the most intensive iron bioavailability was registered in plants in objects where liming was used [11]. In some other research non-directional changes in the iron content in plants were observed irrespective of liming application [12, 13]. There is a considerable interrelation between the metabolic effect of iron and other trace metals. Excessive amounts of metals, particularly Mn, Ni and Co reduce the uptake and translocation of iron, which as a result inhibits chlorophyll formation leading to chlorosis. These interactions occur both in the soil environment and in plant tissues [14]. Such symptoms and a declined chlorophyll content were noticed in plants growing in zinc contaminated soil [15]. Proper calcium supply improved plant resistance to either deficiency or excess of iron [10]. Plants growing in zinc contaminated soil after liming application revealed a much better condition that these which were growing in polluted but non-limed soil (the Author's own unpublished data). A complex character of the antagonism in the Fe–Ca relation, which has been emphasized in literature, depends on different soil conditions and concerning the soil metabolism is sometimes combined with other elements [10].

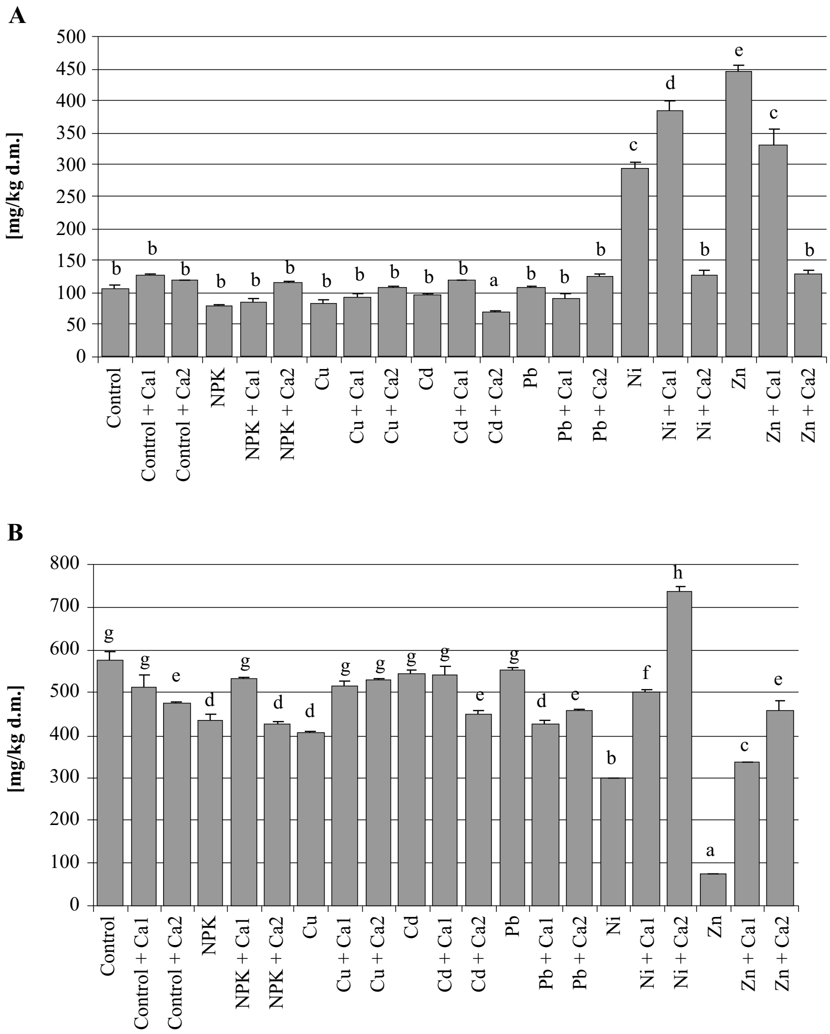

Fig. 3. Iron content in shoots (A) and roots (B) of broad bean (*Vicia faba* L., ssp. *maior*) cultivated in unpolluted soil (Control, Control +NPK) and in soil contaminated with single heavy metals and limed with a dose acc. to 1 Hh (…+Ca1) and acc. to 2 Hh (…+Ca2). Values marked with different letters are statistically different at $p = 0.05$.

Conclusions

1. Soil contamination with heavy metals, such as Cu, Cd, Pb, Ni and Zn causes significant changes of Ca, Mg and Fe contents in broad bean plants.

2. Application of liming contributes to balancing the contents of the analyzed elements in a plant. After liming the content of Ca, Fe and Mg in plants growing in soil

contaminated with individual heavy metals in most cases was on the level similar to the one registered in plants growing in unpolluted soil.

References

[1] Gorlach E. and Curyło T.: Acta Agr. Silv. Ser. Agr. 1990, **29**, 83–92.
[2] Siuta J.: Gleba, diagnozowanie stanu i zagrożenia. Wyd. IOŚ, Warszawa 1995.
[3] Kabata-Pendias A. and Piotrowska M.: Ocena stopnia zanieczyszczenia gleb i roślin metalami ciężkimi i siarką. Wyd. IUNG Puławy, 1993, ser. P, 53 pp.
[4] Gospodarek J.: Ecol. Chem. Eng. 2006, **13**(11), 1231–1240.
[5] Gospodarek J.: Consequent effect of soil contamination with heavy metals on broad bean seed quality (*Vicia faba* L., ssp. *maior*), (Ecol. Chem. Eng. 2008, **15**(1–2), 55–64.
[6] Gospodarek J. and Nadgórska-Socha A.: Ecol. Chem. Eng., 2006, **13**(6), 505–512.
[7] Ostrowska A, Gawliński S. and Szczubiałka Z.: Metody analizy i oceny właściwości gleb i roślin. Katalog. Instytut Ochrony Środowiska. Warszawa 1991.
[8] Azucue J. and Murdoch A.: Int. J. Environ. Chem., 1994, **57**, 151–162.
[9] Jaworska M., Gospodarek J.: Ecol. Chem. Eng. 2005, **12**(8), 803–809.
[10] Kabata-Pendidas A. and Pendias H.: Biogeochemia pierwiastków śladowych, Wyd. Nauk. PWN Warszawa 1993.
[11] Maciejewska M. and Kotowska J.: Fol. Univ. Agric. Stetin. 1998, **190**, Agr. (72), 205–209.
[12] Scheffer K., Koch E. and Vardaskis F.: Landwirtsch. Forsch. 1978, **21**(3–4), 156–161.
[13] Kotowska J.: Wpływ wapnowania i nawożenia mineralnego na plon oraz zawartość Cu, Zn, Fe, Ca, K, N, P w roślinach uprawianych w zmianowaniu. 1992, Rozprawy 146, AR Szczecin, 85 pp.
[14] Bergmann W.: Ernahrungesstorungen bei Kulturpflanzen, 1988, 2nd ed. VEB G. Fisher Verlag, Jena, 762 pp.
[15] Nadgórska-Socha A., Gospodarek J., Jaworska M. and Ciepał R.: Ecol. Chem. Eng., 2005, **12**(4), 421–426.

WPŁYW WAPNOWANIA GLEBY SKAŻONEJ METALAMI CIĘŻKIMI NA ZAWARTOŚĆ MAGNEZU, WAPNIA I ŻELAZA W ROŚLINACH BOBU (*Vicia faba* L. ssp. *maior*)

Katedra Ochrony Środowiska Rolniczego, Uniwersytet Rolniczy w Krakowie
Katedra Ekologii, Uniwersytet Śląski w Katowicach

Abstrakt: Celem podjętych badań było określenie wpływu zróżnicowanych dawek wapna na zawartość mangezu, wapnia i żelaza w roślinach bobu rosnących w warunkach gleby zanieczyszczonej pojedynczymi metalami ciężkimi na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG. Analizie poddano bób (*Vicia faba* L., ssp. maior) odm. Windsor Biały uprawiany w dwóch seriach: na glebie wapnowanej i niewapnowanej. 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 $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona ołowiem w dawce: 530 mg $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona miedzią w dawce: 85 mg $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona cynkiem w dawce: 1000 mg $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona niklem w dawce: 110 mg $\cdot$ kg^{-1} s.m. Wapnowanie przeprowadzono na podstawie analizy kwasowości hydrolitycznej gleby z poszczególnych obiektów. Zastosowano dwie dawki: według 1 i 2 Hh. Dawki wapna wynosiły odpowiednio: Kontrola – 356 i 712 mg CaO $\cdot$ kg^{-1} s.m.; Kontrola + NPK – 420 i 840 mg CaO $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona kadmem – 504 i 1008 mg CaO $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona ołowiem – 420 i 840 mg CaO $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona miedzią – 398 i 796 mg CaO $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona cynkiem 860 i 1720 mg CaO $\cdot$ kg^{-1} s.m.; gleba zanieczyszczona niklem 524 i 1048 mg CaO $\cdot$ kg^{-1} s.m.

Skażenie gleby metalami ciężkimi, takimi jak Cu, Cd, Pb, Ni i Zn powoduje statystycznie istotne zmiany w zawartości Ca, Mg i Fe w roślinach bobu. Zastosowanie zabiegu wapnowania przyczynia się do zrównoważenia zawartości badanych pierwiastków w roślinie – po ich przeprowadzeniu zawartość Ca, Fe i Mg w roślinach rosnących w glebie skażonej poszczególnymi metalami ciężkimi w większości przypadków kształtuje się na podobnym poziomie, jak w roślinach rosnących w glebie niezanieczyszczonej.

Słowa kluczowe: metale ciężkie, wapnowanie, akumulacja, Mg, Ca, Fe

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Magdalena JAKUBOWSKA[1]

OWLET MOTHS (*Noctuidae*) AS BIOINDICATORS OF ECOLOGICAL PROCESSES OCCURRING IN AGROCOENOSES FARMLAND

SÓWKOWATE (*Noctuidae*) JAKO BIOINDYKATORY PROCESÓW EKOLOGICZNYCH ZACHODZĄCYCH W AGROCENOZACH PÓL UPRAWNYCH

Abstract: The aim of the study was to compare the *Noctuidae* moth in the environment of farmland in four places, using ecological indices. The study was conducted from 2003 to 2005 on the fields sown with the winter wheat, sugar beets, the maize and the winter rape. The subject of the study was the imago of butterflies caught by light traps. Light traps (Polish brand) located at the Institute of Plant Protection (IPP), Agricultural Experimental Farm – Winna Gora, Sugar Beet Breeding Research Station in Wieclawice, Experimental Station in Słupia Wielka and IPP – Poznan-research garden. The collected *Noctuidae* imagines were identified and characterized qualitatively and quantitatively. Moreover, the composition of caught of butterflies and its richness was assessed using "abundance relative method", which estimated the number of dominance of species. The *Noctuidae* imagines were analyzed using Shannon-Weaver's diversity index (H'), Simpson's dominance index (SJ) and Sorenson's indices of similarity (J %). The results showed higher similarity between species composition in agrocenoses of Poznan and Wieclawice. The smallest diversity of species, reflected in the lowest value of diversity index and the high value of dominance index, was observed in a biotic community of the Winna Gora which reveals the species compensation of few *Noctuidae* kinds. In this case there were: *Agrotis segetum* (Schiff. et Den.), *A. exclamationis* (L.), *Xestia c-nigrum* (L.), whether *Discestra trifolii* (L.).

Keywords: cutworms, bioindicators, ecological index, light-trap

As many as 150.000 species belong to the *Lepidoptera* order, of which about 25.000 are Owlet moths (*Noctuidae*). The Owlet moths inhabit mainly areas in the moderate climate. Approximately 60 % of their species live on the northern hemisphere, approximately 25 % on the southern hemisphere, while the remaining 15 % in the tropics. In Poland, the Owlet moths family belongs to one of the larger taxonomical groups of insects and is represented by approximately 500 species of butterflies [1, 2].

[1] Department of Method Forecasting and Pest Registration, Institute of Plant Protection, ul. Władysława Węgorka 20, 60-318 Poznań, Poland, email: mjakubowska@echostar.pl

Majority of Owlet moth species known in Poland is characterized by a vast distribution. Due to their environmental valency they accommodate easily to any changes occurring in the environment as a result of human activity. Most Owlet moths are eurybionts that inhabit various forest and meadow biotopes as well as open areas. In agrocenoses they can constitute about 50 % of all species of butterflies; majority of them are economically important pests of cultivations. Within the Owlet moths approx. 70 % of species, of which nearly all *Noctuinae Noctuidae*, *Hadeninae Noctuidae* and many other Owlet moths, are polyphagous with a broad trophic spectrum that covers from a few dozens to several host plants [3, 4].

Large population fluctuations are an important feature of numerous species of *Noctuidae*, and these are particularly visible in agrocenoses. The quantity and quality changes repeated every year in specified seasons result from differences in their biotic cycles [1, 3]. Species with the same biotic cycles constitute phenological groups, the largest of which is composed of summer species that are active in the imago stage in July and August, while the smallest of which includes autumn and winter species flying in the autumn, and even in warmer periods of winter, and in spring following the diapause.

The paper presents barely a small part of vast data obtained in the course of works performed on the areas monitored.

Material and methods

The *Noctuidae* were caught from May to the first half of October each year from 2003 to 2005 using light traps filled with insecticide. Inspections and picking of insects caught from the traps were carried out systematically 2–3 times a week. The areas for research were determined on the following premises: in Poznan in the phonological garden of the Institute of Plant Protection (Wielkopolska province), in Winna Gora in the Agricultural Experimental Station of the IPP (Wielkopolska province, about 70 km far away Poznan) and in Wieclawice (Kujawy-Pomerania province, about 50 km from Torun) area of the former Beet Breeding Station. One trap was installed on each of the tested premises. The insects caught were assigned to species, secured and summarized [5–8].

Based on the number of species and total population of the individuals caught, the following indexes were specified that characterize the *Noctuidae* community:

– structure of the species domination in the *Noctuidae* groups expressed by the so-called relative abundance calculated according to the following formula: $D = s/S^* 100$ [%]; where s – number of individuals belonging to the given species caught within 2 years in one trap on the tested area, S – total number of individuals of all species of the cenotic unit tested;

– Shannon-Weaver index that determines the species diversity. The index value is calculated basing on the following formula: $H' = Spilog2pi$; where S – number of species in the group (total), pi – (n1/N) of i-th species in a group composed of S species, and n1 – population of the i-th species in a team with N total number of individuals;

– species richness index acc. to the Simpson's formula; $d = (S-1)/logN$; where: S – number of species in the community, N – total number of individuals caught.

Results and discussion

Overview of faunistic and ecological characteristics of Noctuidae

Throughout the four years of the research over 14 thousand of individuals were caught to 3 light traps (8411 individuals in 2003; 3722 in 2004 and 2188 in 2005), and they represented species from genera belonging to subfamilies.

Most species belonged to two subfamilies: *Hadeninae* (29 species) and *Noctuinae* (20 species), and they constituted about 92 % of the total number of butterflies caught. An occurrence of a large number of populous species of butterflies typical for this kind of a landscape was detected in the material. Found were also species that are generally rare in Poland such as *Gortyna flavago* Schiff. and species that had not been recorded for the areas of Poznan and Wieclawice – *Agrochola purpurea* L. Generally, 2003 was a year with the largest number of butterflies caught in comparison with 2004 and 2005. Most individuals were collected in Wieclawice (6128 butterflies caught in 2003, 2093 individuals in 2004, and 1327 individuals in 2005); in Poznan (2003 – 1732 individuals, 2004 – 1361 individuals, 2005 – 510 individuals) and the smallest number of butterflies were caught in Winna Gora (2003 – 651 individuals, 2004 – 268 individuals, 2005 – 301 individuals). In the phenological groups for one-generation species a majority consisted of the spring and summer species that are active in the imago stage in June (*eg* species from such genera as *Orthosia* spp., *Cuculia umbratica* L., *Apameamonoglypha* Hufn., *Melanchra pisi* L.) and summer species (*Cosmia trapezina* L., *Mythimna conigera* Schiff., *Noctua pronuba* L.), where the flight of their butterflies took place in July and August. From multi-generation species 11 species need to be mentioned the flight of which lasted uninterruptedly from May to September, and even to the first decade of October. Majority of these species are well-known pests of crop plants of large economic significance (e.g. *Agrotis segetum* Schiff., *A. exclamationis* L., *Xestia c-nigrum* L., *Mythimna pallens* L., *Mamestra brassicae* L., *Lacanobia oleracea* L.). Observed were also two migrating species: *Agrotis ipsilon* Hufn. and *Autographa gamma* L., which occur in Poland, but probably do not winter here.

In the tested *Noctuidae* community, species characteristic for open areas related trophically to synantropic and meadow plants were the most populous group. The second group was composed of species characteristic for forest and scrub plant community connected in terms of feeding with trees and bushes, while the third of the most populous groups included species characteristic for environments linked directly with agrocenoses of fields of: beets, rape, cereal plants.

The determination of the structure of domination was applied for faunistic identification of the Noctuidae community. Over 80 % of all butterflies from the Noctuidae community caught in the phenological garden in Poznan in the period from 2003 to 2005 were recognized as dominants. In Poznan *Xestia c-nigrum* was eudominant, and within the three years of the research it reached the domination level of over 24 %. Another class of dominants at the level of 16.5 % of all moths collected was established by one species *Agrotis segetum*. The class of subdominants was represented by 11 species, the class of recedents by two species at the domination level of approximately 2 %. The class of accompanying species was composed of 36 species. In Winna Gora, two species dominated distinctly: *Agrotis segetum* – 26.4 %, *A. exclamationis* – 15.3 %, with *Xestia c-nigrum* about 12 %, and *Discestra trifolii* L. 10.8 %. They were all accompanied by: *Hoplodrina ambigua* Schiff., *Mythimna pallens* and *Xestia triangulum* Hufn. The class of recedents and subrecedents was composed of approximately 30 species that constituted about 14 % of all the imagines collected. In Wieclawice, alike in Poznan, the domination of a few species of the Noctuidae community was at a similar level, and so the class of eudominants included *Xestia c-nigrum* – 21.7 %, and of dominants: *Mythimna pallens* – 13.4 %. The class of subdominants was composed of 10 species, all of which represented open areas. The classes of recedents and subrecedents were represented in great numbers.

For determining the characteristics of the Noctuidae community, the Shannon-Weaver species diversity index was used. Based on the values obtained it can be shown that the higher the index the larger the species diversity of the tested community (Table 1, 2).

Table 1

Resemblance of *Noctuidae* species of two biotic communities of the Wielkopolska and Kujawy-Pomorania province in 2003–2005.

Location	No of species occurring simultaneously	Semblance of species [J %]
Poznan Winna Gora	45	50.56
Poznan Wieclawice	61	61.62
Winna Gora Wieclawice	46	51.11

Table 2

The richness, diversity and evently of *Noctuidae* species of two biotic communities of the Wielkopolska and Kujawy-Pomorania province in 2003–2005.

Place	Number of specimens	Number of species	Diversity [H']	Richness [dS]	Eaveness [J']
Poznan	3553	79	4.419	21.968	0.701
Winna Gora	1220	55	3.893	17.496	0.673
Wieclawice	9528	81	4.477	20.106	0.706

The enclosed table shows that the highest values were recorded in the areas located in Wieclawice and Poznan. The conclusion from the above is that the species diversity of the given community depends on the natural environment and, according to literature available, probably also on the habitat's humidity.

A slightly different approach that complements the conclusions from the diversity index is presented on the basis of the Simpson's richness index. The index is based on the probability theory in which the species abundance is measured by the sum of probabilities of occurrence of all species in the tested community. When the occurrence of species is considered in view of this approach, the best richness is clearly visible in Poznan and Wieclawice, while it is the worst in the area tested in Winna Gora (idle field).

Conclusions

Monitoring of the Noctuidae community can be an element of a broader environment monitoring system the objective of which is to determine the direction of changes occurring in the biocenoses tested. Despite its significant ecological valency, the Noctuidae community reacts to all anthropogenic and biotic disturbances. From the data available it is known that for the last 50 years significant changes have occurred in the Noctuidae community in the area of Warsaw City Center. Particularly in the last 20 years a considerable impoverishment of the species composition was noted. For these communities durable and constant in time was a group of 25–30 species composed of all very populous and populous species and several less populous species. A group of species having small populations that occur only occasionally in the Warsaw City Center is the element that changes in time. The group's content has decreased by at least 70 %.

Vanishing of rare species seems to be caused primarily by unfavorable environmental changes occurring as a result of intense development of that area (desertification of densely developed areas, decreased surface of urban green areas, changes in plant species composition, great pollution and other similar factors).

Monitoring of the Noctuidae community can disclose changes in the biodiversity that is expressed among others by the number species of organisms co-occurring in the given area. If detected at the right time, changes of various kinds in biocenoses can have a significant influence on undertaking adequate economic steps aiming at maintaining the ecosystem of either open areas or agrocenoses at a balanced level [4, 5, 7, 8].

The three-year-long research and observations of the Noctuidae need to be treated as a pilot study, and the results obtained as the starting point for further observations on the influence of the structure of species in the Noctuidae communities on the increasing environment degradation process.

Acknowledgements

This study was supported by the Ministry of Education and Science (Grant N N310 4315.33).

References

[1] Merzheevskaja O.I.: Larvae of Owlet Moths (Noctuidae). Biology, morphology, and classification, E.J.Brill, New York 1989.

[2] Adamczewski S.: *Zagadnienie regionalizacji faunistycznej Polski na tle fauny owadów*. Polskie Pismo Entomol. (Wrocław), 1955, 2(24 Supl.), 123-155.

[3] Nowacki J.: *Sówkowate (Lepidoptera, Noctuidae) Kotliny Kłodzkiej w dolinie środkowego biegu Warty.* Fragm. Faunis., 1989, **19**(32), 415–444.

[4] Nowacki J.: *Sówki (Lepidoptera, Noctuidae) środowisk naskalnych w okolicy Mirowa na Jurze Krakowsko-Częstochowskiej.* Wiad. Entomol., 2006, **25**(2), 105–124.

[5] Nowacki J.: *Sówkowate (Lepidoptera, Noctuidae) ekosystemów wydmowych na terenie Słowińskiego Parku Narodowego*. Wiad. Entomol., 1993, **12**(4), 273–291.

[6] Nowacki J.: *Fauna wybranych grup owadów (Insekta) Puszczy Bukowej koło Szczecina. 5. Sówkowate (Lepidoptera, Noctuidae).* Wiad. Entomol., 1993, **12**(2), 115–136.

[7] Nowacki J.: *Sówkowate (Lepidoptera, Noctuidae) Karkonoszy Polskich.* Wiad. Entomol., 1998, **16**(3–4), 177–188.
[8] Borowiak M. and Chrzanowski A.: *Inwentaryzacja i analiza faunistyczno-ekologiczna zgrupowania sówkowatych (Lepidoptera, Noctuidae) Polskiej części Karkonoszy*. Nauka Przyr. Technol. 2007, **1**(1)#6, 1–19.

SÓWKOWATE (*Noctuidae*) JAKO BIOINDYKATORY PROCESÓW EKOLOGICZNYCH ZACHODZĄCYCH W AGROCENOZACH PÓL UPRAWNYCH

Zakład Metod Prognozowania i Rejestracji Agrofagów
Instytut Odnowy Roślin – Państwowy Instytut Badawczy

Abstrakt: Celem pracy było porównanie struktury sówkowatych w środowisku pól uprawnych, czterech badanych miejscowości za pomocą wskaźników ekologicznych. Badania przeprowadzono w latach 2003–2005 na polach doświadczalnych obsianych pszenicą ozimą, burakami cukrowymi, kukurydzą oraz rzepakiem ozimym. Przedmiotem badań były odłowione imagines motyli sówkowatych za pomocą pułapek świetlnych zwanych potocznie samołówkami. Określono skład jakościowy i ilościowy odłowionych motyli, obliczając strukturę dominacji gatunków wyrażoną za pomocą tzw. abundancji relatywnej. Do analizy porównawczej materiału zastosowano indeks różnorodności Shannonna-Weavera (H'), dominacji Simsona (SI) oraz wskaźniki podobieństwa Sorensona (J %). Wyniki badań wykazały większe podobieństwo składu gatunkowego agrocenoz Poznania i Więcławic. Najmniejszą różnorodnością gatunków, potwierdzoną małą wartością indeksu różnorodności oraz dużą wartością indeksu dominacji, charakteryzowała się cenoza Winnej Góry, co wskazuje w strukturze dominacji gatunkowej na kompensację tylko kilku gatunków sówkowatych, w tym przypadku np. *Agrotis segetum* (Schiff et Den.), *A. exclamationis* (L.), *Xestia c-nigrum* (L.), *Discestra trifolii* (L.).

Słowa kluczowe: rolnice, bioindykatory, wskaźniki ekologiczne, pułapka świetlna

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Marta KANDZIORA[1], Ryszard CIEPAŁ[1]
and Aleksandra NADGÓRSKA-SOCHA[1]

HEAVY METALS AND SULPHUR ACCUMULATION IN THE *Picea abies* L. Karst. NEEDLES AND SOIL OF THE FOREST PROMOTIONAL COMPLEX "LASY BESKIDU SLASKIEGO"

AKUMULACJA METALI CIĘŻKICH I SIARKI W SZPILKACH *Picea abies* L. Karst I GLEBIE NA TERENIE LEŚNEGO KOMPLEKSU PROMOCYJNEGO „LASY BESKIDU ŚLĄSKIEGO"

Abstract: The investigation of zinc, cadmium, lead, copper and sulphur contents was carried out in the soil and in the needles of *Picea abies* L. Karst. in the Forest Promotional Complex "Lasy Beskidu Slaskiego". Samples of soil (from the level 0–10 cm) and annual, biennial and triennial needles of spruce were collected in autumn 2005 and 2006 from top parts of mountains: Rownica, Barania Gora and Czantoria. The highest heavy metals concentration were determined in triennial needles of *Picea abies* collected from Czantoria and Barania Gora. The lowest content of sulphur and investigated metals was found in soil samples and heavy metals in plant material from the Równica. Cadmium content (1.2 μg/g) was higher than normal level (0.2–0.8 μg/g) only triennial needles collected from Czantoria. The lead concentration (118–165 μg/g) in the soil of all investigated areas was exceed, lead level considered as allowable. Relatively low microelement concentrations in the soil and the needles of *Picea abies* L. Karst. indicated poor plant nutrition.

Keywords: heavy metals, sulphur, *Picea abies* L. Karst., Beskid Slaski

Introduction

Spruce (*Picea abies* L. Karst.) is one of the most endangered species of trees in Europe, with the highest percentage of damaged trees (30 %). There is a close relation between the amount of trees damaged and the level of pollution in a given area. The chemical composition of the assimilation apparatus of spruce does not only indicate the contents of nutrients but also shows the degree of the environmental pollution [1].

[1] Departament of Ecology, Silesian University, ul. Bankowa 9, 40-007 Katowice, Poland, email: marta.kandziora@yahoo.pl

Thus, the most endangered spruce are those from Poland, the Czech Republic and Germany, which is a result of high deposition of pollution in those countries [2].

Strong anthropopressure is characteristic of Forest Promotional Complex "Lasy Beskidu Slaskiego". It results from the vicinity of large industrial areas of the Upper Silesia, Ostrava and Karvina District in the Czech Republic [2], as well as from the pollution emitted by transport and agriculture. On the basis of the analysis of assimilation apparatus, spruce trees from Beskid Slaski have been categorized into the 1st and 2nd classes of damage [2]. It is assumed that the high level of pollution until mid-90s had a huge impact on the condition and vitality of the trees. The first symptoms of the spruce forests dying out were observed in the 50s in Beskid Mały, in the 70s the problem was discovered in Beskid Slaski, and later also in Beskid Zywiecki [4]. In 2006 the amount of dead wood and coarse woody debris was estimated at 118,000 m^3 in the forests of Beskid Śląski, which was ten times more than in the previous years. In addition, every year the number of sanitary cuts increases which is caused by the unfavourable weather conditions and the increasing threat of the insect pests [5].

Dying out of the spruce forests cannot of course be attributed just to one disadvantageous factor. The bad condition of the stands results from a variety of causes, accumulated over a period of time, which overlap and strengthen the final effect – dying out of a forest. Among diverse factors which have a negative influence on the stand, the industrial pollution, mostly heavy metals and sulphur, is the most significant factor in this process.

The research aimed at estimating heavy metals (Cd, Pb, Zn and Cu) as well as sulfur accumulation in the upper levels of the forest soil and in the accumulation apparatus of spruce in the stands of Forest Promotiona Complex "Lasy Beskidu Slaskiego" in relation to dying of the spruce forest.

Material and methods

The investigation was carried out in the soil and needles of *Picea abies* L. Karst. in the Forest Promotional Complex "Lasy Beskidu Slaskiego". Samples of soil (from the level 0–10 cm) and annual, biennial and triennial needles of *Picea abies* were collected in autumn 2005 and 2006 from top parts of mountains: Rownica, Barania Gora and Czantoria). In order to determine the heavy metal concentration, spruce needles were divided into three groups (one-, two- and three-year-old), dried at 105 °C to a constant weight, ground to a powder, then mineralized and dissolved in 10 % HNO_3. After filtration Cd, Pb, Zn and Cu content were determined using flame Atomic Absorption Spectrometry (AAS) [6]. Soil was air dried and extracted with 10 % HNO_3 and measurements were made using the conventional Atomic Absorption Spectrometry (AAS) [6]. The sulphur (total sulphur) content in plants and soil was determined with colorimetric method [6, 7]. The quality of analytical procedures was controlled by using the reference material (Certified Reference Material CTA–OTL–1 Oriental Tabacco Leavs). The data were processed using software Statistica.

Results and discussion

Results are shown in Table 1, 2.

Table 1

Content of cadmium, lead, zinc, cooper [µg/g] and sulphur [%] in the needles *Picea abies* L. Karst. Values with the same letter are statistically the same for p<0,05

Location	Sample	Cd [µg/g]	Pb [µg/g]	Zn [µg/g]	Cu [µg/g]	S [%]
Barania Gora	annual needles	0.27b	0.53a	25.2a	1.75a	0.052a
	biennial needles	0.325a	0.38a	25.045a	1.685a	0.045a
	triennial needles	0.354a	1.2a	24.115a	1.29a	0.026a
Czantoria	annual needles	0.755a	0.535a	22.415a	1.285a	0.087b
	biennial needles	0.49b	1.155a	25.46b	2.298a	0.043a
	triennial needles	1.175b	1.625b	54.225b	4.237a	0.065b
Rownica	annual needles	0.54a	0.32a	15.67b	1.32a	0.063a
	biennial needles	0.43c	1.01a	11.23a	1.14b	0.042a
	triennial needles	0.76c	0.37c	9.435c	1.7b	0.060b

Table 2

Content of cadmium, lead, zinc, cooper and sulphur [µg/g] and in the upper layer of the soil Values with the same letter are statistically the same for p < 0,05

Location	Cd [µg/g]	Pb [µg/g]	Zn [µg/g]	Cu [µg/g]	S [µg/g]
Barania Gora	1.62a	165.6a	37.4a	9.4a	147.3a
Czantoria	1.24b	155.6b	26.9b	6.9b	100.1b
Rownica	0.96c	118.6b	11.5c	6.6b	81.0b

The concentration of cadmium, which is considered highly toxic, fluctuates in the examined material from 0.27 µg/g dry mass in the needles of one-year-old spruce from Barania Gora to 1.175 µg/g in the needles of three-year-old from Czantoria. Cadmium concentration in the soil remains within the normal range, from 0.96 µg/g on Rownica to 1.62 µg/g on Barania Gora. In the plant material from Czantoria and in the soil from Barania Gora the content of cadmium was exceeded, 0.2–0.8 µg/g Cd in plants and to 1 µg/g Cd in the soil [8].

Lead is also considered a very toxic element and its content in the plants shows the influence of anthropogenic factors. The concentration of lead in the analyzed material falls between 0.32 µg/g in one-year-old needles from Rownica and 1.625 µg/g in three-year-old needles from Czantoria, and in the soil between 118.6 µg/g on Rownica to 165.6 µg/g on Barania Gora. Hence, it can be concluded that in all investigated areas, the normal range of lead (100 µg/g Pb) [8] has been exceeded.

Zinc is an element which is indispensable to a proper functioning of living organisms. Its content may fluctuate in a wide range from 10 to100 µg/g [8]. The concentration of zinc in the examined samples of plant material was in the range from 9.435 μg/g (in the three-year-old spruce needles from Rownica) to 54.225 μg/g (in the three-year--old spruce needles from Czantoria) and in the soil from 11.5 μg/g on Rownica to 37.4 μg/g on Barania Gora, which does not exceed the norms and even shows zinc deficiency.

The concentrations of copper in the analysed samples are within the normal ranges from 1.14 μg/g in the two-year-old spruce needles from Czantoria and do not exceed the norms (2–20 μg/g Cu) [8]. Similarly, the content of copper in the soil from 6.6 μg/g on Rownica to 9.4 μg/g on Barania Gora are within the normal range of concentration (1–140 μg/g Cu) [8].

Sulphur, similarly to zinc and copper, is absolutely crucial for the development of living forms. Its higher concentration in the plants is often a result of air pollution caused by sulphur dioxide. The content of sulphur fluctuated between 0.026 % in the three-year-old needles form Barania Gora to 0.087 % in one-year-olds from Czantoria. Sulphur content in the soil falls between 81.0 % (Rownica) and 147.3 % (Barania Gora).

The research showed, that heavy metals concentration in spruce needles and soil was similar to those of Zwolinski study (Cd 0.09–0.46 μg/g, Pb 1.9–2.5Xμg/g, Zn 11.0–28.2 μg/g, Cu 2.0–2.8 μg/g, S 0.096–0.12 % in spruce needles and Zn 20–56 μg/g, Cu 7–13Xμg/g, Cd 0,6–1 μg/g in soil) [3]. Only lead concentrations (118.6–165.6Xμg/g) in soil were considerably higher than Zwolinski results. This study differed from data for 'Male Pieniny [1], only concentration of zinc (12.5–43.5 μg/g) was similar but concentrations of cadmium (0.01–0.1 μg/g) and sulphur (mean 0.023 %) were lower and the content of lead (1.0–2.0 μg/g) was higher than in this study. Similar situation was reported in the research conducted in Podhale Region. The concentrations of zinc (31.8–34.4 μg/g) and lead (1.6–1.7 μg/g) were lower and the content of cadmium (0.24–0.34 μg/g) was higher [1, 9]. The content of sulphur in Forest Promotional Complex "Lasy Beskidu Slaskiego" was lower than previously reported by Barszcz and Malek (annual needles 0.103–0.138 %, biennial needles 0.094–0.121 %) [10]. The concentrations of zinc in annual, biennial and triennial needles were similar to German research results (annual – 30.7 μg/g, biennial 27.6 μg/g, triennial 24.8 μg/g) [11]. The contents of cadmium and lead were higher than in Norway (0.011–0.173 μg/g Cd, 0.03–0.89 μg/g Pb), but lower than in Slovakia (1.19 μg/g Cd, 1.73 μg/g Pb) [11, 12].

The spruce forests in Beskid Slaski do not show an excessive accumulation of heavy metals in the needles. The content of metals both in the soil and in the needles is within the normal range. Only lead displays excessive concentration in the soil, which indicates that the soil is polluted with this metal. It is also noticeable that those forests are deficient in the basic nutrients, which has been noticed also in his studies by Zwolinski [2].

References

[1] Panek E. and Szczepańska M.: *Metale śladowe i siarka w wybranych gatunkach roślin w Małych Pieninach*. Gospod. Surowc. Mineral., 2005, **21**(1) 89–109.

[2] Zwoliński J.: *Ocena zagrożenia lasów świerkowych w Beskidzie Śląskim przez zanieczyszczenia powietrza atmosferycznego*. Pr. Inst. Bad. Leśn. A, 2003, 1(951) 53–68.

[3] Lech P. and Żółciak A.: *Uwarunkowania występowania opieńkowej zgnilizny korzeni w lasach Beskidu Śląskiego*. Leśne Pr. Badaw., 2006, **2**, 33–49.

[4] Fronczak K.: *Dlaczego wymierają świerki*. Przyr. Polska, 2006, **10**, 25–27.
[5] Fronczak K.: *W trosce o beskidzkie świerczyny*. Głos lasu, 2007, **1**(439), 2–5.
[6] Ostrowska A., Gawliński S. and Szczubiałka Z.: *Metody analizy i oceny właściwości gleb i roślin*. IOŚ, Warszawa 1991.
[7] Saalbach E., Essen G. and Judel G.K.: Pflanz. Dung. Bodenkunde, 1961, **93**, 17–26.
[8] Kabata-Pendias A. and Pendias H.: *Biogeochemia pierwiastków śladowych*. PWN, Warszawa 1993.
[9] Panek E. and Józefko U.: *Trace metals (Cd, Cr, Pb, Zn) and sulphur in spruce Picea abies L. of the roadside in the Podhale region, southern Poland*. Macro and Trace Elements, 21 workshop. 18–19.10.2002 Jena, Gemany, s. 249–255.
[10] Barszcz J. and Małek S.: *Stability of Norway spruce (Picea abies L. Karst] stands in the Beskid Śląski and Beskid Żywiecki Mts. From the aspect of their nutrition status*. J. Forest Sci., 2008, **54**(2), 41–48.
[11] Trimbacher C. and Weiss D.: *Needle surface characteristic and element contents of Norway spruce in relation to the distance of emission sources*. Environ. Pollut., 1999, **105**, 111–119.
[12] Čebrus D. and Steinnes E.: *Conifer needles as biomonitors of atmospheric heavy metal deposition comparison with mosses and precipitation, role of the canopy*. Atmos.. Environ. 2000, **34**, 4265–4271.

AKUMULACJA METALI CIĘŻKICH I SIARKI W SZPILKACH *Picea abies L. Karst.* I GLEBIE NA TERENIE LEŚNEGO KOMPLEKSU PROMOCYJNEGO „LASY BESKIDU ŚLĄSKIEGO”

Katedra Ekologii, Uniwersytet Śląski

Abstrakt: Prowadzono badania dotyczące zawartości metali ciężkich (Zn, Pb, Cu, Cd) w glebie i szpilkach *Picea abies* L. Karst. na terenie Leśnego Kompleksu Promocyjnego „Lasy Beskidu Śląskiego”. Próbki gleby (z górnego poziomu) oraz szpilki jedno-, dwu- i trzyletnie świerka pobierano ze szczytowych partii gór: Równicy, Baraniej Góry i Czantorii jesienią 2005 i 2006 r. Stwierdzono najwyższe stężenia metali ciężkich w igłach trzyletnich zbieranych na Baraniej Górze i Czantorii. Najniższe koncentracje badanych metali i siarki zanotowano w próbkach gleby oraz w materiale roślinnym z Równicy. Zawartość Cd (1.2 μg/g) w trzyletnich igłach świerka pobieranych na Czantorii była wyższa od określanych jako stężenie normalne (0.2–0.8 μg/g). Stwierdzono przekroczenie dopuszczalnego poziomu dla Pb w glebie wszystkich badanych powierzchni (118–165 μg/g). Stosunkowo niskie stężenia mikroelementów zarówno w glebie, jak i igłach świerka wskazywać mogą na zły stan odżywienia roślin.

Słowa kluczowe: metale ciężkie, siarka, świerk pospolity *Picea abies* L. Karst., Beskid Śląski

ECOLOGICAL CHEMISTRY AND ENGINEERING

Vol. 16, No. 7 2009

Grażyna KAUP[1] and Magdalena DZIĘGIELEWSKA[2]

INFLUENCE OF SELECTED SOIL SAPROPHYTIC FUNGI ON THE POPULATION OF NEMATODES *Heterodera schachtii* SCHMIDT

WPŁYW WYBRANYCH GRZYBÓW GLEBOWYCH NA POPULACJĘ NICIENI *Heterodera schachtii* SCHMIDT

Abstract: Pot experiments were carried out in 2005–2006. In the first year of the experiment microorganisms were introduced into soil. In the following year the innoculum was not repeated. The goal of the present work was to determine the influence of saprophytic fungi of *Aspergillus versicolor* and *Trichocladium asperum* on the population of *Heterodera schachtii* in the second year of the research. The following parameters were taken into account in the interpretation of the experiment: the concentration of *H. schachtii* cysts in 100 g of soil, the concentration of eggs and larvae in 1 g of soil and the fertility of females. The research was carried out in accordance with the rules adopted in nematology. At the co-occurrence of *H. schachtii* and saprophytic fungi of *Aspergillus versicolor* and *Trichocladium asperum,* in comparison with the test combination (*H. schachtii* without microorganisms), a smaller number of cysts in 100 g of soil, considerably higher fertility of females (the number of eggs and larvae in a cyst) and significantly higher concentration of eggs and larvae in soil were noted.

Keywords: co-occurrence*, Heterodera schachtii, Aspergillus versicolor, Trichocladium asperum*

In ecological agriculture, works concerning possibilities to make use of soil microorganisms, and especially fungi accompanying parasitic nematodes in rhizosphere and having nematodocidal properties, are very valuable. These organisms can constitute an important element of natural reduction of the *Heteroderidae* population: highly specialised nematodes forming cysts [1–4].

The aim of the present work was to determine the influence of saprophytic fungi of *Aspergillus versicolor* (Vuill.) Tiraboschi and *Trichocladium asperum* Harz on the population nematodes of *Heterodera schachtii* Schmidt in the second year of the research.

[1] The West Pomeranian University of Technology, Faculty of Enviromental Management and Agriculture, Department of Agronomy, ul. J. Słowackiego 17, 71-434 Szczecin, phone: 91 425 03 72, email: gkaup@agro.ar.szczecin.pl

[2] The West Pomeranian University of Technology, Faculty of Enviromental Management and Agriculture, Department of Plant Protection, ul. J. Słowackiego 17, 71-434 Szczecin, phone: 91 425 03 72

Material and methods

The research (2005–2006) was carried out in pots (10 dm^3 each) on red beet. In the first year of research the innoculum of microorganisms was added to the combination of respectively: 10 cysts of *H. schachtii*/100 g of soil and 21-day old one-spore cultures of *A. versicolor* or *T. asperum* on the AGZ soil (3 Petri dishes Ø 10 cm) or a pure culture medium of the same amount. Each combination was done in 3 repetitions. In the second year of the research the final concentration of microorganisms from the previous year constituted the initial value.

The following parameters were taken into account in the interpretation of the experimental data the concentration of *H. schachtii* cysts in 100 g of soil, the concentration of eggs and larvae in 1 g of soil and the fertility of females. Confidence semi-intervals were calculated with the use of Newman-Koul test at the significance level of 0.05.

Results and discussion

In earlier research concerning the reduction of harmful phytopathogenic nematodes, the innoculum of fungi was introduced each year [1–3]. The conducted experiment aimed at testing the behaviour of the population of *H. schachtii* in the second year of the research without renewing the innoculum of saprophytic fungi.

In the first year of the research at the presence of saprophytic fungi, a significantly smaller number of *H. schachtii* cysts was noted, as well as a significantly lower number of eggs and larvae in 1 g of soil in comparison with the test combination (a nematode without a fungus) [4].

In the second year of the observation a lower number of cysts in 100 g of soil was noted in the combinations of the co-occurrence of *H. schachtii* and the investigated saprophytic fungi in comparison with the test combination (only *H. schachtii*). On the other hand, in both combinations of the co-occurrence of the researched microorganisms considerably higher fertility of females and significantly higher concentrations of eggs and larvae in soil were determined in comparison with the test combination (*H. schachtii* without microorganisms) (Fig. 1).

Although in the research on the co-occurrence of *Heteroderidae* and saprophytic fungi hyphae were not observed inside cysts, high sensitivity of *H. schachtii* to the presence in soil of *A. versicolor* or *T. asperum* was shown, which manifested itself by lowered fertility and productivity of females. Similar results were obtained in the experiment with *Penicilium frequentans* and *Stachybotrys chartarum* conducted on *Globodera rostochiensis* [5]. Other laboratory research also confirmed a destructive influence of substance excretion by saprophytic fungi on developing larvae of soil nematodes [7, 8].

Population growth coefficients, in combinations with saprophytic fungi were considerably lower in the first year of research. In the second year of the experiment growth coefficients were twice higher in comparison with the combination with only *H. schachti* (Fig. 2). The number of cysts was still lower when compared with the control test. According to Brzeski [9] each species aims at attaining the level of population saturation and in the course of growth of the initial population it decreases. Hence, it

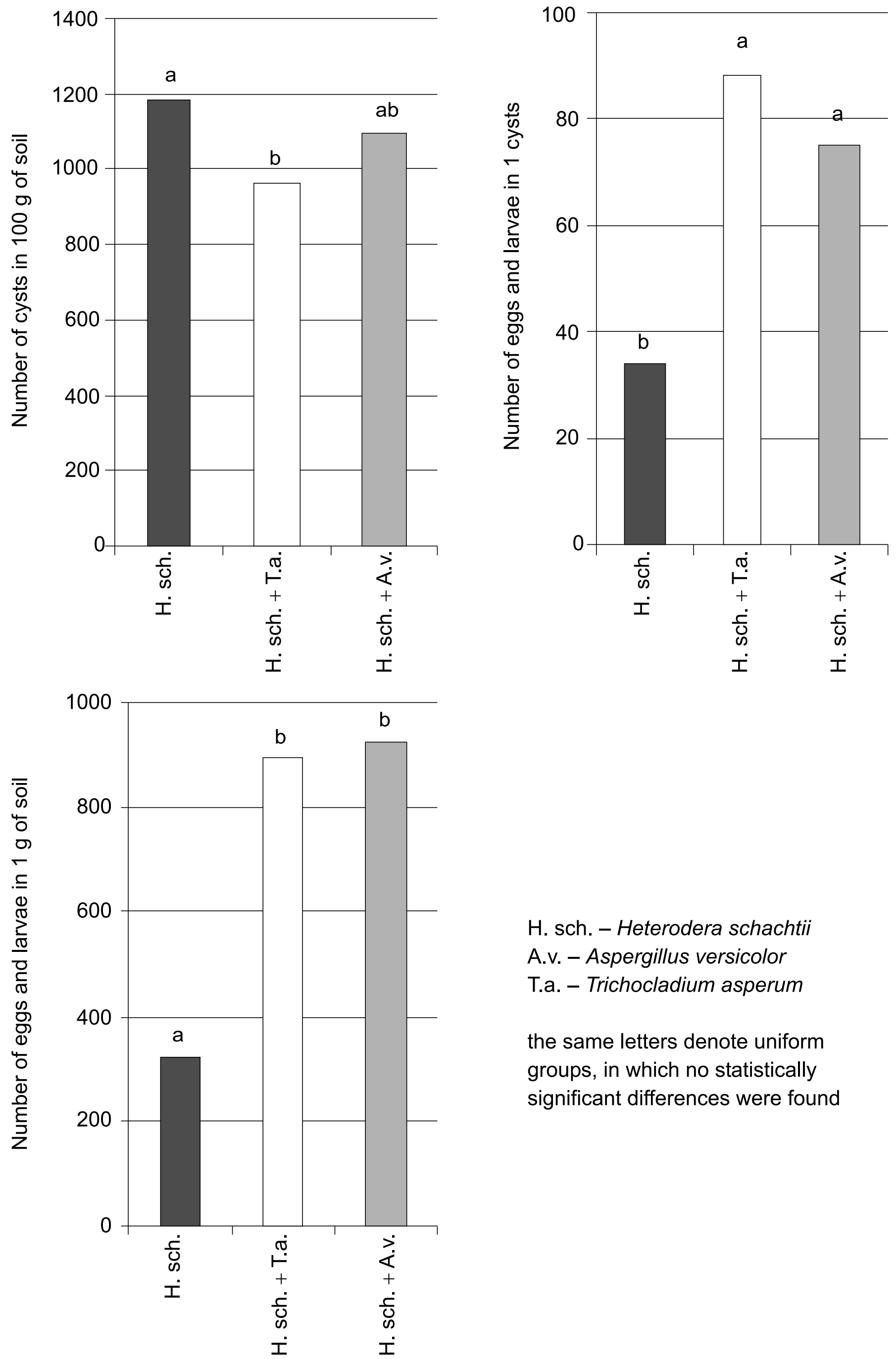

Fig. 1. Effect of of *Aspergillus versicolor* (Vuill.) Tiraboschi and *Trichocladium asperum* Harz on the population nematodes of *Heterodera schachtii* Schmidt

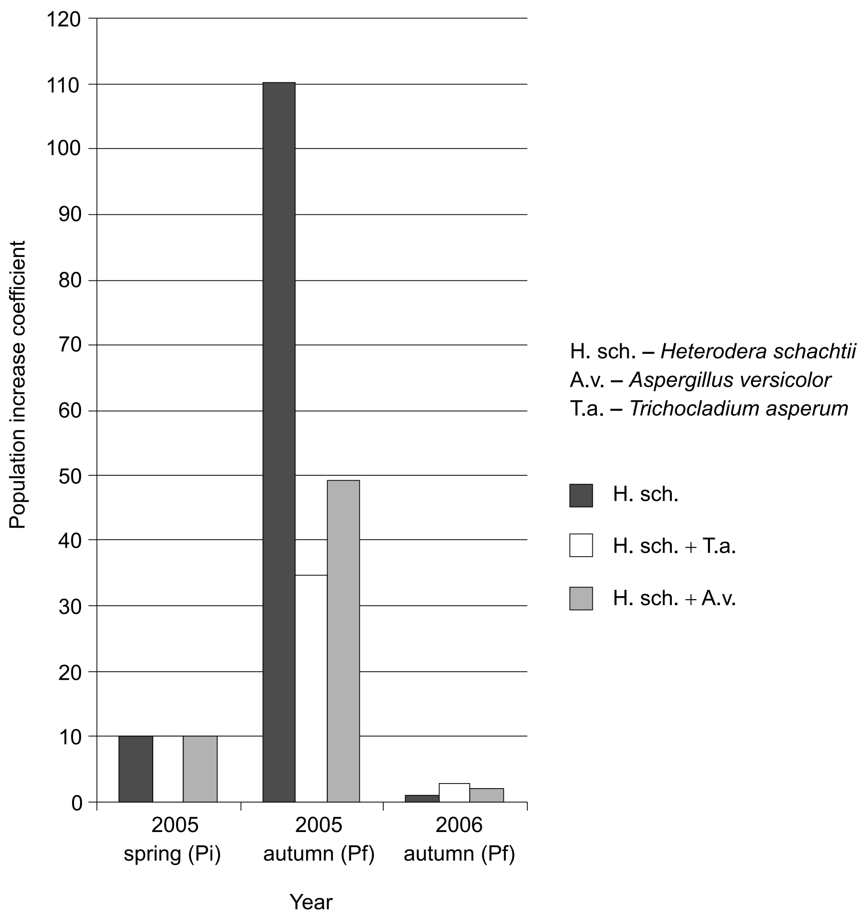

Fig. 2. Effect of *Aspergillus versicolor* (Vuill.) Tiraboschi and *Trichocladium asperum* Harz on the population nematodes of *Heterodera schachtii* Schmidt

could be assumed that the population of *H. schachtii* in the test combination achieved its maximum growth in the first year of the research (Fig. 2). Furthermore, the decreasing number of eggs and larvae in the consecutive year of the observation corroborates the thesis of the population saturation (Fig. 1).

Conclusions

At the co-occurrence of *H. schachtii* and saprophytic fungi of *Aspergillus versicolor* and *Trichocladium asperum,* in comparison with the test combination (*H. schachtii* without microorganisms) was shown:

1. a smaller number of cysts in 100 g of soil,
2. considerably higher fertility of females (the number of eggs and larvae in a cyst),
3. significantly higher concentration of eggs and larvae in soil were found.

References

[1] Kerry B.R. and Crump D.H.: Nematologica 1977, **23**, 193–201.
[2] Wronkowska H. and Janowicz K.: Acta Acad. Agric. Techn. Olst. Ser. Agric., 1988, 343(**47**), 91–99.
[3] Janowicz K., Wronkowska H. and Mazurkiewicz-Zapałowicz K.: Role of soil mikroorganisms i reduction of parastoc nematodes. Biological control of soil-borne and post-harvesst pathogenes. Skierniewice 1995, 121–127.
[4] Kaup. G. and Pogoda K.: Progress i Plant Protection/Postępy w Ochronie Roślin, 2006, **46**(2), 346–348.
[5] Janowicz K., Mazurkiewicz-Zapałowicz K., Kaup G. and Kuźna-Grygiel W.: Russian J. Nematol., 2004, **12**(1),1–7.
[6] Kaup G., Janowicz K., Mazurkiewicz-Zapałowicz K., Paruch K. and Gajewicz P.: Progr. Plant Protect./ Post. Ochr. Rośl., 2004. **44**(2), 799–804.
[7] Chen S.Y., Dickson D. W. and Mitchell D.J.: J. Nematol., 2000, **32**, 190–197.
[8] Janowicz K. and Mazurkiewicz-Zapałowicz K.: Zesz. Nauk. AR, Wrocław 1998, (332), 77–84.
[9] Brzeski M.W.: Ekol. Polska, Ser. A, 1969, **XVII**(13), 227–240.

WPŁYW WYBRANYCH GRZYBÓW GLEBOWYCH NA POPULACJĘ *Heterodera schachtii* SCHMIDT

Katedra Agronomii, Katedra Ochrony Roślin, Wydział Kształtowania Środowiska i Rolnictwa
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

W latach 2005–2006 przeprowadzono badania wazonowe. W pierwszym roku doświadczenia wprowadzono do podłoża badane mikroorganizmy, w roku kolejnym nie ponawiano inokulum. Celem pracy było określenie wpływu grzybów saprofitycznych *Aspergillus versicolor* oraz *Trichocladium asperum* na populację *Heterodera schachtii* w drugim roku badań. W interpretacji doświadczenia uwzględniono następujące parametry: zagęszczenie cyst *H. schachtii* w glebie, zagęszczenie jaj i larw w 1 g gleby oraz płodność samic. Przy współwystępowaniu *H. schachtii* z grzybami saprofitycznymi *Aspergillus versicolor* oraz *Trichocladium asperum* w porównaniu do kombinacji kontrolnej (*H. schachtii* bez mikroorganizmów) znaleziono mniejszą liczbę cyst w 100 g gleby, znacznie większą płodność samic (liczba jaj i larw w cyście) oraz większe zagęszczenie jaj i larw w glebie.

Słowa kluczowe: współwystepowanie nicieni i grzybów saprofitycznych, *Heterodera schachtii, Aspergillus versicolor, Trichocladium asperum*

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Helena KUBICKA, Agnieszka PYZA and Aneta WOLSKA-SOBCZAK[1]

ACTIVITY OF CHOSEN ORGANIC ACIDS ON THE GROWTH OF RYE SEEDLINGS TREATED WITH CADMIUM OR LEAD IONS

DZIAŁANIE WYBRANYCH KWASÓW ORGANICZNYCH NA WZROST SIEWEK ŻYTA TRAKTOWANYCH KADMEM LUB OŁOWIEM

Abstract: The influence of cadmium and lead ions on the growth of seedlings of selected inbred lines was studied after the application of three organic acids. The growth stunt of the researched seedling lines was a result of the harmful effects of the aforementioned elements. The application of ascorbic and gibberellin acids inhibited the stress caused by lead and cadmium ions, which was shown by the growth of shoots and roots of seedlings. However, 2,4-D acid did not minimize the toxic influence of cadmium and lead. It can be concluded that the application of ascorbic and gibberellin acids may lessen the negative effects of stress caused by those heavy metals.

Keywords: lead, cadmium, organic acid, rye, *Secale cereale* L.

There are many factors which pollute the environment. Some are natural, others are caused by humans (industry and agriculture) where *eg* heavy metals are released [1, 2]. Cadmium is especially harmful for plants which actively absorb this substance through their roots and transport it to all organs. A 5 mg/kg content is enough for symptoms of toxicity to appear. These are chlorophylic marks, the browning of leaves and reddening of nerves. The excess of this toxic substance leads to a change of DNA structure in plants, a reduction of membrane permeability and growth stunt [3]. Lead is also absorbed through roots but is transported to shoots to a lesser extent. It is then stored in cellular walls. The excess is manifested through withering, darkening of leaves and smaller roots [4].

For this reason, the aim of the work was to check whether the selected organic acids inhibit the toxic activity of those elements on the growth of seedlings of genetically differentiated rye inbred lines.

[1] Botanical Garden – Center for Biological Diversity Conservation of the Polish Academy of Sciences, ul. Prawdziwka 2, 02-973 Warszawa, Poland, phone: 22 648 38 56, fax: 22 757 66 45, email: helenakubicka@wp.pl

Materials and methods

5 of 11 inbred lines (CH_7/99, L310, L176, M353, L299, L230, M15, 154/6) of the S_{25} generation of winter rye (*Secale cereale* L.) assessed by Kubicka and Pyza [5] were selected for the experiment. They differed on account of their sensitivity to lead and cadmium. The germinated seedlings of the rye lines were treated with ascorbic and dichlorophenoxyacetic acids at concentration of 10^{-2} M as well as gibberellin at concentration of 10^{-3} M for 72 hours, and then placed on the Hoagland nutrient containing lead ions at concentrations of 10^{-3} M and cadmium (10^{-4} M) for 48 hours. Next, 25 seedlings of each combination were placed on the Hoagland nutrient. Measurements of root and shoot lengths as well as the number of roots were conducted on the 14 day-old seedlings. Standard deviations were taken into account.

Results and discussion

In an earlier research [5] it was observed that cadmium at a concentration of 10^{-4} M caused a significant stunt of growth in rye inbred lines. However, a lower concentration of lead had a stimulating influence on the growth of shoots (above that of control) therefore both concentrations of lead 10^{-3} M and cadmium 10^{-4} M were applied.

Rye inbred lines subjected to the influence of lead at a concentration of 10^{-3} M were characterized by a significant reduction of growth. Line M15 occurred to be most sensitive with its shoot and root length decreased by 72 % and a 34 % reduction of root number. L299 line proved most tolerant. Cadmium caused a 9 % reduction of shoots and a 34 % reduction of the length of roots. In lines L230 the greatest reduction in the growth of roots (48 %) was observed (Fig. 1–4).

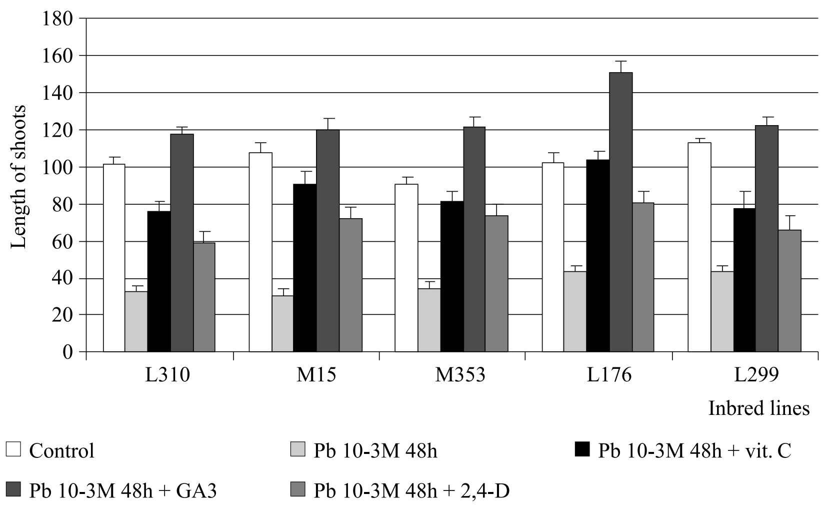

Fig. 1. The influence of lead on length of shoots of rye inbred lines [mm]

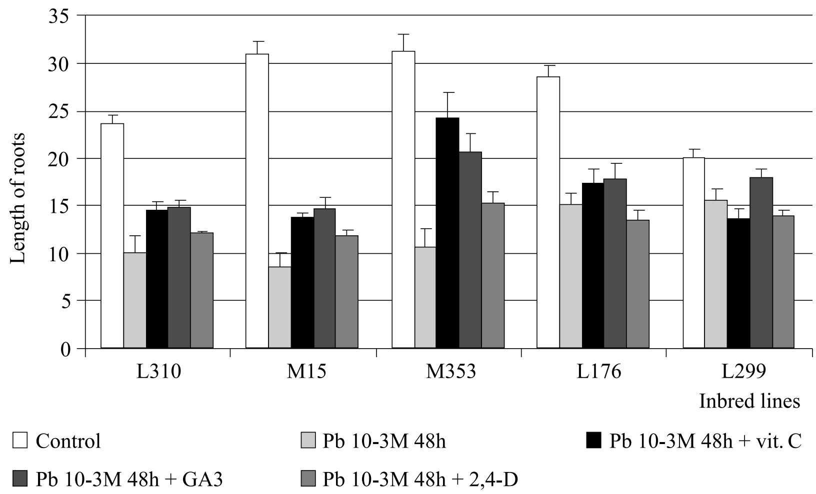

Fig. 2. The influence of lead on length of roots of rye inbred lines [mm]

Heavy metals found in soil are at first absorbed by roots which are at greatest risk to their toxic activity [6]. This is confirmed in the conducted experiments, in which the deformation, browning, reduction of root length and growth stunt of rye inbred line shoots was observed.

The lines selected for the experiment were characterized by a differing sensitivity to ions of those elements, and their reaction to the harmful activity of lead and cadmium was dependent on the genotype. A similar tolerance to cadmium in wheat and barley varieties caused by genetic variability was observed by Ozaruk et al [7] and Tiryakioglu et al [3].

Stress factors influencing the growth and development of organisms may be limited through the use of growth regulators [8] and antioxidants [3, 7]. Gibberellin and 2,4-D acids are commonly used *in vitro* cultures to regenerate plants. Taking this into account, 3 organic acids were used in this experiment: ascorbic, gibberellin and 2,4-D. Their influence on the reduction of stress to the activity of lead and cadmium ions was observed.

The application of organic acids (ascorbic and gibberellin) before the placement of inbred lines seedlings of rye on the lead nutrient reduced their toxic influence, which was observed by the growth of shoots and roots growth in inbred lines (Fig. 1, 2). In all lines a growth of seedlings was observed in combination with gibberellin. The greatest growth of shoots in lines: L176 – fourfold and M15 and M353 – threefold was founded. In the case of roots the greatest growth was observed in line M353 (twofold) in relation to combination with lead. The substances used also increased the number of roots in almost all lines with the exception of L299. 2,4-D had the weakest influence on lead stress reduction. It had little effect on root growth, and even reduced their length and number in lines L176 and L299.

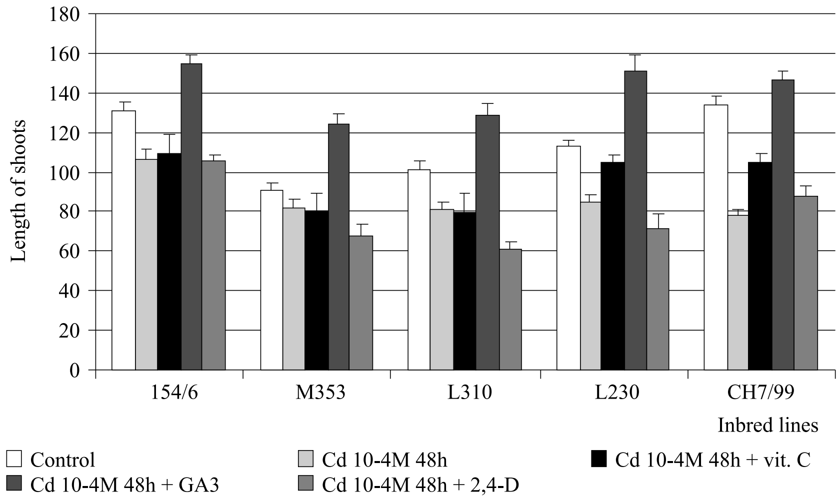

Fig. 3. The influence of cadmium on length of shoots of rye inbred lines [mm]

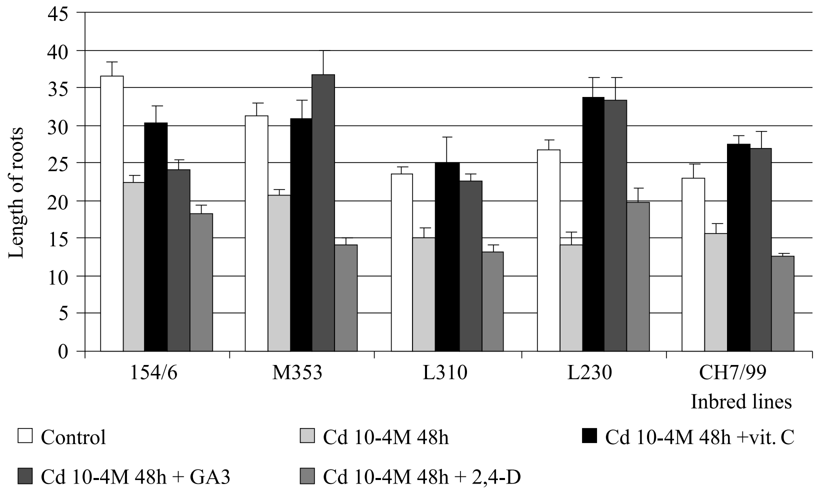

Fig. 4. The influence of cadmium on length of roots of rye inbred lines [mm]

A similar to lead influence of organic acids on seedlings growth was observed in cadmium combinations. Gibberellin acid also appeared most efficient because it caused the greatest seedling growth, almost twofold in lines CH7/99 and L230 in comparison with control (Fig. 3). In line L230 the application of vitamin C resulted in the twofold growth the length of roots in comparison with cadmium combination (Fig. 4). The positive influence of ascorbic acid on the reduction of stress was caused by the action of cadmium on barley varieties [7]. However, gibberellin caused root growth only in line M353. Both substances had a negligible influence on the increase in roots in rye lines. 2,4-D acid almost did not reduce cadmium stress in the researched lines.

Gibberellin acid has an influence on many process accompanying plant development – *eg* elongation of stems [9]. This was proven in the research in which gibberellin caused a greater growth of shoots which were characterized by a brighter colour.

Lead and cadmium have induced the production of reactive forms of oxygen in plants which leads to oxide stress and the increase in antioxidant enzyme activity [3, 7].

In this research it was shown that initial incubation of rye seedlings in a vitamin C solution (antioxidant) before treatment with cadmium and lead ions – neutralized the stress caused by these elements with the exception of L299 (root length). However, the activity of gibberellin acid was more effective. Both substances can be applied to lessen the toxic stress researched in plants which is caused by those heavy metals.

References

[1] Masso E.L., Corredor L. and Antonio M.T.: J. Trace Elements Med. Biol., 2007, **21**, 210–216.

[2] Małkowski E., Kita A., Golas W., Karcz W., and Michael K.: Plant Growth Regul., 2002, **37**, 69–76.

[3] Tiryakioglu M., Eker S., Ozkutlu F., Husted S. and Cakmak I.: J. Trace Elements Med. Biol. 2006, **20**, 181–189.

[4] Yang Y.Y., Jung Ji-Y., Song W-Y., Suh H-S. and Lee Y.: Plant Physiol., 2000, **124**, 1019–1026.

[5] Kubicka H. and Pyza A.: Ecol. Chem. Eng., 2007, **14**, 1–7.

[6] Sharma P. and Dubey R.S.: J. Plant Physiol., 2005, **17**, 35–52.

[7] Ozturk L., Eker S. and Ozkutlu F.: Turk. J. Agric. For., 2003, **27**, 161–168.

[8] Kubicka H., Dec D. and Gilewska M.: [in:] Obieg pierwiastków w przyrodzie, Instytut Ochrony Środowiska, Warszawa 2003, vol. II, 672–676.

[9] Frigerio M., Alabadi D., Perez-Gomez J., Garcia-Carcel L., Philips A.L., Hedden P. and Blazquez M.A.: Plant Physiol., 2006, **14**, 553–563.

DZIAŁANIE WYBRANYCH KWASÓW ORGANICZNYCH NA WZROST SIEWEK ŻYTA TRAKTOWANYCH KADMEM LUB OŁOWIEM

Ogród Botaniczny – Centrum Zasobów Różnorodności Biologicznej PAN, Warszawa

Abstrakt: W przeprowadzonym doświadczeniu analizowano wpływ kadmu i ołowiu na wzrost siewek wybranych linii wsobnych żyta ozimego po uprzednim zastosowaniu trzech kwasów organicznych. Szkodliwe działanie tych pierwiastków ujawniło się zahamowaniem wzrostu siewek badanych linii. Stosowane kwasy – askorbinowy i giberelinowy spowodowały zmniejszenie stresu wywołanego jonami kadmu i ołowiu, co uwidoczniło się zwiększonym przyrostem części nadziemnych i podziemnych siewek. Natomiast kwas 2,4-D nie miał wpływu na niwelowanie szkodliwego działanie kadmu i ołowiu. Wykazano, iż zastosowane kwasy organiczne – askorbinowy i giberelinowy mogą znaleźć zastosowanie do zmniejszania negatywnych skutków stresów wywołanych tymi metalami ciężkimi.

Słowa kluczowe: ołów, kadm, kwasy organiczne, żyto, *Secale cereale* L.

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Agnieszka LIS-KRZYŚCIN[1], Zbigniew J. BURGIEŁ[2]
and Irena WACŁAWSKA[3]

STUDIES OF FUNGISTATIC ACTIVITY OF COPPER-MODIFIED GLASSY FERTILISERS

BADANIA FUNGISTATYCZNEJ AKTYWNOŚCI SZKIEŁ NAWOZOWYCH MODYFIKOWANYCH DODATKIEM MIEDZI

Abstract: The subject of the present work was to study the possibilities of using glassy fertilisers both as mineral slow-release fertilisers and as material showing fungistatic activity. Apart from P, K, Ca, Mg and Si, the chemical composition of glassy fertilisers could contain a combination of microelements (0–10 % mas.). Thanks to the fungistatic activity of copper, glassy fertilisers modified with 2, 5 and 10 % of copper addition could become the subject of the study. Fungistatic activity of copper introduced into glassy fertilisers was studied with the aim of checking its effect on vegetative growth of the mycelium of *Fusarium culmorum*. The experiments covered the behaviour of copper-modified glassy fertilisers in hydroponic and soil cultivation. The results show very low fungistatic activity of glassy fertilisers.

Keywords: glassy fertilisers, copper, fungistatic activity

One of the main factors that pollute the environment, especially soils and waters, is agriculture. With the use of mineral fertilisers and pesticides, unfavourable substances are introduced into the environment. In order to reduce the influence of agriculture on the environment, research aimed to search for new materials which perform fertilising or protective functions has been carried out. In AGH – University of Science and Technology, Krakow, PL, a new type of mineral fertilisers, glassy fertilisers which can perform such a function, has been developed [1].

Glassy fertilisers are silicon-phosphorus-potassium fertilisers providing a plant with basic macroelements, such as phosphorus, potassium, calcium and magnesium, as

[1] Department of Soil Cultivation and Fertilization in Horticulture, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Kraków, Poland, email: alis@ogr.ar.krakow.pl

[2] Department of Plant Protection of the University, email: zjburgiel@ogr.ar.krakow.pl

[3] Department of Advanced Ceramics, AGH – University of Science and Technology, Al. A. Mickiewicza 30, 30-059 Kraków, Poland, email: iwac@interia.pl

well as with microelements (Cu, Fe, Mn, Zn, B and Co). Their chemical composition can be regulated in many ways depending on plants' nutritive requirements, and can be grouped as follows: SiO_2 > 27 % mas., P_2O_5 0–15 % mas., CaO 15–30 % mas., MgO 15–30 % mas., K_2O 0–20 % mas. and microelements 0–10 % mas. The internal structure of glass is similar to the structure of silicon minerals, and has a form of a net composed of silicon and oxygen atoms, where other components remain in free spaces. Mineral glassy fertilisers are produced with the traditional method of melting (1300–1400 °C) the mixture of such materials as: apatite, phosphorite, serpentine marble, potash (anhydrous potassium carbonate), and oxides incorporating appropriate microelements. The glassy mass obtained is calcined and then crushed. Due to vitrification and the appropriate chemical composition, glasses are difficult to dissolve in water, whereas their dissolubility increases with the extent of disintegration [2, 3]. The mechanism of releasing the components of glassy fertilisers resembles natural processes of erosion based on a phenomenon described as incongruent dissolving. In the process, the components of the dissolving solid substance get into the solution in proportions different from those in which they occur [4]. In the soil environment, the crystallization of secondary minerals, such as calcium and potassium silicates, takes place on the glass surface. They gradually decompose, and the cations contained in them are released. The cycle of these changes depends on the environmental conditions (pH, concentration of nutritive components, temperature, soil biological activity) [1, 5–7]. As a result, mineral glassy fertilisers show considerable flexibility of behaviour in the soil environment, depending on the physico-chemical conditions and the resultant nutritive needs. The usefulness of the glasses as a fertilisers in the cultivation of garden plants was examined during pot and field experiments [5, 8].

Inorganic copper compounds are characterised by high fungistatic activity [9]. Taking the above into account, glassy fertilisers with an increased amount of this component were developed.

The aim of the research was to check the fungistatic activity of copper-modified glassy fertilisers, as studies are being made into the possibility of their usage in cultivation mats, which in turn could be applied to hydroponics.

Material and methods

The examined glassy fertilisers were characterised by the unchanging content of SiO_2, P_2O_5 and K_2O. The copper content amounted to: 2 % (object 1), 5 % (obj. 2) and 10 % (obj. 3) of the mass of CuO. In relation to copper, the part of CaO and MgO changed, though the proportion of the MgO/CaO content remained unchanged, and equalled to 1 (Table 1). The glassy fertiliser fraction 0.1–0.3 mm in diameter was used for the research.

The effect of copper released from glassy fertilisers on the growth of *Fusarium culmorum* (W. G. Sm) Sacc. fungus, on a culture medium and in the soil environment was studied in laboratory conditions.

In the first stage of the research, 1 g of glassy fertiliser was shaken out from 100 cm^3 of the 0.1 % solution of Superba Red (N : P_2O_5 : K_2O = 7 : 9 : 25 + micro) for 3 hours on a rotatory shaking machine with the speed of 30 rotations per minute. The reaction of the culture medium was brought to pH 6.5–6.6. The obtained solutions were agar solidified (2 g · 100 cm^{-3}), poured into a Petri scale pan, and instilled with an agar disc (5 mm in diameter) overgrown with a *Fusarium culmorum* (W. G. Sm) Sacc mycelium [10].

Table 1

Chemical content of glassy fertilisers

Glassy fertilisers/objects	CuO	SiO_2	P_2O_5	K_2O	MgO	CaO
	[%]					
1	2.00	49.50	5.00	10.00	16.75	16.45
2	5.00	49.50	5.00	10.00	15.25	15.25
3	10.00	49.50	5.00	10.00	12.75	12.75

For the comparison of fungistatic activity of copper contained in the solution, the experiment also comprised a combination (object 4) with copper fungicide Miedzian 50 P (50 % copper oxychloride), concentrated in the culture medium to 100 mg · dm^{-3}. Scale pans filled with the culture medium with no copper content in it made the control object. After five days of cultivation, the diameter of the fungus colony in two perpendicular directions was measured. On the basis of the measurement, the percentage of the colony's growth inhibition was calculated by means of Abbott's formula [10].

The experiment was conducted in five replications. The results were statistically elaborated with the variance analysis method. The significance of differences among combinations was assessed on the basis of the repeated Duncan test ($p = 0.05$).

At the same time, an experiment in which a test fungus was cultivated on aqueous agar made of water (pH 6.5–6.6) where glasses with a certain copper content were shaken out, was conducted. The pattern of the experiment was analogous to the one described above.

The second stage of the research concerned the effect of a copper-modified glassy fertiliser added to the substrate on the settling of *Fusarium culmorum* fungus on the soil. To study that effect, the incubation of a soil mixture consisting of clay loam and compost acquired from a biological compost supplier was conducted in a ratio of 2 : 1. The compost was introduced in order to increase the mixture's biological activity. A glassy fertiliser containing 2, 5 and 10 % CuO in a dose of 4 g · dm^{-3} was inserted to the prepared substrate. The control object was the substrate with no glassy fertiliser content. The soil incubation was performed for 10 weeks in the temperature of 22–24 °C at the optimum humidity (60 %). The substrate samples were sterilised twice for 30 minutes in an autoclave (1.5 Pa, 110 °C), and next infected with a test fungus cultivated for 4 weeks on a sand-maize culture medium (quartz sand + 3 % maize meal). 10 g of inoculum was added to 100 g of the substrate, and in sterile conditions the components were mixed and ground in a mortar. Such material was incubated in room temperature for two weeks.

The assessment of the ground homing by the test fungus was carried out with Warcup's tabular-sandy method in Mańka's modification [11].

Two parallel series of the experiment were conducted in 10 repetitions each. The results were elaborated statistically with the variance analysis method, assessing the importance of the differences among combinations on the basis of Duncan test ($p = 0.05$).

Results and discussion

In each of the experiment combinations with the hydroponic culture medium, the *Fusarium culmorum* fungus created a delicate netlike aerial mycelium without the pigmentation typical of this species.

In the mediums containing copper, a slight but significant inhibition of the colonies' growth was observed. The degree of inhibition depended on the copper content in the glassy fertiliser (Table 2). The strongest inhibition of the fungus growth was identified in mediums with 10 % CuO. The colonies' diameter was smaller by 13.9 % than in the control object. A slightly stronger inhibition of the fungus' growth (18.8 %) was observed in the mediums containing Miedzian 50 WP [a.i. copper oxychloride].

Table 2

Evaluation of the effect of copper released from glassy fertilisers on the growth of *Fusarium culmorum* fungus

Combination	Diameter of the colony [mm]	Growth inhibition [%]
1	56.7 c*)	4.9
2	55.2 c	7.4
3	51.3 b	13.9
Miedzian 50 WP	48.4 a	18.8
Control	59.6 d	–

* Means indicated by the same letter do not differ significantly at $p = 0.5$.

The introduction of glassy fertilisers with various content of CuO into the substrate did not significantly affect the settling of the *F. culmorum* fungus. The number of units that formed the colonies on 1 g of the substrate in particular combinations is presented in Table 3.

Table 3

Evaluation of the effect of copper released from glassy fertilisers on the settling of *Fusarium culmorum* fungus on the substrate

Combination	Series I	Series II
Control	2575 a	2615 a
1	2515 a	2590 a
2	2485 a	2573 a
3	2494 a	2570 a

Inorganic copper compounds are known to be characterised by high activity and a wide spectrum of fungicidal activity. Many years ago some of them (alkaline copper carbonate) were used to lace seeds, and together with the seed material were introduced into the soil. Due to their phytotoxical activity and poor effectiveness in protection against pathogens causing seedling canker, their application into the soil was abandoned [9].

The research that had been performed showed low fungistatic activity both of the copper introduced with the glassy fertiliser and the Miedzian 50 WP preparation in relation to the *F. culmorum* fungus. In laboratory experiments on fungicides, the effectiveness lower than 49 % is considered insufficient [10]. However, the results achieved in an in vitro observation cannot be directly compared to natural conditions. The root excretions have been noticed to exhibit the ability of modifying both the amount and the pace of releasing mineral components from glassy fertilisers [3, 6]. Thus, supposedly, the plants' presence in such an arrangement could additionally influence mutual interactions of the ions present, and increase the efficiency of the glasses used with the addition of copper.

Conclusions

The achieved results show that using copper-modified glassy fertilisers will not affect the phytosanitary state of substrates and soils. Nevertheless, it may improve plants' provision with this microelement.

References

[1] Stoch L., Stoch Z. and Wacławska I.: Patent, PL 185229131 2003

[2] Stoch L. and Wacławska I.: Zesz. Probl. Post. Nauk Roln., 1996, **429**, 293–299.

[3] Stoch L., Wacławska I. and Lis-Krzyścin A.: Chem. Agricult., 2000, ***2***, 74–80.

[4] Korapetjanc M.C.: Wstęp do teorii procesów chemicznych. PWN, Warszawa 1983.

[5] Ostrowska J, Lis-Krzyścin A. and Wacławska I.: Folia Univ. Agric. Stetin. 190, Agricultura, 1998, **72**, 253–269.

[6] Ostrowska J., Lis-Krzyścin A. and Wacławska I.: Rocz. AR w Poznaniu CCCXLI, Ogrodnictwo, 2000, (35), 133–140.

[7] Wacławska I. and Ostrowska J.: Proc. Int. Congr. Glass 2. Ext., Edinburgh, 990–991, 2001.

[8] Ostrowska J., Lis-Krzyścin A. and Wacławska I.: Chem. Agricult., 2000, **2**, 67–73.

[9] Borecki Z.: Fungicydy stosowane w ochronie roślin. PWN, Warszawa 1984.

[10] Kowalik R. and Krechniak E.: Materiały do metodyki badań biologicznych ocen środków ochrony roślin. IOR, Poznań 1961.

[11] Mańka K.: Zesz. Probl. Post. Nauk Roln., 1964, **160**, 9–23.

BADANIA FUNGISTATYCZNEJ AKTYWNOŚCI SZKIEŁ NAWOZOWYCH MODYFIKOWANYCH DODATKIEM MIEDZI

Katedra Uprawy Roli i Nawożenia Roślin Ogrodniczych, Uniwersytet Rolniczy
Katedra Ochrony Roślin, Uniwersytet Rolniczy
Katedra Ceramiki Specjalnej, Akademia Górniczo-Hutnicza

Abstrakt: Przedmiotem prezentowanej pracy było zbadanie możliwości wykorzystania szkieł nawozowych nie tylko jako nawozów mineralnych o wydłużonym działaniu, lecz także materiałów odznaczających się fungistatyczną aktywnością. Do składu chemicznego szkieł nawozowych można wprowadzić oprócz P, K, Ca, Mg i Si, zestaw mikroelementów w przedziale 0–10 % mas. Znając fungistatyczną aktywność miedzi, za przedmiot badań obrano szkła nawozowe, do których składu wprowadzono 2, 5, 10 % mas. tego mikroelementu. Oddziaływanie miedzi sprawdzano, badając jej wpływ na wzrost wegetatywny grzybni *Fusarium culmorum*. Badania dotyczyły zachowań szkieł nawozowych modyfikowanych miedzią w uprawach hydroponicznych i glebowych. Badane szkła nawozowe wykazują bardzo słabe działanie fungistatyczne.

Słowa kluczowe: szkła nawozowe, miedź, fungistatyczna aktywność

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Stefan MARTYNIUK[1] and Jadwiga OROŃ[1]

INTERACTIONS BETWEEN PHYSICO-CHEMICAL CHARACTERISTICS OF SOILS AND POPULATIONS OF BACTERIA FIXING ATMOSPHERIC NITROGEN

INTERAKCJE POMIĘDZY FIZYCZNO-CHEMICZNYMI WŁAŚCIWOŚCIAMI GLEB A POPULACJAMI BAKTERII WIĄŻĄCYCH AZOT ATMOSFERYCZNY

Abstract: Population densities of bacteria from the genus *Azotobacter* strongly depend on soil reaction. These bacteria are usually absent in acid soils (pH below 6). Our studies have shown that among the researched soil physical and chemical characteristics, mechanical soil structure (particularly clay content) and soil reaction have the greatest influence on numbers of the symbiotic bacteria in the soils. Symbionts of red clover and alfalfa were usually more abundant in soil richer in clay and having pH about 7. On the contrary, such soils are not conducive for lupine and serradella symbionts.

Keywords: *Azotobacter*, symbiotic bacteria, soil properties, agricultural practices

The process of biological nitrogen fixation (BNF) is carried out by various physiological groups of bacteria. From an ecological point of view these bacteria can be divided into: - free-living diazotrophic bacteria (*Azotobacter*, *Clostridium*), fixing atmospheric N in soil, or in associations with plant roots (*Azospirillum*), and – symbiotic bacteria (*Bradyrhizobium*, *Rhizobium*, *Sinorhizobium*), fixing N_2 in root nodules of leguminous plants or some trees (*Frankia*) [1, 2]. On the global scale, BNF contributes a major source of nitrogen to the biosphere. Total world biological N_2 fixation is estimated to be as high as $17.2 \cdot 10^7$ tons per year. Particularly, symbiotic nitrogen fixation is an important source of N to leguminous plants and soil. For example, a field alfalfa crop may fix up to 460 kg N/ha, of which 60–70 % may be derived from the symbiosis with specific rhizobia [1]. Amounts of N_2 fixed by free-living diazotrophic bacteria in soil are much lower and range from 1 kg to 30 kg N/ha [1–3]. In this short review soil and agrotechnical factors affecting populations of free-living Azotobacter spp. and root-nodule bacteria in soils are discussed.

[1] Departament of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation – State Research Institute, ul. Czartoryskich 8, 24-100 Puławy, Poland, email: sm@inung.pulawy.pl

Occurrence of Azotobacter in soils

Important ecological feature of bacteria from the genus *Azotobacter* is their sensitivity to soil reaction; in acid (pH < 6.0) soils these bacteria are generally absent or occur in very low numbers [3–5]. Soil populations of *Azotobacter* spp. in neutral or alkaline soils rarely exceed several thousands cells per gram of soil. With respect to Polish soils, in 1923 Ziemięcka [3] published results of her pioneer studies on the occurrence of *Azotobacter* spp. in soil samples collected in 1917 and 1918 from 28 locations in the former Polish Kingdom. These studies showed that 50 % of the examined soils contained *Azotobacter* spp.. Most of the soils studied by Ziemięcka were also characterized for their chemical properties, such as soil reaction and contents of: humus, total N, P_2O_5 and CaO. Among these parameters, soil reaction was found to be the most important environmental factor influencing the occurrence and numbers of *Azotobacter* spp. in the soils studied. In 2000 Martyniuk & Martyniuk [5] examined population densities of *Azotobacter* spp. in 31 soils from Poland and compared their results with those reported by Ziemięcka [3], to see whether intensification of agricultural practices that took place during the course of the past century has caused any changes in the colonization of Polish soil by *Azotobacter* spp. In this study various populations of *Azotobacter* spp. were detected in 16 out of 31 soils examined, thus the percentage of soils with *Azotobacter* (51.6 %) was slightly higher than that (50 %) reported by Ziemięcka. This comparison indicates that intensification of agricultural practices during the course of the past century did not change significantly colonization of Polish soils by the bacteria studied. Detailed discussion on changes in Polish agriculture taking place during the course of the 20th century, and on the effects of these changes on soil quality, is beyond the scope and volume of this paper. However, the use of mineral fertilizers and its effect on soil chemical properties, particularly soil pH, deserves short consideration in relation to *Azotobacter*. It has been well documented in long-term field experiments that mineral N fertilizers may cause substantial acidification of soil, particularly when these fertilizes are used in high doses without liming. Since *Azotobacter* is very sensitive to soil acidity one could expect that intensification of the use of mineral N fertilizers might cause acidification of agricultural lands resulting in a reduction of soil populations of *Azotobacter* spp. Condensed description of the changes in Polish agriculture, including the use of mineral fertilizers, in the 19th and 20th centuries, is given by Krasowicz [6]. For example, application of mineral N increased in Poland (on average) from less than 5 kg N ha^{-1} in the forties of the 20th century to about 70 kg N in the eighties of that century. However, data presented by Lipiński [7] prove that acidification of Polish soils did not increase between 1955 and 1999. On the contrary, slight improvement of this soil property could be seen in that period. For example, the area of acidic soils (pH < 5.5) decreased from 58 % in the decade 1955–1964 to 55 % in the period of 1994–1999, and simultaneously, the area of soils having the pH > 6.5 increased from 17 % to 19 % in the respective periods [7]. Thus, these data seem to correspond with a slightly higher percentage of soils colonized by *Azotobacter* spp. found in the year 2000, as compared with that presented by Ziemięcka [3].

Populations of rhizobia in soils as influenced by environmental factors and agrotechnical practices

Root-nodule bacteria, fixing atmospheric nitrogen in the symbiosis with leguminous plants, are members of the following genera: *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium* and *Allorhizobium* within the family *Rhizobiaceae* [8]. These bacteria, commonly known as rhizobia, survive in soil between symbiotic phases as saprophytes and their populations depend on many physical and chemical properties of the soil environment and on the frequency of planting of legumes in a given area or field [9–12]. Based on a long-term plot experiment Martyniuk et al [11] assessed numbers of various species of rhizobia in two soils cropped to cereals and treated with different rates of mineral fertilisers (NPK), with or without liming. In the limed soils relatively high populations of rhizobia nodulating red clover or pea were found, though these legumes were not grown in this experiment for over 20 years, but in the unlimed soils populations of the rhizobia were markedly lower. Contrary to pea and clover rhizobia, almost no microsymbionts of alfalfa were detected in the studied soils, both limed and unlimed, indicating that populations of alfalfa rhizobia in soil strongly depend on cultivation of their host plant. Similar results were obtained by Nutman and Hearne [9] for rhizobia occurring in soils of the UK. In France, Amarger [13] analysed populations of various species of root-nodule bacteria in 60 different soils and related numbers of these bacteria to soil pH. It was found in this study that populations of alfalfa rhizobia were much smaller in soils with the pH below 6.0 than in neutral or alkaline soils, but the reverse was true for lupine rhizobia. Similar studies were conducted for 80 soil samples collected from different sites in Poland [2]. Rhizobia nodulating lupine and serradella, (*Bradyrhizobium* sp.) were not detected in 19 out of 80 Polish soils analyzed and in the other 27 soils populations of these bacteria were low. Moderate numbers of *Bradyrhizobium* sp. were found in 10 soils and 24 soils contained high numbers of these rhizobia. Among the soils tested, only 4 soils originated from fields in which lupine or serradella were grown in the year of sampling or in the previous growing season. These soils contained the highest numbers ($1.7–2.8 \cdot 10^4$) of *Bradyrhizobium* sp., proving that cultivation of the host-plants had beneficial effect on soil populations of these root-nodule bacteria. It should be added, however, that many soils harbouring moderate or high populations of *Bradyrhizobium* sp. were not planted to lupine or serradella for long period of time, in some cases even more than 30 years. This fact indicate that bradyrhizobia have the capacity to survive in soil for many years in the absence of their host-plants in crop rotation. The numbers of *Bradyrhizobium* sp. were significantly (negatively) correlated with the contents of silt-clay fractions, with the total N contents and with the pH (in water) of the examined soils, indicating that the light-textured, acid soils are beneficial for the proliferation and survival of these root-nodule bacteria. Rhizobia forming the symbiosis with roots of vetch, pea and faba-bean, (*Rhizobium leguminosarum* bv. *viciae*) were found almost in all the examined soils and most of these soils (70) contained high or moderate populations of *R.l. viciae*. Common occurrence of these bacteria in Polish soils could be explained, at least partially, by relatively frequent cultivation of the host plants of *R.l. viciae* by farmers, since in

23 cases the studied soil originated from fields on which these crops (pea or faba-bean) were included in the crop rotation. These soils contained generally high populations of *R.l. viciae*, but also many soils not planted to peas or faba-bean were also rich in these root nodule bacteria. The soils with no detectable or very low populations of *R.l. viciae* were very acid (pH = 3.7–4.2). Highly significant correlation coefficients between populations of these rhizobia and pH of the soils indicate that soil reaction is an important factor influencing the occurrence of *R.l. viciae* in soils [2]. Symbionts of clover, (*R. leguminosarum* bv. *trifolii*), similarly to *R.l. viciae*, were found in high or moderate populations almost in all Polish soils examined. Only 4 soils contained no detectable populations of *R.l. viciae* and in 7 soils these populations were assessed as very low. The majority of the researched soils were not planted to red clover for many years (with the exception of 3 soils) indicating that rhizobia nodulating this crop can survive in soils for a long period of time, even in the absence of the host plant. Highly significant correlation coefficients with soil pH and with the contents of silt-clay fractions suggest that medium or heavy soils heaving slightly acid or neutral pH are beneficial for these rhizobia [2, 9, 12].

Rhizobia nodulating beans (*R. leguminosarum* bv. *phaseoli*), were examined in 76 soils and of this number in 15 soils these bacteria were not detected. In the other soils tested populations of the bean rhizobia varied from very low (in 3 soils) to high in 25 soils. Field bean was grown only on 3 of the tested soils and the numbers of *R.l. viciae* in these soils were high. Soil populations of the bean rhizobia showed significant correlation with the contents of total N as well as with the contents of 0.02 mm soil mechanical fraction in the soils. Root nodule bacteria of alfalfa (*Sinorhizobium meliloti*), was the only species of the rhizobia which was not detected in the majority of the studied soil. Soils with no, very low and low populations of the alfalfa rhizobia made up almost 93 % of all the studied soils. Only 3 soils originated from fields with the stands of alfalfa and all these soils contained high numbers of *S. meliloti*. These results clearly indicate the presence and population levels of the alfalfa rhizobia in Polish soils are, similarly to soils in other countries [2, 9, 12, 13] strongly dependent on the cultivation of the host crop. Soil texture as represented by the contents of silt-clay fractions is also an important factor influencing the survival of *S. meliloti* in soil.

References

[1] Ishizuka J.: *Trends in biological nitrogen fixation research and application*. Plant Soil 1992, **141**, 197–209.

[2] Martyniuk S.: *Systemy biologicznego wiązania azotu*. Naw. Nawoż./Fert.Fertil. 2002, **1**(10), 264–277.

[3] Ziemięcka J.: *Występowanie azotobaktera w glebach polskich*. Rocz. Nauk Roln. 1923, **10**, 1–78.

[4] Geinley P.L.: *Influence of the absolute reaction of a soil upon Azotobacter flora and nitrogen fixing ability*. J. Agric. Res. 1923, **24**, 907–928.

[5] Martyniuk S. and Martyniuk M.: *Occurrence of Azotobacter spp. in some Polish soils*. Pol. J. Environ. Stud. 2003, **12**, 371–374.

[6] Krasowicz S.: *Produkcja roślinna na ziemiach polskich w XIX i XX wieku – rys historyczny*. Pamięt. Puław. 2002, **130**, 11–18.

[7] Lipiński W.: *Odczyn i zasobność gleb w świetle badań stacji chemiczno-rolniczych*. Naw. Nawoż./ Fert. Fertil. 2000, **3**(**4**), 89–105.

[8] Małek W. and Sajnaga E.: *Current taxonomy of the rhizobia*. Acta Microb. Pol. 1999, **48**(2), 109–122.

[9] Nutman P.S. and Hearne R.: *Persistence of nodule bacteria in soil under long-term cereal cultivation*. Rothamsted Raport for 1979, Part 2, 1979, 77–90.
[10] Sadowsky M.J. and Graham P.H.: *Soil biology of the Rhizobiaceae*, [in:] The Rhizobiaceae, Kluwer Academic Publishers, Dordrecht 1998, pp. 155–172.
[11] Martyniuk S., Woźniakowska A. and Martyniuk M.: *Effect of agricultural practices on populations of Rhizobium in some field experiments*. Bot. Lithuan. 1999, **3**, 99–102.
[12] Martyniuk S., Oroń J. and Martyniuk M.: *Diversity and numbers of root-nodule bacteria (rhizobia) in Polish soils*. Acta Soc. Bot. Polon. 2005, **74**, 83–86.
[13] Amarger N.: *Aspect microbiologique de la culture des legumineuses*. Le Selectionneur Francais 1980, **28**, 61–66.

INTERAKCJE POMIĘDZY FIZYCZNO-CHEMICZNYMI WŁAŚCIWOŚCIAMI GLEB A POPULACJAMI BAKTERII WIĄŻĄCYCH AZOT ATMOSFERYCZNY

Zakład Mokrobiologii Rolniczej, Instytut Uprawy, Nawożenia i Gleboznawstwa
– Państwowy Instytut Badawczy, Puławy

Abstrakt: Liczebność populacji bakterii z rodzaju *Azotobacter* w glebie uzależniona jest przed wszystkim od odczynu środowiska glebowego. Bakterie te na ogół nie występują w glebach o odczynie kwaśnym (poniżej pH 6,0). Przedstawione badania wykazały, że spośród analizowanych właściwości fizycznych i chemicznych gleb, skład granulometryczny, a zwłaszcza zawartość części spławianych, oraz odczyn gleb mają największy wpływ na liczebności glebowych populacji bakterii symbiotycznych (brodawkowych). Symbionty koniczyny i lucerny są na ogół liczniejsze w glebach zwięźlejszych i odczynie obojętnym lub zbliżonym do obojętnego, natomiast takie gleby nie sprzyjają symbiontom łubinu i seradeli.

Słowa kluczowe: *Azotobacter*, bakterie symbiotyczne, właściwości gleby, zabiegi agrotechniczne

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Monika Anna MICHAŁOWSKA[1], Stefan RUSSEL[2]
and Józef CHOJNICKI[3]

INFLUENCE OF SOME ABIOTIC FACTORS ON THE OCCURRENCE OF MYXOBACTERIA IN SELECTED FOREST SOILS OF PUSZCZA BIALA

WPŁYW CZYNNIKÓW ABIOTYCZNYCH NA WYSTĘPOWANIE MYKSOBAKTERII W WYBRANYCH GLEBACH LEŚNYCH PUSZCZY BIAŁEJ

Abstract: The aim of present paper was to establish the effect of some physical and chemical factors on the number and species composition of myxobacteria. The following chemical and physical factors were studied: pH, carbon and nitrogen content as well as terms of samples collection and depth of soil profile of selected forest soils (muck, gley) of Puszcza Biala The number of myxobacteria was determined by plate method using appropriate microbiological media. Isolated and purified strains were identified on the basis of their macro- and micromorphology using stereoscopic, light and scanning electron microscope.

The results showed that the highest number of myxobacteria was found in upper horizons of the examined soils. Higher pH and higher content of carbon as well as moderate humidity of muck soil have significant influence on higher number and species diversity of myxobacteria than in gley soil. It was found that two species of the cellulolytic myxobacteria: *Sorangium cellulosum* and *Polyangium compositum* dominated in whole profile of the investigated soils.

Keywords: myxobacteria, slime bacteria, soil microflora, forest soils, Puszcza Biala

Myxobacteria are microorganisms which have maintained, probably due to their unusual and complex life cycle as well as fascinating appearance, an unremitting attraction for many microbiologists all over the world. They are aerobic, unicellular, Gram-negative gliding bacteria with rod-shaped vegetative cells that mostly occur in many

[1] Division of Microbial Biology, Faculty of Agriculture and Biology, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warszawa, Poland, email: monika_michalowska@sggw.pl

[2] Department of Rural Sanitation, Institute for Land Reclamation and Grassland Farming, Falenty, Al. Hrabska 3, 05-090 Raszyn, Poland, email: stefan_russel@sggw.pl

[3] Division of Soil Science, Department of Soil Sciences, Faculty of Agriculture and Biology, Warsaw, University of Life Sciences, ul. Nowoursynowska 159, 02-776 Warszawa, Poland, email: jozef_chojnicki@sggw.pl

soil types. Myxobacteria frequently develop on decomposing plant material, the bark of living and dead trees or dung of herbivorous animals such as rabbit, hare, goat, deer and sheep [1–3].

They are unique among prokaryotes for their complicated multicellular behaviour. They use intercellular communication to engage in cooperative morphogenesis from which they produce unusual structures called fruiting bodies which contain myxospores [4]. Under starvation conditions the cells aggregate within the swarm, pile up and form fruiting bodies that allow the community to survive unfavourable environmental conditions [5–7]. Inside the maturing fruiting body the rod-shaped vegetative cells are converted into myxospores by shortening and fattening. Thick-walled myxospores are asexual, dormant cells that are responsible for the survival under hostile conditions such as desiccation, high and low temperature, high salt concentrations, anaerobic conditions, ultrasound and UV irradiation. They can survive in the environment for 10–25 years [2, 4, 8]. Myxospores germination is induced by favourable environmental conditions and in the laboratory by transfer to a suitable growth medium.

Myxobacteria also display other interesting features like social behaviour expressed by collective food uptake and cooperative motility [2, 9]. Their cells move by gliding over the surface of or within the substrate, so that colonies spread over the culture plate [10]. Cell motility plays an important role in development and morphogenesis in myxobacteria, especially in formation of fruiting bodies [11–13].

Myxobacteria are strictly aerobic organotrophs which prefer moderate temperature in the range between 9–38 °C. Myxobacteria specialize in the biodegradation of biomacromolecules. These organisms are divided into two sharply separated metabolic groups. Group 1 holds cellulose degraders. This group is capable of utilizing inorganic nitrogen compounds while growing on cellulose and glucose. Group 2, by far the majority of myxobacterial species, depend on an amino acid-containing medium such as peptone for growth. They obtain the required oligopeptides by hydrolyzing proteins [5]. In nature, these myxobacteria feed on other microorganisms like eubacteria or yeasts, that therefore have been called micropredators [2, 14].

Myxobacteria have some potential for various applications. They play a substantial role in natural environment in solubilizing large macromolecules, cell carcasses and other biological detritus. Their predatory activity can be very useful to control cyanobacterial water blooms by destroying cells of cyanobacteria in aqueous environments. Myxobacteria can also be used as pollution indicators which can inform us about environmental problems. A good opportunity for application may lie in the field of myxobacterial enzymes such as restriction endonucleases and special proteases which can be very useful in biochemistry and medicine. The most promising opportunities for biotechnological applications with myxobacteria is the production of secondary metabolites, especially compounds with biological activity such as antibiotics and other inhibitors like epothilon with antitumor activity. These substances are promising candidates for the development of useful drugs [2, 4].

The aim of present paper was to establish the effect of some physical and chemical factors on the number and species composition of myxobacteria. The following abiotic factors were studied: pH, carbon and nitrogen content as well as terms of samples collection and depth of soil profile of selected extremely acid, forest soils of Puszcza Biala.

Materials and methods

The soil samples were taken from Puszcza Biala from June to September 2006. The investigation was carried out on the muck and gley soils. The samples collected from all genetic horizons of investigated soils were analysed. The number of myxobacteria was determined by plate method by inoculation of appriopriate microbiological media with clumps of soil and soil suspensions 10–1, 10–2, 10–3. Inoculated microbiological media were incubated at 30 °C for 1–4 weeks and checked daily, beginning with the second and third day, under a dissecting microscope for the appearance of myxobacterial swarms and fruiting bodies. After an incubation period the colonies and fruiting bodies zones were counted to determine the number of myxobacteria in examined soils. Obtained results were expressed as CFU (colony forming units) in 1 g dry mass of soil.

For isolation and purification of bacterial strains the following media were used: VY/2 containing autoclaved yeasts (*Saccharomyces cerevisiae*) as the nourishment and B12 source [15, 16], water agar with mineral salts and filter paper as carbon source and CY containing casitone (Difco) as rich source of nitrogen [4]. To inhibit fungal growth 100 $\mu g \cdot cm^{-3}$ cycloheximide or nystatine to media, sterile water used to moisten clumps of soil and soil suspensions was added.

In order to obtain pure cultures isolated strains were purified by following methods [5]:

– direct purification from swarm or fruiting bodies to a suitable agar medium: predatory myxobacteria-CY, VY/2, MD1, CAS, CT, water agar with streaks of E. coli [5, 16]; cellulolytic myxobacteria-water agar with filter paper and mineral salts, ST21CX, CEL3, STAN-6 [5].

– heating of the suspension of the mature fruiting bodies at 58 °C for 10, 20 and 40 min.

– purification of the suspension of the mature fruiting bodies with mixture of antibiotics such as chloramphenicol, streptomycin, tetracycline, kanamycin, erythromycin, gentamycin (20–30 $mg \cdot 50\ cm^{-3}$ of water).

Isolated and purified strains of myxobacteria were identified on the basis of morphology of vegetative cells, myxospores, fruiting bodies and colonies using stereoscopic, light and scanning electron microscope. Furthermore for the identification of pure and enrichment cultures the determination keys of McCurdy and of Reichenbach and Dworkin were applied [5, 17].

Statistical methods were used to determine the influence of some abiotic factors of selected forest soils of Puszcza Biala (terms of samples collection, depth of soil profile as well as pH, carbon and nitrogen content) on the occurrence of myxobacteria. There were conducted one and two-factor analysis of variance as well as Tukey's studentized range test using SAS 9.1 software (SAS Institute INC., Cary, NC).

Results and discussion

Investigated soils from Puszcza Biala (muck and gley) have different chemical and physical properties which affected on the number and species composition of the population of myxobacteria (Table 1).

The higher number and higher biodiversity of myxobacteria were found in muck soil than in gley soil (Fig. 1, Table 2).

The results showed that myxobacteria mostly occurred in surface layers of soil-AM horizon. In deeper horizons the number of myxobacteria rapidly decreased reaching the lowest values in Dg horizon. These results are in good agreement with those of Krzemieniewska and Krzemieniewski [18], Gorny [19] and Reichenbach [3]. This fact was probably caused by shortage of oxygen and organic matter in deeper horizons of investigated soil which make unfavourable living conditions for this group of microorganisms.

Table 1

Characteristics of soil profiles of muck and gley.

Profile	Systematics of investigated soils	Genetic horizons	Depth [cm]	pH		%		C:N
				H2O	KCl	C	N	
21a	Type: muck soil Order: post-bog soils Division: hydrogenics soils	AM	5–10	5.1	4.6	36.50	2.09	17.46
		Mt	20–30	5.2	4.9	17.40	0.95	18.31
		Dg	50–60	6.3	5.8	1.10	0.07	15.71
35	Type: gley soil Order: bogged soils Division: semi-hydrogenics soils	Ol	0–1	4.8	4.2	18.59	1.77	10.50
		A	1–17	4.1	3.4	6.65	0.63	10.44
		Gor	17–50	6.4	5.2	0.09	0.01	6.92
		Gr	50–65	6.7	5.6	0.02	–	–

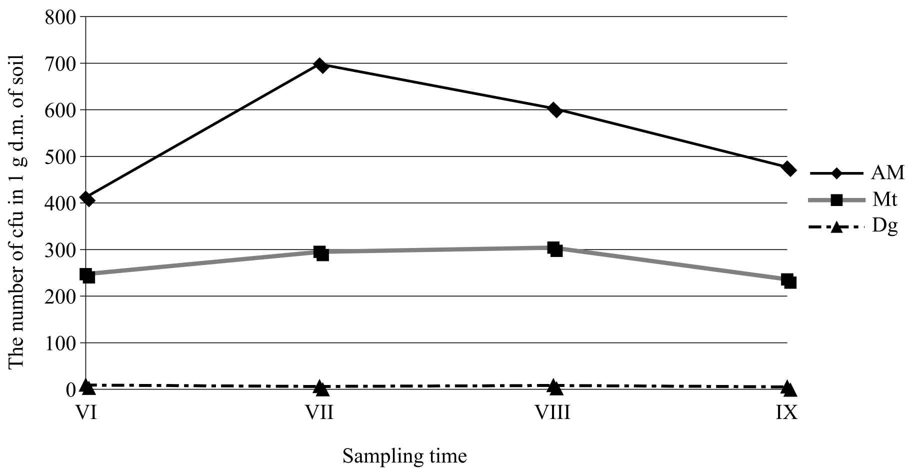

Fig. 1. The occurrence of myxobacteria in muck soil

Qualitative analyses also comprised dynamics of the number of myxobacteria in various seasons of the year. It was confirmed that the number of myxobacteria has increased from June with the maximum in July (AM horizon) and August (Mt horizon). The fall of the number of examined microorganisms was observed in September what

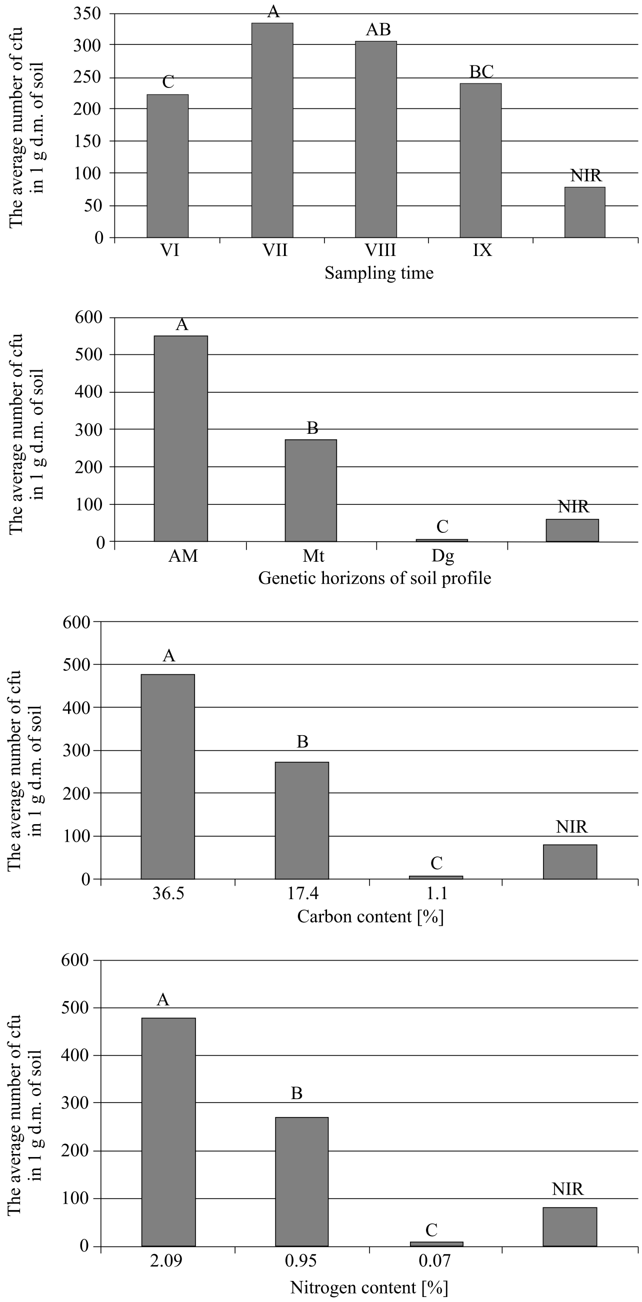

Fig. 2. Results of Tukey's test concerning the influence of abiotic factors on the number of myxobacteria in muck soil (Tukey's homogenous groups are represented by letters A through C. Bars in group A have the highest number of myxobacteria whereas bars in groups B to C have successively lower number of myxobacteria. Bars that have letters in common do not differ significantly from one another

was connected with drop of the air temperature as well as higher intensity of atmospheric falls.

Results of two-factor analysis of variance showed a significant influence of season and depth of soil profile on the number of myxobacteria in muck soil. Furthermore one-factor analysis of variance showed a significant influence of carbon and nitrogen content on the number of myxobacteria in examined soil.

Results of Tukey's test showed a statistically significant differences of the averages of the number of myxobacteria depending on season. There were identified three homogenous groups with the highest average of the number of myxobacteria in July and the lowest average in June.

Taking into consideration the influence of depth of soil profile there were observed a significant differences in each of three genetic horizons of muck soil. Tukey's test identified three homogenous groups with the highest average of the number of myxobacteria in AM horizon and the lowest average in Dg horizon.

Results of Tukey's test also showed a significant differences of the number of myxobacteria depending on carbon and nitrogen content. Tukey's test identified three homogenous groups with the highest average of the number of myxobacteria at the highest carbon and nitrogen content in muck soil.

Table 2

Species composition and frequency of isolation of myxobacteria from muck soil

Species of myxobacteria	Frequency of isolation [%]
Polyangium compositum	43.00
Sorangium cellulosum	34.67
Myxococcus fulvus	11.67
Angiococcus disciformis	6.33
Corallococcus coralloides	1.33
Polyangium septatum	1.00
Polyangium sorediatum	0.67
Cystobacter fuscus	0.67
Polyangium aureum	0.33
Polyangium luteum	0.33

The most often occurring myxobacteria were representatives of the *Sorangineae* suborder such as cellulolytic species *Polyangium compositum* and *Sorangium cellulosum* isolated from whole genetic horizons of examined soil profile. There were also observed myxobacteria belonging to the *Cystobacterineae* suborder such as *Myxococcus fulvus* and *Corallococcus coralloides*, especially in surface layers of soil. As pointed out by Krzemieniewska and Krzemieniewski [18, 20] and Dawid [2] in acid soils occur characteristic flora of myxobacteria with the majority of representatives belonging to *Sorangium* and *Polyangium* genuses as well as some species of *Myxococcus* genus, especially *Myxococcus fulvus*.

The second type of investigated soil-gley have dissimilar properties to muck soil. This soil was formed on the area with high level of groundwater which cause high humidity and anaerobic conditions creating negative influence on the occurrence of myxobacteria (Fig. 3, Table 3).

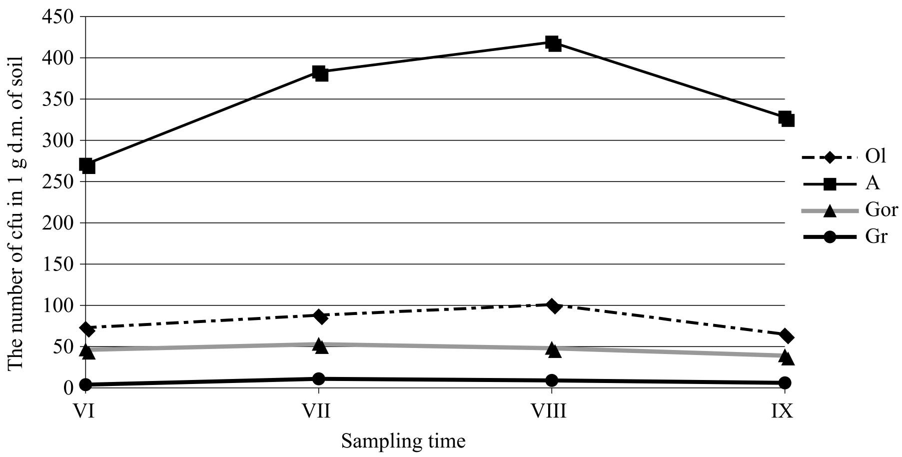

Fig. 3. The occurrence of myxobacteria in gley soil

Table 3

Species composition and frequency of isolation of myxobacteria from gley soil

Species of myxobacteria	Frequency of isolation [%]
Sorangium cellulosum	49.00
Polyangium compositum	21.33
Myxococcus fulvus	12.33
Corallococcus coralloides	9.33
Angiococcus disciformis	7.00
Cystobacter minus	0.67
Polyangium septatum	0.67
Polyangium luteum	0.34

The results showed that distinct majority of myxobacteria occurred in A horizon. There was observed the considerably lower number of myxobacteria in Ol horizon, even though it is upper horizon. It is directly connected with fact that dead leaves are not major and most convenient environment of living of these microorganisms, so that this horizon is settled by selected species. Furthermore, similar to muck soil, the number of myxobacteria decreased in deeper horizons of soil profile because of high saturation of soil with water causing anaerobic conditions as well as low content of organic matter, which is in agreement with the previous observations of Krzemieniewska and

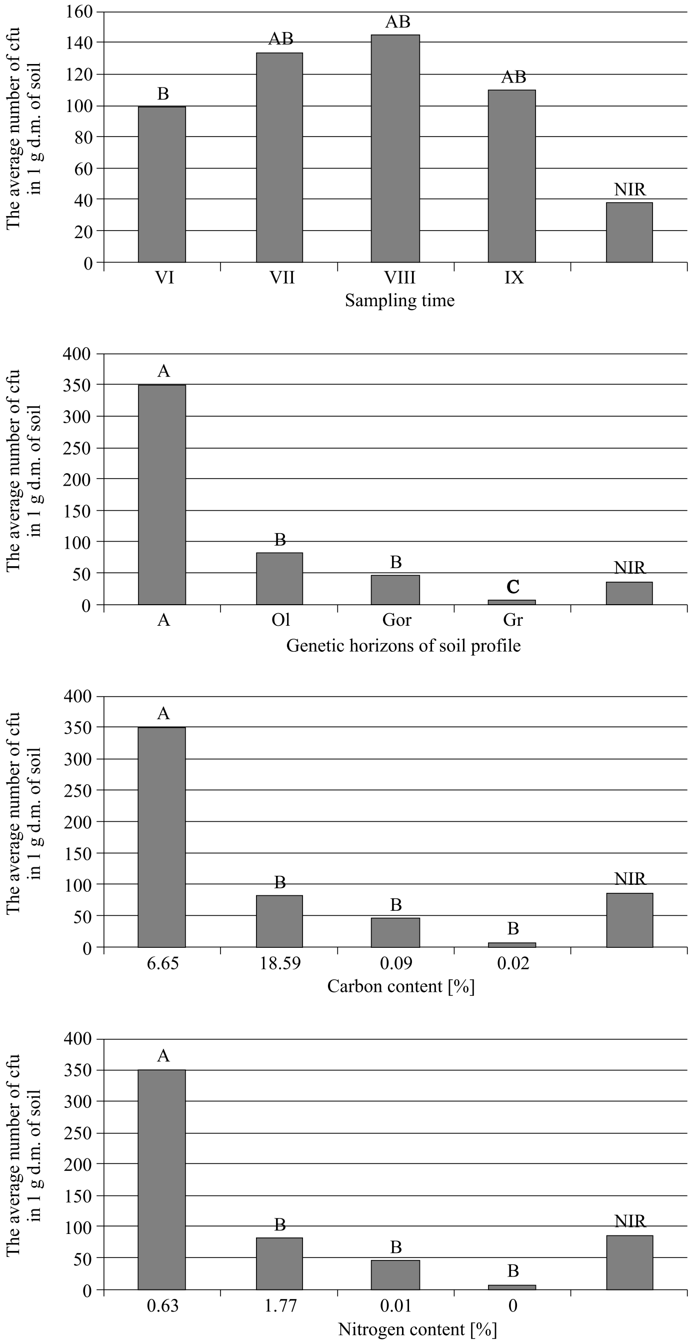

Fig. 4. Results of Tukey's test concerning the influence of abiotic factors on the number of myxobacteria in gley soil.

Krzemieniewski [18, 20] and Reichenbach [3].

The number of myxobacteria in gley soil, similar to muck soil, increased from June reaching maximum in August. With early autumn in September there was observed the lower number of myxobacteria than in summer, what is probably connected with cooling and intensification of atmospheric falls causing high humidity of soil.

Similar to muck soil results of two-factor analysis of variance showed a significant influence of season and depth of soil profile on the number of myxobacteria in gley soil. Furthermore one-factor analysis of variance showed a significant influence of carbon and nitrogen content on the number of myxobacteria in examined soil.

Results of Tukey's test showed a statistically significant differences of the averages of the number of myxobacteria depending on season. There were identified two homogenous groups with the highest average of the number of myxobacteria in August and the lowest average in June.

There were also observed a significant differences of the number of myxobacteria depending of depth of soil profile. Tukey's test identified three homogenous groups with the highest average of the number of myxobacteria in A horizon and the lowest average in Gr horizon.

Taking into consideration the influence of carbon and nitrogen content in examined soils there were identified two homogenous groups. The influence of these factors on the number of myxobacteria were not so significant as in muck soil.

The results showed that dominating myxobacteria were *Sorangium cellulosum* and *Polyangium compositum*. There were also commonly found *Myxococcus fulvus*, *Corallococcus coralloides* and *Angiococcus disciformis* mainly isolated from Ol and A horizons. Obtained results confirm data published by Krzemieniewska and Krzemieniewski [18, 20] and Dawid [2].

Summarizing muck soil is more favorable environment of living of myxobacteria than gley soil. This fact is the result of higher reaction of upper horizons of muck soil, higher content of carbon, insignificant higher content of nitrogen and moderate humidity in comparison with gley soil.

Conclusions

1. Myxobacteria are strictly aerobic microorganisms occurring in surface layers of examined soils.

2. The number and species composition of myxobacteria are strictly dependent on type of soil, physicochemical properties of soil and atmospheric factors.

3. Dominating myxobacteria were representatives of cellulolytic species which play important role in carbon cycle.

References

[1] Dworkin M. and Kaiser D.: Myxobacteria, 2ed., Am. Soc. Microbiol. Press, Washington DC 1993.

[2] Dawid W.: *Biology and global distribution of myxobacteria in soils*, FEMS Microbiol. Rev. 2000, 24, 403–427.

[3] Reichenbach H.: *The ecology of the myxobacteria*, Environ. Microbiol. 1999, **1**, 15–21.
[4] Bull C.T., Shetty K.G. and Subbarao K.V.: *Interactions between myxobacteria, plant pathogenic fungi, and biocontrol agents*, Plant Dis. 2002, **86**, 889–896.
[5] Reichenbach H. and Dworkin M.: *The myxobacteria*. [In:] The prokaryotes (Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.H., Eds.), Springer-Verlag, New York 1992, 3416–3487.
[6] Kaiser D.: *Multicellular development in myxobacteria*. [In:] Genetics of bacterial diversity, (Hopwood A., Chater K.F., Eds.), Acad. Press, London 1989, 243–263.
[7] Reichenbach H.: *The myxobacteria: common organisms with uncommon behavior*, Microbiol. Sci. 1986, **3**, 268–274.
[8] Shimkets L. J.: *Social and developmental biology of the Myxobacteria*, Microbiol. Rev. 1990, **54**, 473–501.
[11] Dworkin M.: *Recent advantages in the Social and Developmental Biology of the Myxobacteria*, Microbiol. Rev. 1996, **60**, 79.
[9] Drews G.: Mikrobiologisches Praktikum, 2ed., Springer-Verl., Berlin 1974.
[10] Dworkin M.: Developmental biology of the bacteria, Benjamin/Cummings Publ. Co., Reading M.A., California 1985.
[12] Burchard R.P.: *Gliding motility of bacteria*, Bio Sci. 1980, **30**, 157–162.
[13] Burchard R.P.: *Gliding motility of prokaryotes: ultrastructure, physiology and genetics*, Ann. Rev. Microbiol. 1981, **35**, 497–529.
[14] Reichenbach H. and Höfle G.: *Biologically active secondary metabolites from Myxobacteria*, Biotechnol. Adv. 1993, **11**, 219–277.
[15] Gerth T., Trowitzsch W., Piehl G., Schulze R. and Lehmann J.: *Inexpensive media for mass cultivation of myxobacteria*, Appl. Microbiol. Biotechnol. 1984, **19**, 23–28.
[16] Rice S.A. and Lampson B.C.: *Phylogenetic comparison of retron elements among the Myxobacteria: Evidence for vertical inheritance*, J. Bacteriol. 1995, **177**, 37–45.
[17] McCurdy H. D.: *Order Myxococcales*. [In:] Bergey's Manual of Systematic Bacteriology (Staley J.T., Bryant M.P., Pfennig N, Holt J.G., Eds.), Williams and Wilkins, Baltimore, MD, 1989, 3, 2139-2170.
[18] Krzemieniewska H. and Krzemieniewski S.: *Rozsiedlenie miksobakteryj*, Acta Soc. Bot. Polon. 1927, **5**, 104–128.
[19] Górny M.: Zooecology of forest soils, PWRiL, Warsaw 1975.
[20] Krzemieniewska H. and Krzemieniewski S.: *Miksobakterje Polski. Uzupełnienie*, Acta Soc. Bot. Polon. 1927a, **5**, 79–98.

WPŁYW CZYNNIKÓW ABIOTYCZNYCH NA WYSTĘPOWANIE MYKSOBAKTERII W WYBRANYCH GLEBACH LEŚNYCH PUSZCZY BIAŁEJ

Samodzielny Zakład Biologii Mikroorganizmów, Wydział Rolnictwa Biologii
Szkoła Główna Gospodarstwa Wiejskiego

Abstrakt: Celem pracy było określenie wpływu czynników fizykochemicznych, tj. pH, zawartości węgla i azotu oraz terminu pobrania próbek i głębokości profilu glebowego wybranych gleb leśnych Puszczy Białej (murszowa, gruntowo-glejowa) na liczebność oraz skład gatunkowy myksobakterii. Liczebność myksobakterii określono za pomocą metody płytkowej. Wyizolowane i oczyszczone szczepy zidentyfikowano na podstawie charakterystyki makro- i mikromorfologicznej z wykorzystaniem mikroskopii stereoskopowej, świetlnej i skaningowej mikroskopii elektronowej.

Wyniki analiz ilościowych wykazały, że myksobakterie najliczniej zasiedlają powierzchniowe poziomy genetyczne badanych profilów glebowych. Większe pH, większa zawartość węgla oraz umiarkowana wilgotność gleby murszowej wpływają znacząco na większą liczebność oraz większą różnorodność gatunkową myksobakterii w porównaniu z glebą gruntowo-glejową. W badanych glebach dominowały myksobakterie celulolityczne z gatunku *Sorangium cellulosum* i *Polyangium compositum*.

Słowa kluczowe: myksobakterie, bakterie śluzowe, mikroflora gleby, Puszcza Biała

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Aleksandra NADGÓRSKA-SOCHA[1] and Ryszard CIEPAŁ[1]

PHYTOEXTRACTION OF ZINC, LEAD AND CADMIUM WITH *Silene vulgaris* MOENCH (GARCKE) IN THE POSTINDUSTRIAL AREA

FITOEKSTRAKCJA CYNKU, OŁOWIU I KADMU PRZEZ *Silene vulgaris* MOENCH (GARCKE) NA TERENACH POPRZEMYSŁOWYCH

Abstract: For the purpose of our study we have selected the perennial herb *Silene vulgaris* (*Caryophyllaceae*) which is characteristic for many metal-enriched soils in Europe to measure the *in situ* phytoextraction of zinc, lead and cadmium in the postindustrial area. *Silene vulgaris* belongs to the metalollophytes. The plant material and the soil samples (from upper layer) were collected from the vicinity of the non-ferrous metal smelter Szopienice at the distances of 50, 250, 450 m from zinc waste heap in Katowice and from the former calamine site in Dabrowa Gornicza in September 2003–2005. The soil located near the smelter Szopienice was the most polluted area and the site exhibited differences in the heavy metal concentration (metals extracted with 10 % HNO_3) in the upper layer (66640–6455 mg/kg Zn, 119–39 mg/kg Cd, 1280–1011 mg/kg Pb). The heavy metal bioavailability was low and connected with high pH values (6.7–7.8). Phytoextraction was calculated from the biomass and its concentration of metal. It was at a maximum at the distance of 450 m from the smelter (1119 g/ha Zn, 11 g/ha Pb and 6 g/ha Cd) for all the investigated metals. Only Zn amount accumulated in the aboveground plant parts seems to be promising for phytoextraction.

Keywords: heavy metals phytoextraction, *Silene vulgaris*, metallophytes

Phytoextraction is the use of plants to remove toxic elements from contaminated environments [1]. Some plant species were reported to accumulate metals from the soil in their aboveground biomass. The ideal plant to use in phytoextraction should have the ability to accumulate the metals intended to be extracted, preferably in aboveground parts, but above all it has to tolerate very high metal concentrations in soils [1–3]. The efficacy of phytoextraction is related to the ability of hyperaccumulating plants to grow and develop their root systems and to take up and accumulate the available metals in the upper parts. The aerial parts would be dried and burnt to ashes. Unfortunately very often the production of biomass by the hyperaccumulators is slow and the root systems, with the exception of *Silene vulgaris*, small [4]. *Silene vulgaris* Moench (Garcke) (*Caryophyllaceae*) is a perennial herb, which is common in many metal-enriched soils in Europe. It belongs to metallophytes, which are tolerant of high heavy metals concen-

[1] Depatment of Ecology, The University of Silesia, Bankowa 9, 40-007 Katowice, Poland, phone 32 359 11 18, email: olan@hoga.pl

tration and have high heavy metal accumulation ability [4–9]. This plant is widespread in the Upper Silesian Industrial Region on the old calamine heaps, in the smelter surroundings or the metallurgical waste dumps [7–11]. Such sites are characterized by the continuous and natural phytoextraction of metals, except for the fact that plants are not harvested. The absence of agricultural management (eg fertilization) is characteristic of these sites [1]. The aim of this work was to determine the *in situ* phytoextraction of zinc, cadmium and lead with metallophyte – *Silene vulgaris* Moench (Garcke) growing in the postindustrial areas: in the nearest vicinity of non-ferrous plant, on the zinc smelter spoil heap in Katowice and in the former calamine site in Dabrowa Gornicza. These sites exhibited varied heavy metal contamination level.

Material and methods

The investigation was carried out in September in 2003–2005. The plants of *Silene vulgaris*, Moench (Garcke) were collected in the nearest vicinity of nonferrous plant Szopienice at the distances of 50, 250, 450 m, on the zinc smelter spoil heap in Katowice-Welnowiec and from the former calamine site in Dabrowa Gornicza. In each place the aerial plant parts from 20 individuals from an area of 40 m^2 of 2 m in dia-meter were collected. In order to determine the heavy metal concentration, the plant material (aerial parts) was washed in tap and in distilled water, dried at 105 °C to a constant weight and ground to fine powder, then mineralized and dissolved in 10 % HNO_3. After filtration Zn, Pb and Cd contents were determined using flame Atomic Absorption Spectrometry (AAS) [12]. The quality of the analytical procedure was controlled by using the samples of the reference material in each series of analysis. (Certified Reference material CTA-OTL-1 Oriental Tobacco Leaves). The phytoextraction was calculated from the biomass and its concentration of metal. The soil samples from the 0–10 cm layer were collected in the same investigated places. Heavy metal contents were estimated according to the method of Bowman et al [13] and Ostrowska et al [11], in the air dried soil samples, which were sieved. Heavy metals were extracted using 0.01 M $CaCl_2$ (bioavailable fraction) and 10 % HNO_3. The analysis was conducted using the atomic absorption spectrometry. Soil pH was measured in water (1:2.5 soil:water ratio) using a pH meter, and organic matter content [%] was estimated by the Ostrowska's methods [12]. All plants and soil samples were determined in six replications. The data was processed using the software Statistica to compute significant statistical differences between samples ($p < 0.05$) according to Tukey's multiple range test.

Results and discussion

The previous researches showed a high decrease in the soil enzymes activity at the distance of 250 m from the smelter, where the highest heavy metals bioavailability was found. The reduction of enzymatic soil microorganism activity can cause inhibition of organic matter decomposition and the reduction of most biochemical processes in the soil. This effect leads to soil degradation [14]. Tables 1 and 2 present heavy metals content in the soil upper layer in the investigated area. The highest heavy metals bioavailability was noted in the soil collected in the distance of 250 m from the smelter. The chemical properties of the soils in the investigated areas are shown in Table 3. The low heavy metals bioavailability can be connected with relatively high pH values, which were within the range 6.7–7.8. The highest Cd (79 mg/kg dm) and Pb (109.7 mg/kg dm) Zn (8316 mg/kg dm) accumulation was noted in aerial plant parts collected at the distance of 250 m. The amount of Zn was almost 4.5 times higher than the content of this metal in the plants in the other areas (Tab. 4). All of the investigated metals were above or within the range (Cd) of toxic level similarly to previous study [7, 15, 16]. In pot experiment in EDTA presence in acid soil Zn, Cd and Pb (in Szopienice plant) were accumulated in higher amounts in the shoots. The higher translocation of Zn and Cd to shoots of *Silene vulgaris* was observed [16]

Table 1

Mean heavy metal contents in soil (HNO_3 extracted) in the investigated areas
Values with the same letter are statistically the same for $p < 0.05$.

Investigated area	Mean heavy metal contents in the soil (HNO_3 extracted) [14]					
	Cd	SD	Pb	SD	Zn	SD
50 m	119.3a	55.1	1280.3a	558.6	66638.4a	19184.8
250 m	40.5b	9.8	1011.1a	245.1	8513.7b	625.8
450 m	38.9c	11.8	1157.2a	115.9	6454.6b	1554.1
Heap	101.9ab	56.7	1404.2b	388.8	45732.2c	3377.1
C	54.7c	4.3	1139.03a	135.1	24578.9d	1965.6

Table 2

Mean heavy metal contents in soil ($CaCl_2$ extracted) in the investigated areas
Values with the same letter are statistically the same for $p < 0.05$.

Investigated area	Mean heavy metal contents in soil ($CaCl_2$ extracted)					
	Cd	SD	Pb	SD	Zn	SD
50 m	12.2a	1.9	43.8a	20.9	296.1a	22
250 m	13.2a	1.1	92.3a	37.8	431.9b	43.8
450 m	9.6b	0.6	41.3a	18.6	321.9a	84.6
Heap	6.3c	1.9	35.8a	17.9	211a	44.6
C	2.3d	0.8	53a	10.6	35.5c	20.1

Table 3

Soil properties (organic matter contents [%] and pH values); average ± SD
Values with the same letter are statistically the same for $p < 0.05$.

Mean organic mater contents [%] and pH values in soil				
Investigated area	Mean organic matter content	SD	Mean pH value	SD
50 m	4.4a	0.8	6.91a	0.02
250 m	2.7b	0.9	6.89a	0.14
450 m	4.6a	1.03	6.65a	0.11
Heap	8.1c	0.5	6.71a	0.19
C	9.7b	0.7	7.77b	0.11

Table 4

Heavy metal bioaccumulation in *Silene vulgaris* aerial parts in the investigated area
Values with the same letter are statistically the same for $p < 0.05$.

Investigated area	Mean heavy metal content in aerial plant parts [mg/kg d.m.]					
	Cd	SD	Pb	SD	Zn	SD
50 m	87	3.8	261.7	3.9	7019	90.8
250 m	78.8	1.2	109.7	4.7	8316	111.3
450 m	30.6	1.9	59	8.5	5843	375
Heap	6.7	0.5	69.8	5.2	6733.6	118
C	13.4	0.5	34.6	1.9	1875.8	204.6

The plant populations differed at various distances from the emitor. The highest biomass of *Silene vulgaris* aboveground parts was noted for the plants collected at the distance of 450 m from the smelter (194 kg d.m./ha Fig. 1). Lower biomass of the investigated plants in the calamine site was caused most likely by the competition between other plant species. Phytoextraction demands a reasonable aboveground, thus harvestable biomass. Most metal-hyperaccumulators have a low biomass production of less than 4 Mg per ha and per year [3]. Ernst pointed out [4] that only a few plant species have evolved metal specific ecotypes at different metal enriched soils and among these plants is *Silene vulgaris*. Various metals in polymetalic soils strongly affect the productivity of even the metal resistant plants. Metallophyte – *Silene vulgaris* biomass is also too low (Fig. 1). The obtained biomass results were lower than the biomass estimated for *Thlaspi caerulescens*

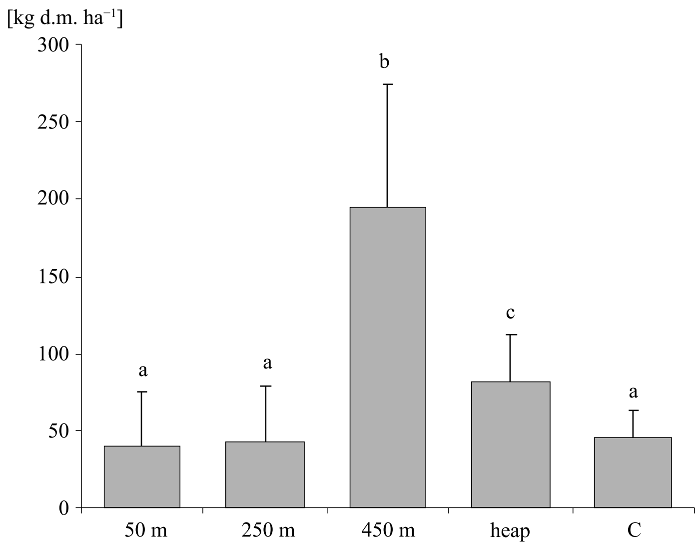

Fig. 1. *Silene vulgaris* biomass on the investigated stands

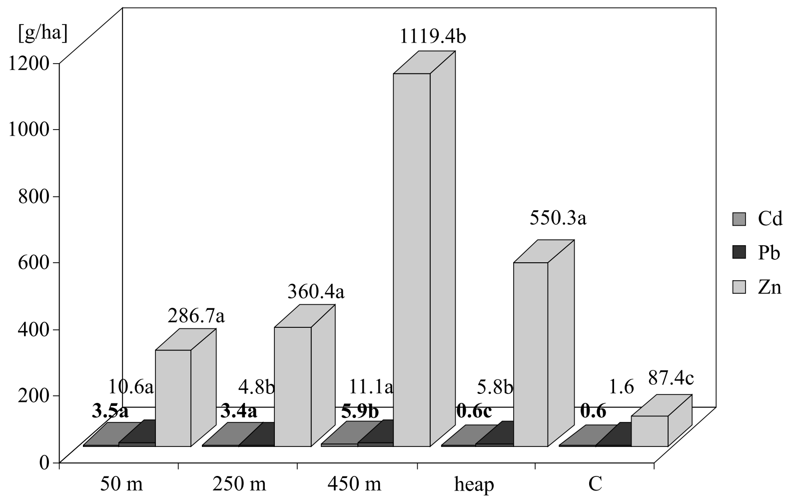

Fig. 2. Phytoextraction of Zn, Pb, Cd with *Silene vulgaris*

0.6–0.9 Mg/ha by Keller [18]. The plants collected at the distance of 450 m from the emitor exhibited the highest Zn, Cd and Pb *in situ* phytoextraction (1119 g/ha Zn, 11 g/ha Pb and 6 g/ha Cd, Fig. 2). Van Nevel pointed out that phytoextraction efficiency will decline under the increasing soil metal concentrations [19]. The quantity of Zn extracted by plants was 3 times higher than in the plants collected at the distance 250 m, where the highest heavy metals bioavailability and highest Zn accumulation in the plant aboveground parts were found and almost 13 times higher than the Zn extracted by *Silene vulgaris* on the calamine site. In Schwartz et al investigations [1] phytoextraction values ranging from 1.5 to 10 kg Zn ha^{-1} were recorded, involving the hyperaccumulator *A. halleri*. The examined *Silene vulgaris* does not have the ability to phytoextract Cd and Pb with high efficiency. Compared to *Rumex crispus* (high biomass plant) extracted 0.16 Cd kg ha^{-1} and below 1.6 Pb kg ha^{-1} and investigators pointed out that the plant used for the field experiment did not prove to have ability to phytoextract Pb with high efficiency [20]. Comparable amount of Zn extracted by *Silene vulgaris* indicated that it could participate only in this metal phytoextraction. On the other hand especially in extremely polluted sites the use of local vegetation may give an opportunity to create a soil cover. Polluted site related species have already proven its ability to survive under pollution stress. It is also recommended to use indigenous species for phytoextraction, as they are considerably cheaper than exotic species and do not create adaptation problems [21–22].

Conclusions

The heavy metals phytoextraction with *Silene vulgaris* was at a maximum at the distance of 450 m from the smelter (1119 g/ha Zn, 11 g/ha Pb and 6 g/ha Cd) for all investigated metals. Only Zn amount accumulated in the aboveground plant parts seems to be promising for phytoextraction. *Silene vulgaris* vegetation may give an opportunity to create a soil cover of the polluted site.

References

[1] Schwartz Ch., Gerard E., Perronet K. and Morel J.: *Measurment of in situ phytoextraction of zinc by spontaneous metallophytes growing on a former smelter site*. Sci. Total Environ., 2001, **279**, 215–221.

[2] Marchiol L., Asslari S., Sacco P. and Zerbi G.: *Phytoextraction of heavy metals by canola (Brassica napus) and radish (Raphanus sativus grown on multicontaminated soil*. Environ. Pollut., 2004, **132**, 21–27.

[3] Ernst W.: *Phytoextraction of mine wastes – Options and Impossibilities*. Chem. Erde, 2005, **65**(S1), 29–42.

[4] Ernst W.: *Bioavailability of heavy metals and decontamination of soils by plants*. Appl. Geochem., 1996, **11**, 163–167.

[5] Wierzbicka M. and Panufnik D.: *The adaptation of Silene vulgaris to growth on calamine waste heap (S. Poland)*, Environ. Pollut., 1998, **101**, 415–426.

[6] Wierzbicka M. and Rostański A.: *Microevolutionary changes in ecotypes of calamine waste heap near Olkusz, Poland: A Review*, Acta Biol. Cracov., Ser. Bot., 2002, **44**, 7–19.

[7] Heflik M., Nadgórska-Socha A. and Ciepał R.: *Heavy metals accumulation and its effects on Silene vulgaris plants grown in metal contaminated sites*, Ecol. Chem. Eng., 2006, **13**(7), 657–663.

[8] Rostański A.: *Zawartość metali ciężkich w glebie i roślinach z otoczenia niektórych emitorów zanieczyszczeń na Górnym Śląsku*. Arch. Ochr. Środow., 1997, **23**(3–4), 181–189.

[9] Koszelnik-Leszek A.: *Budowa blaszki liściowej oraz zawartość chromu, niklu i cynku w Silene vulgaris (Moench) Garcke i w glebie na hałdzie odpadów serpentynitowych w Wirkach (Dolny Śląsk)*. Zesz. Probl. Post. Nauk Roln., 2007, **520**, 227–234.

[10] Tokarska-Guzik B., Rostański A. and Klotz S.: *Roślinność hałdy pocynkowej w Katowicach Wełnowcu*. Acta Biol. Siles., Florystyka: geografia roślin. 1991, **19**(36), 94–101.

[11] Gucwa-Przepióra E. and Turnau K.: *Arbuscular Mycorrhiza and Plant Succession on Zinc Smelter Spoil Heap in Katowice-Wełnowiec*. Acta Soc. Bot. Polon., 2001, **70**, 153–158.

[12] Ostrowska A., Gawliński S. and Szczubiałka Z.: *Metody analizy i oceny właściwości gleb i roślin*. Instytut Ochrony Środowiska. Warszawa 1991, 334–340.

[13] Bouwman L., Bloem J., Römkens P., Boon G. and Vangronsveld J.: *Beneficial effects of the growth of metal tolerant grass on biological and chemical parameters in copper- and zinc contaminated sandy soils*. Minerva Biotec., 2001, **13**, 19–26.

[14] Nadgórska-Socha A., Łukasik I., Ciepał R. and Pomierny S.: *The activity of selected enzymes in soil loaded with varied heavy metals level*. Acta Agrophys., 2006, **8**(3), 713–726.

[15] Heflik M., Kandziora M., Nadgórska-Socha A. and Ciepał R.: *Aktywność kwaśnych fosfataz u roślin występujących na terenach o podwyższonej zawartości metali ciężkich*, Ochr. Środow. Zasob. Natural., 2007, **32**, 151–154.

[16] Kandziora M., Heflik M., Nadgórska-Socha A. and Ciepał R.: *Synteza związków bogatych w grupy -SH jako odpowiedź na podwyższone stężenie metali ciężkich u roślin Silene vulgaris (Caryophyllaceae)*. Ochr. Środow. Zasob. Natural., 2007, **33**, 69–72.

[17] Nadgórska-Socha A., Łukasik I., Ciepał R. and Falis K.: *Wpływ EDTA na akumulację Cd, Zn, Pb przez Silene vulgaris (Moench) Garcke*. Zesz. Probl. Post. Nauk Roln., 2006, **509**, 197–208.

[18] Keller C.: *Alternatives for Phytoextraction: Biomass Plants versus Hyperaccumulators*. Geophysical Research Abstracts, 2005, **7**, 03285.

[19] Van Nevel L. Mertens J. Oorts K. and Verheyen K.: *Phytoextraction of metals from soils: How far from practice?* Environ. Pollut., 2007, **150**, 34–40.

[20] Zuang P., Yang Q.W., Wang H.B. and Shu W.S.: *Phytoextraction of Heavy Metals by Eight Plant Species In the Field*. Water Air Soil Pollut., 2007, **184**, 235–242.

[21] Sas-Nowosielska A, Kucharski R, Pogrzeba M. and Malkowski E.: *Soil remediation scenarios for heavy metal contaminated soil*. [in:] Soil Chemical Pollution, Risk Asssment, Remediation and Security. Ed.: L. Simeonov, V. Sargsyan, Springer, 2007, 113–319.

[22] Sas-Nowosielska A., Kucharski R. and Malkowski E.: *Feasibility Studiem for Phytoremediation of Metal-Contaminated Soil*. [in:] Soil Biology. Manual for Soil Analysis Vol. 5, Ed.: Ł.R. Margensin, F. Schinner, Springer-Verlag, Berlin 2005, 163–179.

FITOEKSTRAKCJA CYNKU, OŁOWIU I KADMU PRZEZ *Silene vulgaris* MOENCH (GARCKE) NA TERENACH POPRZEMYSŁOWYCH

Katedra Ekologii, Uniwersytet Śląski

Abstrakt: Do badań wybrano rośliny zielne *Silene vulgaris*, które są charakterystyczne dla gleb o zwiększonym stężeniu metali ciężkich w Europie. Mierzono fitoekstrakcję cynku, ołowiu i kadmu na terenach poprzemysłowych. Roślina należy także do metalofitów. Materiał roślinny i próbki gleby pobierano we wrześniu 2002–2005 w najbliższych sąsiedztwie Huty Metali Nieżelaznych „Szopienice", na hałdzie pocynkowej w Katowicach i terenu po eksploatacji galmanu w Dąbrowie Górniczej. Zanotowano największe zanieczyszczenie gleby (metale ekstrahowano 2M HNO_3) z najbliższego sąsiedztwa HMN „Szopienice", gdzie wykazano również różnice w zawartości metali ciężkich w górnym poziomie gleby (66640–14000 mg/kg Zn, 119–39 mg/kg Cd, 1280–1100 mg/kg Pb). Biodostępność metali ciężkich była mała i związana z dość dużymi wartościami pH gleby (6,8–7,8). Fitoekstrakcję oznaczono na podstawie biomasy i koncentracji w niej metali ciężkich. Największą fitoekstrakcje zanotowano dla roślin w odległości 450 m od emitora (1119 g/ha Zn, 10 g/ha Pb i 6 g/ha Cd) dla wszystkich badanych metali. Tylko ilość Zn akumulowana w nadziemnych częściach roślin wydaje się być obiecująca dla procesu fitoekstrakcji.

Słowa kluczowe: metale ciężkie, fitoekstrakcja, *Silene vulgaris*, metalofity

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Paweł NICIA[1], Paweł ZADROŻNY[1] and Tomasz LAMORSKI[2]

GENERAL CHARACTERISTICS OF SELECTED SOIL PROFILES UNDER THE *Caltho-Alnetum* ASSOCIATION IN THE BABIOGORSKI NATIONAL PARK

OGÓLNA CHARAKTERYSTYKA WYBRANYCH PROFILI GLEB POD ZBIOROWISKIEM OLSZYNY GÓRSKIEJ (*Caltho-Alnetum*) W BABIOGÓRSKIM PARKU NARODOWYM

Abstract: *Caltho-Alnetum* associations belong to priority biotopes listed in the annex to 1st Habitats Directive of the European Union. Most of these habitats in the area of present Babiogorski National Park were reclaimed in the seventies. The present research compared the properties of soils and waters in the *Caltho-Alnetum* habitats, which were drained in the seventies with the analogous properties of habitats which were transformed by the anthropogenic factors only to a small degree. The studies revealed a significant effect of reclamation works conducted in the seventies on such parameters as organic carbon and mineral contents, degree of organic matter decomposition, degree of moorshing and groundwater level.

Keywords: *Caltho-Alnetum,* fens, organic soils, swamps

Hydrogenous habitats among which *Caltho-Alnetum* association may be counted fulfill important ecological functions, especially in the mountain areas. Thanks to conditions in the natural *Caltho-Alnetum* association with respect to the floristic composition, they belong to the richest plant associations of the Babiogorski National Park [1, 2].

A high groundwater level in the soils of these habitats, specific properties of waters and soils create favourable habitat conditions for the development of numerous stenotypic animal and plant species, among which many are rare and protected ones. However, the state of dynamic balance which occurs in these habitats may be easily disturbed by eg lowering of the groundwater level. Such a situation occurred on a majority of *Caltho-Alnetum* patches in the area of the Babiogorski National Park, which prior to their putting under protection as a national park, were reclaimed in the seventies in order to increase timber production in these areas.

[1] Department of Soil Science and Soil Protection, University of Agriculture in Krakow, Al. A. Mickiewicza 21, 31-120 Kraków, Poland, phone: 12 662 43 70, email: rrnicia@cyf-kr.edu.pl

[2] Babiogorski National Park, 34-223 Zawoja Barańcowa, 1403, Poland, phone: 33 877 51 10

According to the Decree of the Minister of the Natural Environment dated 16 May 2005 on the types of natural habitats, plant and animal species which require protection in the frame of designed Nature 2000 areas, *Caltho-Alnetum* associations should be put under protection and the degraded habitats should be renaturized.

Before the proper renaturization measures could be taken a detailed inventory must be conducted in these habitats. It should comprise identification and comparison of the chemical and physical properties of soils and waters, as well as species composition of the vegetation covering *Caltho-Alnetum* which was not drained, with the analogous properties of the habitats, which were reclaimed in the seventies.

The conducted research aimed at determining the influence of the land reclamation conducted in *Caltho-Alnetum* associations in the area of the Babiogorski National Park on the essential properties of these habitats.

Materials and methods

Seven habitats under the *Caltho-Alnetum* association were selected in the area of the Babiogorski National Park. One soil pit, located in the central part was made on each of the selected habitats. Soil samples were collected from individual genetic horizons appointed for the soil profile studies. From among habitats selected for the analyses, five were in the decession stage (profiles 2–5), whereas two (profiles 1–2) were in the organic matter accumulation stage.

In the collected soil material, mineral content, organic carbon and nitrogen concentrations were assessed using methods suggested by Sapek and Sapek [3], pH with the potentiometric method in H_2O and 1 mol · dm^{-3} KCl solution. The groundwater level was measured in the soils of biotopes chosen for the research. The degree of peat decomposition was determined during the soil sampling on a ten degree von Post scale [4].

Results and discussions

Due to its specific habitat conditions, the *Caltho-Alnetum* association occurs rarely and usually covers small areas therefore it was considered a priority in the Nature 2000 network (*Poradnik ochrony siedlisk i gatunków Natura 2000. Bory i Lasy. Tom. 5 2004*). The habitats selected for the analyses may be divided into two groups. The first is constituted of habitats only slightly transformed by human activity (profiles 1 and 2) (Table 1). The other group comprises the habitats where water-air relationships were disturbed by the reclamation works conducted in the seventies (profiles 3–7) (Table 1).

The occurrence of the *Caltho-Alnetum* association is strictly connected with the presence of water in their soil profiles. On the basis of their hydrological feeding the analyzed habitats from the area of the Babiogorski National Park may be counted among soligenic fens fed by the underground waters flowing out of the aquifer outlets. The soils of the swampy *Caltho-Alnetum* association with natural water-air relationships (profiles 1, 2) were characterized by a peat-forming process of organic matter accumulation occurring in the surface horizons under conditions of high moisture. A characteristic feature of these soils was a high groundwater level, fluctuating about 3–5 cm from the soil surface. The rate of organic matter accumulation in habitats of this type is very slow. A low rate of organic matter accumulation should be associated with high level of organic matter decomposition caused by a considerable oxygenation of waters feeding fens of this type. Nicia and Miechowka [5] found similar relationships between the oxygenation and a high degree of organic matter decomposition in the soils of low sedge mountain fens.

Table 1

Selected properties of analyzed soils

Profile	Fen name	Sample No.	Horizon	C [g · kg^{-1}]	Decomposition degree	pH	
						H_2O	KCl
Fens at accumulation stage							
1	Mokry Kozub	POtni	0–10	310.5	H_9	6.4	6.0
		Otni	10–19	183.8	H_8	5.2	4.8
		D	19–45	40.5	–	5.4	4.3
2	Pod Orawską Drogą	POtni	0–15	281.7	H_9	6.3	5.9
		Otni	15–30	128.6	H_8	6.0	5.6
		Otni	30–65	161.9	H_8	5.5	5.3
		D	< 65	87.0	–	6.2	5.7
Fens at decession stage							
3	Olszynka Markowa 1	Mtni	0–20	251.7	–	5.7	5.2
		Aa	20–35	103.6	H_8	5.8	5.1
		Aa	35–55	101.4	–	5.8	5.0
		D	55–80	33.6	–	6.0	5.1
4	Mokry Kozub 2	Mtni	0–16	233.5	–	4.6	3.9
		Otni	16–36	181.9	H_8	5.9	5.2
		Otni	36–55	260.7	H_8	5.9	5.6
		Otni	55–75	220.1	H_8	5.9	5.2
5	Cyrhlańska droga	Mtni	0–7	160.6	–	4.4	3.7
		Aa	7–18	114.0	–	5.0	4.1
		D	> 18	4.5	–	5.7	4.3
6	Olszynka Markowa 2	Mtni	0–25	100.6	–	5.2	4.4
		Aa	25–40	71.0	–	6.4	5.7
		D	< 40	16.8	–	6.6	5.1
7	Rybna rampa	Aa	0–15	33.5	–	5.8	4.4
		Agg	15–45	23.0	–	5.7	4.6
		ADgg	> 45	8.0	–	6.0	4.5

Most of the discussed *Caltho-Alnetum* associations from the area of the present Babiogorski National Park were drained in the seventies in order to increase forest productivity. The reclamation was realized by means of drainage ditches crossing the fen surface. A similar drainage system was used also on the second group of fens with transformed water-air relationships (profiles 3–7), (Table 1). The depth of drainage ditches in these habitats was diverse and ranged from 0.3 to 0.9 m depending on the thickness of accumulated organic matter and the depth on which the mineral substratum, from which the discussed soil developed, was found. Depending on the surface of the drained fens and the physiographic conditions of the terrain on which they were dug, the spacing between the drainage ditches was between several and several dozens of meters. A dense and relatively deep network of the ditches draining water from the fens led to a decline of their groundwater level from 0.2 to about 0.75 m. Because the discussed habitats occur on slope locations, considerable velocity of water flow drained by the drainage ditches did not allow for their overgrowing and silting. Even after several dozen years these ditches drain considerable amounts of water, particularly during heavy rain, contributing to further degradation of these habitats.

Lowering the groundwater level caused inhibition of organic matter accumulation to the decession stage (profiles 3–7). The consequence of the groundwater level lowering in these profile was a change of pedogenic process direction. The moorshing process was in progress in the surface horizons of 3–7 profiles. The intensity of moorshing process in the soils of the analyzed habitats was diversified within each habitat and depended on the depth of drainage ditches, distance from them, area layout and yield of fen feeding springs. The highest intensity of the moorshing process was detected in each of the drained habitats, in places situated closest to the deepest drainage ditches, on the slopes with greatest inclination, fed with underground waters outflow of small capacity. Lower contents of organic carbon were determined in the soils of the *Caltho-Alnetum* associations which were involved in the moorshing process, in comparison with the soil at the stage of organic matter accumulation. It may be explained by an increased oxygenation of soil profiles caused by a lowering of the groundwater level, which stimulated the process of organic matter mineralization.

Fen soils which were drained revealed lower pH values in their surface horizons in comparison with fen soils in which the peat-forming process of organic matter accumulation was occurring under conditions of high moisture (Table 1). Lower pH values in the surface horizons of drained fens may be explained by a lowering of the groundwater level containing Ca^{2+} and Mg^{2+} ions, which reduced the possibility of neutralizing acid products of organic matter decomposition.

A change of essential chemical and physical properties of soils and waters of the *Caltho-Alnetum* associations caused by lowering the groundwater level influenced a change of habitat conditions leading to their degradation. A change of habitat conditions may threaten the occurrence in these habitats of many stenotypic animal and plant species, which are characterized by a very narrow tolerance range.

One of the measures for reducing degradation of *Caltho-Alnetum* is raising the groundwater level in the previously drained soil patches under grey alder. As has been demonstrated by the research conducted in the lowland areas, rising the groundwater level may restore the natural direction of plant succession in these areas [6, 7]. This process occurs slowly and should be monitored with respect to the most important properties of soils and waters determining the relationship between the vegetation and the habitat.

Conclusions

1. Reclamation of *Caltho-Alnetum* associations conducted in the seventies caused passing of these habitat soils from the accumulation stage to decession stage, at which the moorshing process occurred.

2. Due to the location of the of reclaimed habitats on slopes, sometimes with several degrees inclination, considerable water velocity drained by the drainage ditches prevented their overgrowing and silting.

3. The intensity of the moorshing process which occurred in the drained habitat soils depended on their distance from drainage ditches, their depth and capacity of underground water outflow feeding the fens.

4. Raising the groundwater level in *Caltho-Alnetum* associations through filling the drainage ditches might limit the degradation of these valuable habitats.

5. Elaborating methods of renaturization of *Caltho-Alnetum* associations requires further, detailed habitat research comprising the properties of soils and waters and their relationship between vegetation and the habitat.

References

[1] Parusel J.: Leśne zbiorowiska roślinne i ich przestrzenne rozmieszczenie na obszarach projektowanych do włączenia w skład Babiogórskiego Parku Narodowego. Praca doktorska. Uniwersytet Śląski, Wydział Biologii i Ochrony Środowiska, Katowice. Maszynopis, 2003, pp. 180.

[2] Parusel. J.B., Kasprowicz M. and Holeksa J.: Zbiorowiska leśne i zaroślowe Babiogórskiego Parku Narodowego. [in:] Babiogórski Park Narodowy. Monografia przyrodnicza, Wyd. Drukarnia Towarzystwa Słowaków w Polsce, Kraków 2004, 429–477.

[3] Sapek A. and Sapek B.: Metody analizy chemicznej gleb organicznych. Wyd. IMUZ, Falenty 1997, pp. 97.

[4] Ilnicki P.: Torfowiska i torf. Wyd. AR Poznań, Poznań 2002, pp. 408.

[5] Nicia P. and Miechówka A.: *General characteristics of eutrophic fen soils*. Polish J. Soil Sci., 2004, **XXXVII**(1), 39–47.

[6] Czerepko J. and Haponiuk-Winiczenko K.: *Zmiany roślinności na zmeliorowanym torfowisku Biele w Puszczy Augustowskiej w latach 1979–2002*. Leśne Prac. Badaw., 2005, 1, 31–42.

[7] Czerepko J., Wróbel M. and Boczoń A.: *Próba określenia reakcji olsu jesionowego na podniesienie poziomu wody w cieku*. Leśne Prac. Badaw., 2006, 4, 7–16.

OGÓLNA CHARAKTERYSTYKA WYBRANYCH PROFILI GLEB POD ZBIOROWISKIEM OLSZYNY GÓRSKIEJ (*Caltho-Alnetum*) W BABIOGÓRSKIM PARKU NARODOWYM

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Babiogórski Park Narodowy

Abstrakt: Siedliska bagiennej olszyny górskiej należą do siedlisk priorytetowych wymienionych w załączniku I Dyrektywy Siedliskowej Unii Europejskiej. Większość tych siedlisk z obecnego terenu Babiogórskiego Parku Narodowego została zmeliorowana w latach siedemdziesiątych. W ramach badań porównano podstawowe właściwości gleb i wód siedlisk bagiennej olszyny górskiej, które w latach siedemdziesiątych zostały odwodnione, z analogicznymi właściwościami siedlisk w małym stopniu przekształconych przez czynniki antropogenne. Badania wykazały duży wpływ melioracji przeprowadzonych w latach siedemdziesiątych na takie parametry gleb, jak zawartość węgla organicznego i popielność, stopień rozkładu materii organicznej, stopień murszenia oraz poziom wód gruntowych.

Słowa kluczowe: olszyna bagienna, młaki, gleby organiczne, mokradła

Vol. 16, No. 7 2009

Elżbieta PISULEWSKA[1], Halina PUCHALSKA
Tomasz ZALESKI[2] and Zbigniew JANECZKO[3]

EFFECT OF ENVIRONMENTAL CONDITIONS ON YIELD AND QUALITY OF NARROW-LEAVED LAVENDER (*Lavandula angustifolia* MILL)

WPŁYW WARUNKÓW ATMOSFERYCZNYCH NA PLON I JAKOŚĆ LAWENDY WĄSKOLISTNEJ (*Lavandula angustifolia* MILL)

Abstract: The objective of this study was to determine the effect of soil and weather conditions during three vegetation seasons (2001, 2003 and 2004) on the yield of the narrow-leaved lavender herb as well as on the content and composition of volatile oils in the flowers of this plant. Field experiments were conducted on three 4-year old plantations of lavender in the 3rd year of cultivation. The site of this study was Krepa near Golcza, located in Miechow Upland. The yields of both the fresh and dry matter of inflorescences and flowers (spikes) of lavender were affected by: weather conditions during the vegetation seasons, size of plants, number of flowering branches and number of flower rings in spikes. At the same time, the weather and soil conditions had no effect on the total content of volatile oils in flowers of lavender. However, the relative content of major terpenes ie linalool, linalyl acetate, geraniol and borneol in lavender oil was altered

Keywords: narrow-leaved lavender, vegetation season, yield of inflorescences, number of flowering branches, volatile oils, luvisol, loess

Narrow-leaved lavender is an evergreen perennial shrub growing originally at high altitudes in the Mediterranean region (eg France, Italy, Spain, North Africa). Large-scale commercial cultivation of lavender was first started in France, Italy and Spain in the 17th century. It was later developed in Bulgaria, Croatia, Hungary and Poland in the 20th century [1].

[1] Department of Plant Production, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, phone: +48 12 662 4385, email: Elzbieta.Pisulewska@ar.krakow.pl

[2] Department of Soil Science and Soil Protection of the University

[3] Department of Pharmacognosy, Jagiellonian University, Kraków, Poland

The environmental needs of lavender are relatively high. The plant grows well on soils rich in nutrients and with pH ranging from neutral to alkaline. It resists dry periods thanks to a well developed root system. On the other hand it is sensitive to large amplitude of day and night temperatures, especially in the beginning of vegetation in spring [2–6].

This paper analyses the responses of lavender plants, grown in Miechow Upland, to varying soil and weather conditions during three vegetation seasons (2001, 2003 and 2004). The first aim was to determine yield of lavender herb (inflorescences and flowers). Second, to determine the content and composition of volatile oils in flowers of the plants.

Material and methods

The site of this study was Krepa near Golcza (50°19′16.8″E 19°56′45.7″N, 360 m a.s.l), located in Miechow Upland. The soil is classified as a Haplic Luvisol [7], derived from loess and consists of 13 % sand, 75 % silt and 12 % clay. The humus horizon this soil characterized by pH 6.5–6.5, C/N ratio – 8.1 and high-level water field capacity (Table 1). Soil samples were taken twice a year in spring (before it was fertilized) and in autumn (after harvest) from two horizons; A – 0–15 cm and B – 15–30 cm. The following soil analyses were carried out: organic carbon content; total nitrogen by the Kjeldahl method [8]; total porosity [9], water field capacity as a soil water potential −15.5 kPa; pH in 1M KCl (1/2.5 v/v); plant-available phosphorous P and K by the Egner–Riehm method, and plant-available Mg by the Schachtschabel method [8]. The basic conditions of the soil are shown in Table 1.

Soil field experiments were conducted on three 4-year old plantations of lavender in the 3rd year of cultivation. The forecrop for each lavender plantation in the first year of cultivation (1998, 2000 and 2001) was wheat grown on manure (30 Mg/ha).

At present there are no registered Polish cultivars of lavender. Therefore, lavender grown on plantations in the Gołcza region was of local origin. Lavender seedlings were produced from locally available seeds and planted in the three permanent sites. Consequently, the lavender plants in the studied plantations were of different origin and varied in shape and size.

Two experimental factors were studied: the vegetation season (years 2001, 2003 and 2004) and the size of lavender plants (S – small plants with the diameter of 45–64 cm; M – medium plants with the diameter of 65–84 cm; L – large plants with the diameter of 85–115 cm). Each year, eighteen plants (6S, 6M and 6L) from each plantation were evaluated to determine: (1) fresh matter of inflorescences (g/plant), (2) number of flowering branches per plant, (3) number of flower rings in spikes, (4) number of flowers in a flower ring, (5) dry matter of inflorescences (g/plant) and (6) dry matter of flowers (g/plant). Lavender inflorescences were collected manually (with a sicle) on the following harvest dates: 15 July 2001, 8 July 2003 and 3 July 2004. These biometrical data were subjected to two-factorial analysis of variance.

Samples of dried lavender flowers were steam-distilled in a steam distillation Deryng apparatus [10] to extract volatile oils, and their content was expressed in g/100 g of the dry material. The extract was further analyzed for individual volatile oils on a gas chromatograph (Pye Unicam) equipped with a capillary column (Supelco). This analytical data were subjected to one-factorial analysis of variance.

Results and discussion

This soil represents typical Haplic Luvisol derived from loess at the Miechow Upland. Soils in this region are characterized by silty texture with a very good physical condition to cultivate plants, especially physical conditions like bulk density and water field capacity (Table 1). The studied soil had a very good water capacity like most of the silty soils from loess. We observed the highest yield of lavender in 2001. Exactly in this year precipitation was the highest throughout the whole experiment and the soil absorbed most of this rainfall and next could supply pants with water for the longer time than in 2003 and 2004. Presumably it was the most important factor that influenced the yield of lavender throughout the experiment.

Table 1

Selected properties of the soil

Horizon symbol	Depth	pH in KCl	% of particle size of the fine soil fraction [µm]			Organic C	Bulk density	Total porosity	Field capacity moisture
	[cm]		2000–63	63–2	< 2	$[g \cdot kg^{-1}]$	$[Mg \cdot m^{-3}]$	$[m^3 \cdot m^{-3}]$	$[m^3 \cdot m^{-3}]$
A	0–30	6.52	13	75	12	17.1	1.44	0.44	0.35
E1	30–40	6.18	13	74	13	4.4	1.47	0.44	0.33
E2	40–65	6.19	13	76	11	2.3	1.36	0.49	0.36
E2/Bt	65–104	6.18	12	79	9	2.6	1.43	0.45	0.37
Bt	104–135	4.67	11	63	16	1.3	1.52	0.41	0.37
C	135–160	4.32	8	76	16	1.4	1.72	0.34	0.33

The humus horizon of the analyzed soil was rich in organic carbon. That is one of the reasons for a high content of plant available P, K and Mg. In most soils the upper horizon of 0–15 cm had a higher content of plant available P, K and Mg (Table 2). A tendency towards an increased content of organic carbon was observed (Table 2). These soils were characterized by very stable pH and small standard deviations throughout the whole experiment (Table 2). An insignificant decrease of plant available P, K and Mg was observed in 2003 and 2004 comparing to 2001, especially in Mg (Table 2). The changes of analyzed soil conditions were not significant, but small tendency increasing and decreasing were observed. It means that soil conditions were similar in each year of this experiment and a lavender had not influence of on soil conditions. It means that weather condition together with correct fertilizer had the most important influence on yield of the lavender.

Table 2

Changes in soil pH, organic carbon, and plant- available potassium, phosphorus and magnesium during years experiment (average concentration, ± standard deviation).
A – horizon 0–15 cm, B – horizon 15–30 cm

Date	pH in KCl		Organic C		K_2O		P_2O_5		MgO	
			[$g \cdot kg^{-1}$]		[$mg \cdot kg^{-1}$]					
	A	B	A	B	A	B	A	B	A	B
2001 spring	6.9 ± 0.3	7.0 ± 0.1	7.7 ± 0.4	7.3 ± 0.9	200.8 ± 12.0	192.8 ± 13.3	259.2 ± 38.9	230.7 ± 13.0	151.8 ± 19.8	150.9 ± 31.0
2001 autumn	6.8 ± 0.1	6.9 ± 0.1	7.8 ± 0.6	7.0 ± 1.0	170.0 ± 20.5	183.3 ± 40.3	222.7 ± 31.9	214.0 ± 48.1	136.5 ± 17.3	122.5 ± 18.1
2003 spring	6.7 ± 0.1	6.9 ± 0.1	8.6 ± 1.0	8.7 ± 0.5	194.4 ± 40.6	146.0 ± 14.3	252.6 ± 34.9	198.1 ± 24.2	67.0 ± 7.2	82.7 ± 17.4
2003 autumn	6.9 ± 0.1	6.8 ± 0.2	8.5 ± 0.4	7.2 ± 0.4	178.5 ± 20.2	130.3 ± 23.0	219.4 ± 21.1	190.6 ± 38.1	114.9 ± 21.2	99.1 ± 15.2
2004 spring	6.8 ± 0.1	6.9 ± 0.1	9.0 ± 1.5	8.7 ± 0.8	194.4 ± 40.6	146.0 ± 14.3	247.4 ± 23.2	178.2 ± 14.1	92.9 ± 24.7	88.9 ± 4.7
2004 autumn	6.8 ± 0.1	6.8 ± 0.1	9.0 ± 0.8	8.5 ± 1.5	178.5 ± 20.2	130.3 ±23.0	242.4 ± 30.4	183.1 ± 20.2	99.1 ± 14.8	92.3 ± 14.6

The results obtained in this study confirmed the effect of weather conditions (Fig. 1, 2) in three vegetation seasons (2001, 2003 and 2004), on both the yield and the quality of the lavender herb.

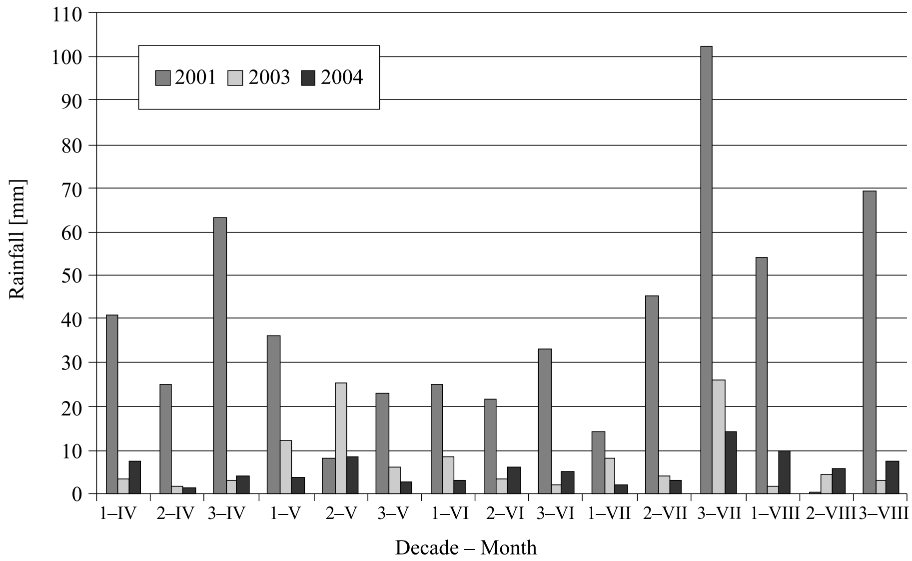

Fig. 1. Amount of rainfall per decade in analyzed seasons

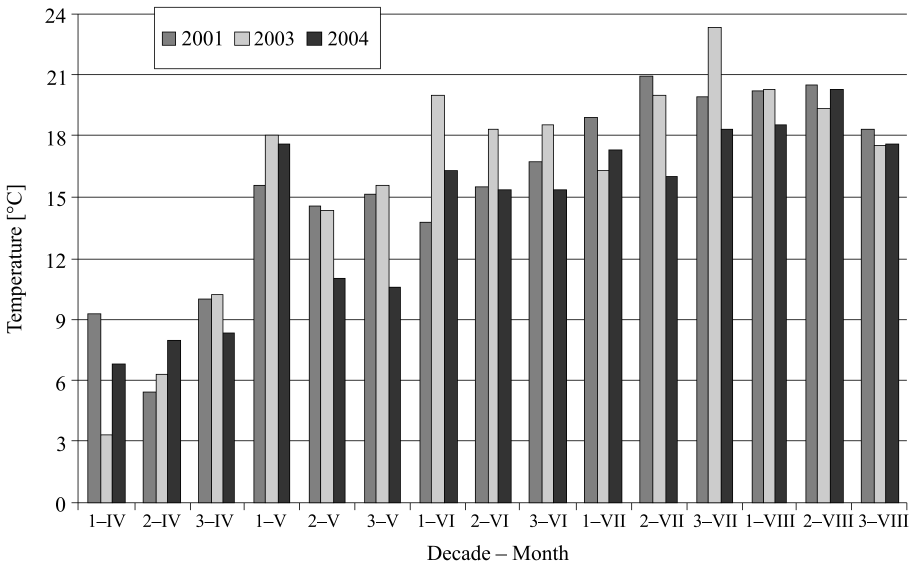

Fig. 2. Average air temperature per decade in analyzed vegetation seasons

Table 3

Inflorescence yield (fresh matter) of lavender [g/plant] depending on vegetation season and plant size

Years	Plant size			Means for years	LSD for years
	S	M	L		
2001	469	728	1090	762	93.07
2003	147	314	508	323	
2004	132	399	727	419	
Means for plant size	249	480	775		
LSD for plant size	93.07				

Table 4

Dry matter lavender inflorescence [g] yield depending on vegetation season and plant size

Years	Plant size			Means for years	LSD for years
	S	M	L		
2001	127	219	281	209	29.7
2003	41.3	87.0	149	92.6	
2004	32.7	108	219	119	
Means for plant size	67.2	138	216		
LSD for plant size	29.7				

Table 5

Dry matter lavender flowers [g] yield depending on vegetation season and plant size

Years	Plant size			Means for years	LSD for years
	S	M	L		
2001	52.8	74.0	109.0	78.8	10.6
2003	17.0	35.5	58.8	37.1	
2004	12.3	39.5	77.8	43.2	
Means for plant size	27.4	49.7	82.1		
LSD for plant size	10.6				

Table 6

Number of inflorescences developed on lavender plants [piece] depending on vegetation season and size of plants

Years	Plant size			Means for years	LSD for years
	S	M	L		
2001	632	1041	1444	1039	136.7
2003	196	416	642	418	
2004	167	548	1007	574	
Means for plant size	332	668	1031		
LSD for plant size	136.7				

The highest yields of the fresh and dry matter of inflorescences (Table 3 and 4), dry matter of flowers (Table 5), and also, the highest numbers of flowering branches (Table 6), and flower rings in inflorescences (Table 7), were recorded in the first year of the study ie 2001.

On the other hand, the lowest and the highest numbers of flowers in rings were recorded in 2001 and 2004, respectively (Table 8). The first vegetation season (April–August 2001) was warm and its average monthly temperatures were close to long-term temperatures for this site, although they were lower than those recorded during the seasons of 2003 and 2004 (Fig. 1 and 2). In addition, intensive rainfall (> 60 mm) which doubled the long-term average and were recorded in the 3rd decade of April could increase the number of flowering branches and thus yields. In 2001 inflorescences were harvested in mid July. The flowering phase was equilibrated and the yield of fresh matter of inflorescences was 5.8 Mg/ha.

Table 7

Number of flower rings developed on lavender inflorescences [piece] depending on vegetation season and plant size

Years	Plant size			Means for years	LSD for years
	S	M	L		
2001	6.7	6.3	6.7	6.6	0.7
2003	5.2	5.7	6.3	5.7	
2004	5.5	5.3	6.0	5.6	
Means for plant size	5.8	5.8	6.3		
LSD for plant size	0.7				

Table 8

Number of lavender flowers developed in a flower ring [g] depending on vegetation season and plant size

Years	Plant size			Means for years	LSD for years
	S	M	L		
2001	8.20	9.00	7.80	8.30	2.10
2003	10.3	10.7	9.50	10.2	
2004	11.3	10.7	11.8	11.3	
Means for plant size	10.1	9.7	8.2		
LSD for plant size	2.1				

In contrast, different environmental conditions were noted in 2003. Plant vegetation was delayed and snowless winter resulted in frost damages of lavender plants. The plants produced flowering branches from root crowns and not from tops, thus resulting in lower number of the branches and lower yields of fresh and dry matter of inflorescences (Table 3, 4, and 6). Temperatures recorded in the first and the second decade of April was below the long time average (2 °C). On the other hand, a significant increases of temperature was recorded in May (the first and the third decade), reaching 16.5 °C and exceeding the long-time average by 23 %.

The amount of rainfall in April was 34.3 mm, amounting to 69 % of the long-time average, whereas in May the rainfall was largely scattered. The first and the second decade of June were warm, with the average temperature of 13 % higher than in May and reaching 19.1 °C. In contrast, the amount of monthly rainfall was only 38 % of the long-term average. In spite of warm and dry June, flowering branches of lavender plants were of different length, flowering was extended in time, underdeveloped flower rings dried early and finally all this affected yield of plants. The plants were harvested in the 1st decade of July yielding 2.04 Mg/ha of inflorescences. In the first decade of May 2004, temperatures were stable and warm. However, in the next two decades a 5 °C decrease in temperature was recorded. In June and July, air temperatures were similar. In 2004 (June–August), total rainfall was 234 and 39 mm higher than in 2001 and 2003, respectively. The plants were harvested in the beginning of July and the yield of inflorescences was 3.3 Mg/ha.

Available data on the yields of narrow-leaved lavender plants recorded in Poland are scarce and imprecise. According to Ruminska [2], the yield of lavender flowers in the second year of cultivation is low and amounts to 100 kg/ha. In next years it increases to 300–500 kg and 600–1000 kg of thredshed and unthreshed flowers, respectively. Also Nowak [3] indicates that the yield of lavender flowers amounts to 1000–1200 kg/ha. In our experiment the yield of dry threshed flowers varied from 615 kg/ha in 2003 to 1295 kg/ha in 2001, and was similar to the results reported earlier.

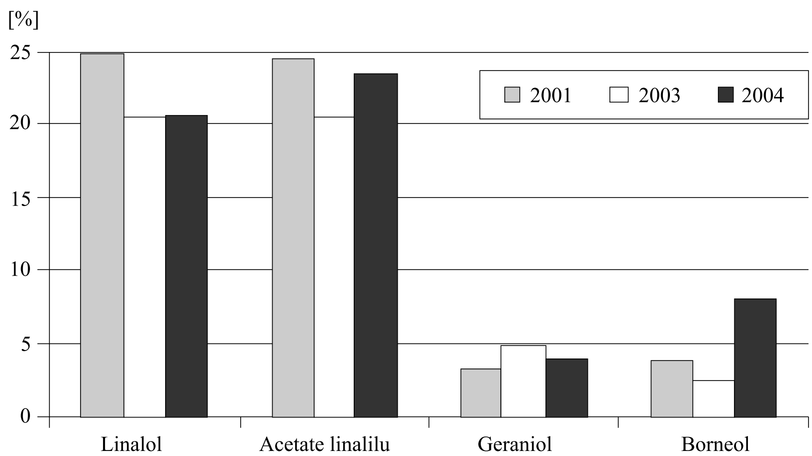

Fig. 3. Content of terpens in lavender flowers in analyzed vegetation seasons

The weather conditions during vegetation seasons (2001, 2003 and 2004) had no effect on the total volatile oil content in lavender plants. However, relative proportions of individual volatile oil were affected. The average content of volatile oils in lavender flowers was 2.39 % and varied from 2.53 to 2.58 % in the third year of cultivation. According to Ruminska [11], the volatile oil contents is affected by a number of factors, eg a plant origin, a season, an extraction method and may vary from 0.7 to 3 %. Similarly, the oils contents reported by Turowska and Stepien [1] and Cybulska et al [12] was 1.33–1.73 and at least 1.7 %, respectively. In contrast, Czikow and Łaptiew [13] obtained the range of 4–5 %. Therefore, it can be concluded that the volatile oils content in lavender, in our experimental conditions, was on a fairly high level. The individual volatile oils in lavender oil determined in our analyses were: linalool, linalyl acetate, geraniol and borneol. Among three vegetation seasons, the highest levels of linalool and linalyl acetate were recorded in 2001, as resulting from suitable weather conditions (Fig. 3). In 2003, the contents of linalool and linalyl acetate were slightly lower than in 2001, and in 2004, the level of lianool remained the same as in 2003 but the level of lianalyl acetale rose. The content of geraniol in lavender oil in the three analyzed vegetation seasons was the highest in 2003, and of borneol in 2004.

In Poland, lavender is mainly cultivated for its inflorescences (bunches) or flowers (potpourri or lavender oil). In the case of lavender oil use in cosmetology, the high level of lianalyl acetale is especially important [14, 15], because of its characteristic scent, as well as lianool. In the conducted experiments, the content of both terpenes has been high and similar to the top level in lavender oil [16].

Conclusions

1. The analyzed soil was characterized by very good conditions to cultivate narrow--leaved lavender. An insignificant decrease of plant available P, K and Mg was observed in 2003 and 2004 as compared with 2001.

2. The effect of weather conditions during three vegetation seasons (2001, 2003 and 2004), was significant and influenced the amount of fresh and dry matter of inflorescences yield, flowers and the number of flowering branches and flower rings developed on the narrow-leaved lavender plants. The highest yield was obtained in 2001, during the vegetation season with high total rainfall (578 mm) and the optimum average (April–May).

3. Two components of the harvest structure had a significant influence on the yield of lavender inflorescences and flowers, namely, the number of flowering branches developed on plants and the number of flower rings. In the analyzed years 2001, 2002 and 2004, the plants developed 1039, 418 and 574 flowering branches on the plants, respectively, and 6.6, 5.7 and 5.6 flower rings in spikes.

4. Among three vegetation seasons the weather conditions were different, which did not influence the content of the oil significantly but the percentage of every terpen. The highest contents of lianool and lianalyl acetale were recorded in the vegetation season of the year 2001.

References

[1] Turowska I.: Dotychczasowe obserwacje nad aklimatyzowaną lawendą. Wiad. Farmaceut., Wyd. PTFa, Warszawa 1936.

[2] Rumińska A.: Poradnik plantatora ziół, PWRiL, Poznań 1991, 208–214.

[3] Nowak T.: *Uwagi doświadczonego plantatora o uprawie lawend,*. Wiad. Zielar., 1995. **11**(37), **4**.
[4] Pisulewska E., Puchalska H. and Janeczko Z.: *Charakterystyka morfotypów lawendy wąskolistnej (Lavandula angustifolia* Mill.*) występujących na plantacjach produkcyjnych*, Folia Horticult., supl. 1, 2003, **1**, 139–141.
[5] Puchalska H. and Janeczko Z.: *Zawartość i skład olejku eterycznego pochodzącego z kwiatów lawendy (Lavandula angustifolia) uprawianej w okolicy Gołczy w województwie małopolskim*, Herba Polon., 2003, **49**(1/2), 11–16.
[6] Pisulewska E., Puchalska H. and Zaleski T.: Uprawa lawendy wąskolistnej (*Lavandula angustifolia* Mill) na Wyżynie Miechowskiej, Wyd. Akademia Rolnicza w Krakowie, Kraków 2004.
[7] FAO-ISRIC-SICS: World reference base of soil resources. World soil resources Reports 84, FAO, Rome 1998.
[8] Lityński T, Jurkowska H. and Gorlach E.: Analiza chemiczno-rolnicza. Przewodnik metodyczny do analizy gleby i nawozów (in Polish), PWN, Warszawa 1976.
[9] Skopp J.M.: Physical properties of primary particles, [in]: M.E. Sumner, Editor, Handbook of Soil Science, CRC Press, Boca Raton, FL, 2000, A3–A17.
[10] Farmakopea Polska, PTFarm, Warszawa (in Polish) 2002.
[11] Rumińska A: Rośliny lecznicze. Podstawy biologii i agrotechniki, PWN, Warszawa 1983, 286–297.
[12] Cybulska H., Janicka H., Karpała Z., Olesiński A., Rajkowski Z. and Rumińska A.: Uprawa i zbiór ziół, PWRiL, Warszawa 1956, 265–274.
[13] Czikow P. and Łaptiew J.: Rośliny lecznicze i bogate w witaminy, Warszawa 1987, 188–191.
[14] Kohlmunzer S.: Farmakognozja, PZWL, Warszawa 1977, 354–376.
[15] Ożarowski A. and Jaroniewski W.: *Działanie i zastosowanie lecznicze lawendy,* Wiad. Zielar., 1995, **11**(37), 1–2.
[16] Pisulewska E., Puchalska H. and Janeczko Z.: *Wpływ roku użytkowania na plon i zawartość olejków eterycznych w kwiatach lawendy wąskolistnej (Lavandula officinalis* Mill.*)*, Mat. III Krajowego Sympozjum „Naturalne i syntetyczne produkty zapachowe", Łódź 2003.

WPŁYW WARUNKÓW ATMOSFERYCZNYCH NA PLON I JAKOŚĆ LAWENDY WĄSKOLISTNEJ (*Lavandula angustifolia* MILL)

Katedra Szczegółowej Uprawy Roślin, Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Badano wpływ warunków glebowych oraz przebiegu pogody w trzech sezonach wegetacyjnych (2001, 2003 i 2004) na plony lawendy wąskolistnej, a także zawartość i skład olejków eterycznych w kwiatach tego gatunku. Badania polowe prowadzono na trzech, czteroletnich plantacjach lawendy, w trzecim roku użytkowania roślin. Plantacje doświadczalne położone były w miejscowości Krępa, koło Gołczy na Wyżynie Miechowskiej. Plony zarówno świeżych, jak i suchych kwiatostanów i kwiatów lawendy były uzależnione od przebiegu pogody w badanych sezonach wegetacyjnych, wielkości roślin, liczby wykształconych kwiatostanów oraz liczby nibyokółków. Natomiast warunki pogodowe i glebowe nie miały wpływu na zawartość olejku w kwiatach lawendy wąskolistnej. Jakkolwiek widoczna była zależność udziału głównych terpenów, takich jak linalolu, octanu linalolu, geraniolu i borneolu w olejku lawendowym w poszczególnych latach badań.

Słowa kluczowe: lawenda wąskolistna, sezon wegetacyjny, olejki eteryczne, gleba płowa, less

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Adam RADKOWSKI[1] and Paweł NICIA[2]

CHEMICAL EVALUATION OF TWO TIMOTHY GRASS (*Phleum pratense* L.) CULTIVARS AS AFFECTED BY THE HARVESTING DATE PART II. MICROELEMENT CONTENTS[3]

CHEMICZNA OCENA DWÓCH ODMIAN TYMOTKI ŁĄKOWEJ (*Phleum pratense* L.) W ZALEŻNOŚCI OD TERMINU ZBIORU CZ. II. ZAWARTOŚĆ MIKROELEMENTÓW

Abstract: The goal of the present study was an assessment of microelements content in timothy grass in dependence on the time of the first swath collection. Two timothy grass cultivars were examined *ie*: Skala and Skald. They were mown three times during the vegetation period. The sward of the first swath was collected six times: the first time during the plant tillering and then at 7-day intervals. In collected green grass the dry matter content was evaluated by drying at 105 °C. After mineralization of hay samples Cu, Fe, Mn, Ni and Zn content was assessed by the ICP-AES method. The research was run in the years 2004–2006 on the pseudopodzol soil formed from light, dusty clay located shallow on the medium clay of good wheat complex.

The time of sample collection significantly affected the microelement content in the examined plants. Retardation of the first swath resulted in a lower level of copper, manganese and zinc. In the plants derived with the second and third swath copper, iron, manganese and zinc level decreased depending on the time of collection. Both cultivars were characterised with a slightly different chemical composition; higher iron, manganese, and zinc level was stated for Skala, whereas the Skald cultivar contained higher amounts of copper and nickel. It was found that average microelement content, with exception of nickel, in both cultivars of timothy grass exceeded the values assumed as optimal.

Keywords: harvesting time, timothy grass (*Phleum pratense*), microelement content

Retardation of the harvesting date of grasses affects a significant reduction of their feeding value [1]. Visible decrease of nutritional value is observed with plant ageing, therefore the right time of mowing is very important, especially in the case of the first

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

[2] Department of Soil Science of the University, phone 12 662 43 70, email: rrnicia@cyf-kr.edu.pl

[3] Radkowski A.: *Chemical Evaluation of two Tomothy Grass* (*Phleum pratense* L.). *Cultivars as Affected by the Time of Collection. Part I. Macroelement Content*, Ecol. Chem. Eng. A 2008, **15**(9), 951–955

swath because during this period grasses create large numbers of generative shoots, which after heading quickly get old and hard. Moreover, chemical composition is also affected by the fertilization, soil type and weather conditions [2, 3].

Among trace elements an important role is played by such microelements as: Fe, Mn, Zn and Cu, which are necessary for living organisms. On the other hand, these elements are required in small amounts and exceeding of their optimal level can have a toxic effect on living organisms [4, 5].

The aim of the present study was to evaluate the changes of the microelement content in timothy grass during the vegetation period depending on the time of the first swath collection.

Material and methods

The research was run in the years 2004–2006 in the Agricultural Experimental Station in Pawłowice near Gliwice, at the altitude of 250 m. The experiment was located on the pseudopodzol soil made from light, dusty clay located shallow on the medium clay of good wheat complex, on the standing after spring wheat. Acidity expressed as pH_{KCl} amounted to 6.3 and the contents of assimilable forms of macroelements were as follows: P_2O_5 = 8.1; K_2O = 11.8 and Mg = 10.2 mg · 100 g^{-1}.

During the vegetation period (April–September), rainfall amounted to 306.8 mm and 306.4 mm, respectively in 2005 and 2006, whereas average temperatures were: 14.9 °C and 16.0 °C, respectively.

Two timothy grass cultivars were taken into account, ie: Skala and Skald, which were mown three times during the vegetation period. The sward of the first swath was harvested six times: the first time during the plant tillering and then at 7-day intervals. The crops were collected, in dependence of the year, between 01.V–24.VI. The second swath was collected every time 6 weeks after the previous one. The last swath was collected at the same time for all variants. The grasses were sown on September 9, 2004 in four replicants, the area of each experimental field amounted to 10 m^2. In the same year fertilization was applied which comprised: 40 kg N, 100 kg P_2O_5 and 165 kg $K_2O \cdot ha^{-1}$. In the years of full utilization nitrogen fertilizer was utilized in the amount of 200 kg N (80 kg N · ha^{-1} for the first swath, 60 kg N · ha^{-1} for the second and third swath), phosphorus – in a dose of 120 kg $P_2O_5 \cdot ha^{-1}$ (once in the spring) and potassium – in a dose of 120 kg $K_2O \cdot ha^{-1}$ (in two equal parts: in the spring and after the first regrowth).

The collected plant material was subjected to the analysis of forage chemical composition. The dry matter content was determined by drying at 105 °C. On the basis of the dry matter content in timothy grass the yields of dry matter per 1 ha were calculated. The plant samples underwent dry mineralization in muffle furnace at 450 °C [6]. Zn, Cu, Ni, Fe and Mn contents were determined using ICP-AES method (atomic emission spectrophotometry equipped with inductively coupled plasma torch).

Presentation of the results was limited to the average values from all investigated years. Results of field experiments were subjected to statistical analysis of variance and the significance of differences between average values was estimated on the basis of the confidence interval according to Tukey at the significance level of $\alpha = 0.05$.

Results and discussion

Time of harvesting had an important influence on the level of microelements in examined plants. Retardation of the first swath harvesting affected reduction of the copper, manganese and zinc concentration in plants (Table 1). After six weeks the copper content decreased by 21.5 % in the Skala cultivar and by 26.5 % in the Skald cultivar. A decline of manganese and zinc contents amounted to: 29.1 % – Skala, 32.3 % – Skald, and 60.8 % – Skala, 49.2 % – Skald, respectively. The highest iron content was achieved with the third harvesting time (heading stage of plants). During the following harvesting dates gradual decrease in its content was observed. However, nickel content in timothy grass was unaffected by the time of first swath harvesting. In the plants derived with the second and third swath the copper, iron, manganese and zinc level was slightly decreasing along with the following harvesting dates.

Table 1

Microelement content in two timothy grass cultivars as affected by the swath and harvesting date [$mg \cdot kg^{-1}$ d.m.] – mean values for 2005–2006

Swath	Time of harvest	Cu		Fe		Mn		Ni		Zn	
		A*	B*	A	B	A	B	A	B	A	B
I	1	13.8	14.7	180.4	251.8	118.6	89.5	2.6	3.1	222.8	197.0
	2	12.4	12.7	227.3	155.2	102.9	80.8	3.2	3.1	195.6	115.1
	3	12.2	12.5	435.2	353.4	99.8	74.7	2.8	3.1	132.1	114.7
	4	12.2	12.4	329.5	290.9	98.4	73.8	6.1	4.0	119.2	106.0
	5	11.0	10.9	286.9	176.5	95.8	73.1	4.0	3.1	114.9	100.2
	6	10.8	10.8	190.2	172.1	84.1	60.6	2.4	3.1	87.3	100.0
Mean		12.0	12.3	274.9	233.3	99.9	75.4	3.5	3.2	145.3	122.2
V [%]		8.9	11.6	8.9	8.9	11.2	12.7	39.4	11.5	36.0	30.5
$LSD_{0.05}$		1.73		106.32		23.49		1.43		60.96	
II	1	14.9	15.8	595.8	355.5	131.5	115.7	3.0	3.9	252.8	200.3
	2	12.9	14.0	424.6	338.8	122.2	98.9	3.5	3.0	227.0	134.2
	3	12.8	13.2	318.1	314.8	116.6	94.5	3.1	3.3	195.2	130.0
	4	12.5	11.2	267.0	313.3	106.5	87.0	4.6	2.8	164.5	118.0
	5	11.1	10.6	213.2	255.1	92.1	76.3	1.4	4.0	135.4	117.4
	6	10.9	10.2	209.4	250.6	89.8	74.0	3.1	4.5	102.3	97.3
Mean		12.5	12.5	274.9	233.3	109.8	91.1	3.1	3.6	179.5	132.9
V [%]		11.6	17.6	8.9	8.9	15.2	17.1	33.1	18.3	31.5	26.7
$LSD_{0.05}$		2.52		118.99		26.42		1.25		68.90	
III	1	13.5	14.3	351.3	339.9	138.7	147.6	2.3	3.4	164.1	137.8
	2	13.2	13.9	317.7	307.8	137.6	131.2	3.0	3.0	132.4	125.4
	3	12.8	13.3	280.1	256.6	120.6	129.2	3.0	3.8	126.0	113.4
	4	12.1	13.2	233.7	246.3	115.9	107.3	3.0	3.7	120.9	106.2
	5	11.6	12.9	230.4	213.6	113.2	104.3	3.2	3.1	102.3	100.9
	6	11.3	11.4	132.6	162.7	106.7	93.8	3.4	3.7	90.4	97.0
Mean		12.4	13.2	274.9	233.3	122.1	118.9	3.0	3.4	122.7	113.4
V [%]		7.1	7.5	8.9	8.9	10.8	17.1	13.2	10.0	20.9	13.7
$LSD_{0.05}$		1.40		75.10		23.80		0.61		27.98	

A – Skala cultivar; B – Skald cultivar; V [%] – coeffiecient of variation.

The nickel content in II and III cutting was also unaffected by the time of harvesting. According to established recommendations the amounts of microelements in forage covering nutritional requirements of animals are: Zn – 50 mg; Cu – 10 mg; Fe – 50 mg and Mn – 60 mg · kg^{-1} d.m. [7–9]. Assessment of the microelement content in both timothy grass cultivars revealed that the level of copper, iron, manganese and zinc was higher than the optimal one listed above. The limiting nickel content assumed for the plant evaluation as regards forage value amounts to Ni ≤ 50 mg · kg^{-1} d.m. [10]. In our study it was found that the content of the above-mentioned element in timothy grass cultivars did not exceed the limiting value.

Reduction of copper, iron, manganese and zinc content due to retardation of the first cutting can be explained with the dilution of components [11]. The obtained results revealed only slight differences in microelement content between both investigated cultivars. Higher iron, mangenese and zinc levels were reached in plants of the Skala cultivar, whereas plants of the Skald cultivar contained higher amounts of copper and nickel.

Taking into consideration all harvesting dates one can find that the highest diversification occurred in the nickel content in the first swath of the Skala cultivars and in the zinc content in both cultivars in all analysed swaths. The opposite phenomenon (the lowest degree of diversification) was observed for the copper concentration in the third swath of both timothy grass cultivars and for the iron content in all swaths of both cultivars.

Conclusions

1. Retardation of the first swath harvesting affected a reduction of the copper, manganese and zinc level in plants.
2. In the plants derived with the second and third swath copper, iron, manganese and zinc level decreased in dependence on the time of collection.
3. Only slight diversification was observed in chemical composition between both cultivars. A higher iron, manganese and zinc content was found for the Skala cultivar, whereas the Skald cultivar was richer in copper and nickel.
4. It was stated that microelement content, with the exception of nickel, in examined timothy grass cultivars exceeded the optimal values.

References

[1] Staniak M.: *Wpływ częstotliwości koszenia i rodzaju gleby na plonowanie i jakość suchej masy festulolium odmiany Felopa*. Ann. UMCS, 2004, Sec. E, **59**(4), 2001–2008.

[2] Borowiecki J. and Staniak M.: *Wpływ terminu koszenia pierwszego pokosu na poziom plonowania i zawartość białka Festulolium odmiany Felopa*. Zesz. Probl. Post. Nauk Rol. 2001, (474, 235–239.

[3] Czeladzka M. and Urbaniak K.: *Trwałość odmian życicy trwałej przy różnej częstotliwości koszenia*. Wiad. Odmianozn. COBORU, 1997, **68**, 3–21.

[4] Czuba R.: *Celowość i możliwość uzupełnienia niedoborów mikroelementów u roślin.* Zesz. Probl. Post. Nauk Rol., 1996, (434), 55–64.

[5] Gorlach E.: *Zawartość pierwiastków śladowych w roślinach pastewnych jako miernik ich wartości.* Zesz. Nauk. AR w Krakowie, 1991, **262**, Sesja Nauk. (34), 13–22.

[6] Ostrowska A., Gawliński S. and Szczubiałka Z.: Metody analizy i oceny właściwości gleb i roślin. Katalog. Wyd. IOŚ Warszawa 1991, 334 pp.
[7] Falkowski M., Kukułka I. and Kozłowski S.: Właściwości chemiczne roślin łąkowych. Wyd. AR Poznań 2000, 132 pp.
[8] Gorlach E.: *Zawartość pierwiastków śladowych w roślinach pastewnych jako miernik ich wartości*. Zesz. Nauk. AR w Krakowie, 1991, **262**, Sesja Nauk. (34), 13–22.
[9] Preś J. and Kinal S.: *Aktualne spojrzenie na sprawę zaopatrzenia zwierząt w mikroelementy.* Zesz. Probl., Post. Nauk. Roln., 1996, (434), 1043–1061.
[10] Kabata-Pendias A., Motowicka-Terelak T., Piotrowska M., Terelak H. and Witek T.: *Ocena stopnia zanieczyszczenia gleb i roślin metalami ciężkimi i siarką*. Ramowe wytyczne dla rolnictwa. Pamięt. Puław., 1993, **53**, IUNG, 20.
[11] Maciejewska M. and Kotowska J.: *Zawartość mikroelementów w sianie w warunkach zróżnicowanego NPK*. Biul. Magnezol. 2001, **6**(3), 295–303.

CHEMICZNA OCENA DWÓCH ODMIAN TYMOTKI ŁĄKOWEJ (*Phleum pratense* L.) W ZALEŻNOŚCI OD TERMINU ZBIORU CZ. II. ZAWARTOŚĆ MIKROELEMENTÓW

Katedra Łąkarstwa, Uniwersytet Rolniczy w Krakowie
Katedra Gleboznawstwa i Ochrony Gleb, Uniwersytet Rolniczy w Krakowie

Abstrakt: Celem badań była ocena zawartości mikroelementów w tymotce łąkowej w zależności od terminu zbioru I pokosu. W doświadczeniu uwzględniono dwie odmiany tymotki łąkowej: Skala i Skald, które koszono trzykrotnie w sezonie wegetacyjnym. W I pokosie ruń zbierano w sześciu terminach: pierwszy wykonano w fazie krzewienia się roślin, a następne w odstępach siedmiodniowych. W próbkach zielonki oznaczono zawartość suchej masy metodą suszarkową w temperaturze 105 °C. Po mineralizacji próbek siana oznaczono zawartość Cu, Fe, Mn, Ni i Zn, metodą ICP-AES. Badania przeprowadzono w latach 2004–2006 na glebie pseudobielicowej wytworzonej z gliny lekkiej pylastej, zalegającej płytko na glinie średniej zaliczanej do kompleksu pszennego dobrego.

Termin zbioru statystycznie istotnie wpływał na zawartość mikroelementów w roślinach. Opóźnienie zbioru pierwszego pokosu powodowało obniżenie się w roślinach zawartości miedzi, manganu i cynku. W roślinach II i III pokosu koncentracja miedzi, żelaza, manganu i cynku zmniejszała się w zależności od terminu zbioru. W składzie chemicznym stwierdzono niewielkie zróżnicowanie między odmianami, większe zawartości żelaza, manganu i cynku odnotowano u odmiany Skala, a miedzi i niklu u odmiany Skald. Stwierdzono, że zawartości mikroelementów w badanych odmianach tymotki łąkowej przewyższały wartości optymalne, poza zawartością niklu.

Słowa kluczowe: termin zbioru, tymotka łąkowa, zawartość, mikroelementy

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Katarzyna SZAFRAŃSKA[1], Urszula KOWALSKA[2], Krystyna GÓRECKA[2], Milena CVIKROVÁ[3], Małgorzata M. POSMYK[1] and Krystyna JANAS[1]

INFLUENCE OF COPPER IONS ON PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN PLANT MATERIAL REGENERATED FROM EMBRYOS OBTAINED IN ANDROGENIC CARROT CULTURE

WPŁYW JONÓW MIEDZI NA FIZJOLOGICZNE I BIOCHEMICZNE ZMIANY W MATERIALE ROŚLINNYM REGENEROWANYM Z ZARODKÓW ANDROGENNYCH MARCHWI

Abstract: Androgenic embryos of 3 different carrot genotypes were cultivated on the medium containing different Cu^{2+} concentrations: 0.1 μM (control), 1 μM, 10 μM and 100 μM. Carrot sensitivity to Cu^{2+} was evaluated on the basis of growth inhibition, changes in peroxidation of membranes and proline content. The cultivation on the medium supplemented with Cu^{2+} resulted in dose-dependent inhibition of the growth and organogenic ability of Narbonne carrot embryos, while in the genotype 1014 treated with 10 μM Cu^{2+} the significant regeneration ability was observed. The same Cu^{2+} concentration greatly increased the level of free proline in the genotype 1014 which was accompanied by relatively low TBARS content. In the genotype Feria 100 μM Cu^{2+} concentration triggered large increase in proline content associated with high regeneration capacity of carrot embryos.

Keywords: copper ions, anther cultures, lipid peroxidation, proline

Copper (Cu^{2+}), an essential microelement for growth and plant development in higher doses can become very toxic and disturb basic physiological processes [1]. Acting as a cofactor in many enzymatic reactions in cells it causes the formation of harmful reactive oxygen species (ROS) and induces oxidative damage of important macromolecules such as DNA, proteins and lipids [2]. Although plants have evolved a variety of

[1] Department of Ecophysiology and Plant Development, University of Łodz, ul. S. Banacha 12/16, 90-237 Łódź, Poland, email: keysi@biol.uni.lodz.pl

[2] Research Institute of Vegetable Crops, Laboratory of Biotechnology, ul. Konstytucji 3-Maja, 96-100 Skierniewice, Poland

[3] Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojová 135, Praha 6, Lysolaje CZ-165 02, Czech Republic

mechanisms for metal tolerance, species and cultivars vary widely in this respect, and closely related genotypes can be valuable tools in studying the mechanisms of toxicity or tolerance.

Carrot is one of the most important vegetables and therefore it has become an object of intensive research aimed at obtaining new cultivars. Production of haploids in carrot through another culture allows carrot breeders to release new lines more quickly and screen for metal accumulation more efficiently. Carrot easily accumulates toxins that is why it is important to use *in vitro* cultures in selecting and eliminating the cultivars which accumulate excessive amounts of metals.

Differences in plant responses to Cu^{2+} seem to depend not only on its concentration but also on the ability of plants to increase the antioxidative protection against negative consequences of heavy metal stress. One of the first symptoms of oxidative stress in plant tissues is lipid peroxidation of cell membranes. Proline, which accumulates in response to various kinds of abiotic stresses eg non-optimal temperatures, heavy metals, wounding [3], contributes to osmotic adjustment in cells and it has been shown that this amino acid can be an effective antioxidant [4]. Also phenolic compounds play an important role in protecting plants against biotic and abiotic stresses [5], and the enhancement of their metabolism is one of the responses to heavy metal stress. The purpose of the current work was to characterize the influence of Cu^{2+} on the regeneration of androgenic embryos of 3 different carrot genotypes and on the extent of lipid peroxidation.

Material and methods

The roots of three carrot (*Daucus carota* L.) genotypes, 1014, Feria and Narbonne were the donors of experimental material. The plants developed from the roots were kept in a greenhouse at about 20 °C. The detailed description of another culture procedure was previously given in [6]. The carrot cultures were kept in darkness at 27 °C and after emerging of the embryos, they were transferred to continuous light at the same temperature. When the embryos became green they were transferred onto the regeneration medium B5 [7] supplemented with Cu^{+2} in the form of $CuSO_4 \cdot 5H_2O$ at concentrations: 0.1 μM (control), 1 μM, 10 μM and 100 μM. The embryos were incubated under light (30 μmol m^{-2} sec^{-1}, 20 °C, photoperiod 16/8) for 24 weeks.

TBARS contents in carrot rosettes were measured according to [8], proline was extracted and estimated according to [9], whereas phenolic acids were extracted as described by [10] and analysed according to [11].

Results and discussion

Table 1 shows the effect of Cu^{2+} on the numbers of regenerated carrot rosetes after 24 weeks. Cultivation on the regeneration medium supplemented with Cu^{2+} resulted in dose-dependent inhibition of the growth and organogenic ability of Narbonne carrot embryos, while in the genotype 1014 treated with 10 μM Cu^{2+} the significant regeneration ability was observed. The same Cu^{2+} concentration greatly increased the level of free proline in the genotype 1014 which was accompanied by relatively low TBARS content in comparison with the control (0.1 μM) (Fig. 1A). It is known that TBARS levels can be a good marker of stress and plants which accumulate more TBARS are more sensitive to stressor than those which accumulate less [12]. However, in the genotype Feria Cu^{2+} at 100 μM triggered large increase in proline content (Fig. 1B) associated with high regeneration capacity of carrot embryos in comparison with the genotypes 1014 and Narbonne (Table 1). It seems that accumulation of free proline may be involved in a protective mechanisms against Cu^{2+} stress during regeneration of androgenetic embryos of carrot [13]. On the other hand, increased level of free phenolic acids in the Narbonne genotype presented in Fig. 2 seems to be not sufficient for carrot rosetes to protect them against damaging influence of Cu^{2+}.

The results presented here show the significant differences in responses of 3 carrot genotypes regenerated from another cultures to Cu^{2+}. Narbonne seems to be the most sensitive to harmful impact of Cu^{2+}, while Feria appears tolerant even to extremely high Cu^{2+} concentration. We suggest the protective role especially of free proline against Cu^{2+} stress during regeneration of androgenetic embryos of carrot.

Table 1

Effect of Cu^{2+} on the numbers of normally rooted and non-rooted carrot rosettes of genotypes 1014, Feria and Narbonne after 24 weeks of cultivation on B5 medium [6]. Control (C) – 0.1 μM of Cu^{2+}.

Cu^{2+} concentration [μM]	Total No. of regenerated rosettes (with and without roots)		
	1014	Feria	Narbonne
C	5.9 a	12.6 a *	8.7 a *
Cu-1	8.8 a	9.6 a	2.1 a
Cu-10	24.1 b	10.6 a	1.0 a
Cu-100	2.7 b	8.4 a	0.9 a

Asterisks (*) indicate the results obtained from Cu^{2+} – treated carrot cultures that significantly differ from the corresponding control values at $p \leq 0.05$

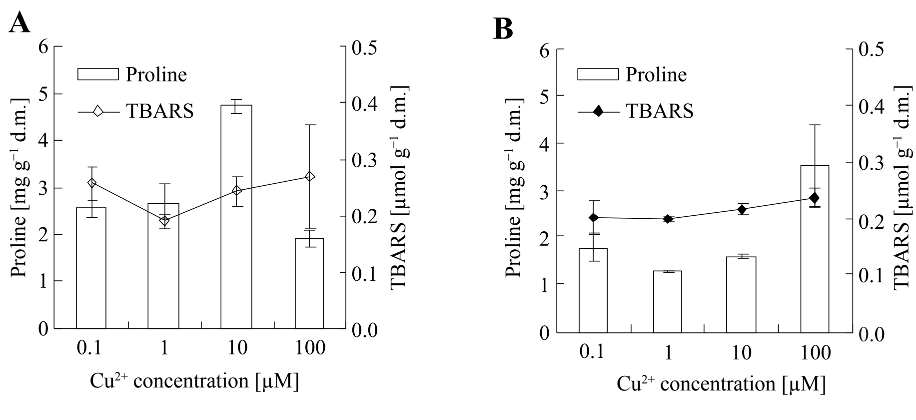

Fig. 1. Proline and TBARS contents in carrot rosetes of genotype 1014 (A) and Feria (B) after 24 weeks of cultivation on medium B5.

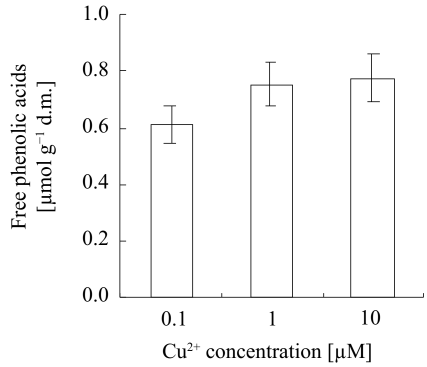

Fig. 2. Contents of free phenolic acids in the rosetes regenerated from carrot tissues of genotype Nar bonne

Acknowledgements

Study financed by the Polish Ministry of Sciences and Higher Education in 2006–2009 as a research project No. N305 040 31/1576.

References

[1] Maksymiec W.: *Effect of copper on cellular processes in higher plants.* Photosynthetica 1997, **34**, 321–342.
[2] DeVos C.H., Schat H., Vooijs R. and Ernst W.H.O.: *Cd induced damage to permeability barrier in roots of Silene cucubalus,* J. Plant Physiol. 1989, **135**, 164–169.
[3] Hare P. and Cress W.: *Metabolic implications of stress-induced proline accumulation in plant,* Plant Growth Regul. 1997, **21**, 79–102.
[4] Matysik J., Alia, Bhalu B. and Mohanty P.: *Molecular mechanisms of quenching of reactive oxygen species by praline under stress in plants,* Current Sci. 2002, **82,** 525–532.
[5] Dixon R.A. and Paiva N.L.: *Stress-induced phenylpropanoid metabolism.* Plant Cell 1995, **7**, 1085–1097.
[6] Górecka K., Krzyżanowska D. and Górecki R.: *The influence of several factors on the efficiency of androgenesis in carrot,* J. Appl. Genet. 2005, **46**, 265–269.
[7] Gamborg O.L., Miller R.A. and Ojima K.: *Nutrient requirements of suspensions culture of soybean root cells,* Exp. Cell Res. 1968, **50**, 151–158.
[8] Heath R.L. and Packer L.: *Photoperoxidation in isolated chloroplast I. Kinetics and stechiometry of fatty acid peroxidation*, Arch. Biochem. & Biophys. 1968, **125**, 189–198.
[9] Bates I.S., Waldren R.P. and Teare I.D.: *Rapid determination of free proline for water stress,* Plant Soil 1973, **39,** 205–207.
[10] Cvikrová M., Meravý L., Macháčkova I. and Eder J.: *Phenylalanine ammonia-lyase, phenolic acids and ethylene in alfalfa (Medicago sativa L.) cell cultures in relation to their embryogenic ability,* Plant Cell Rep. 1991, **10,** 251–255.
[11] Górecka K., Cvikrová M., Kowalska U., Eder J., Szafrańska K., Górecki R. and Janas K.M.: *The impact of Cu treatment on phenolic and polyamine levels in plant material regenerated from embryos obtained in anther culture of carrot,* Plant Physiol. Biochem. 2007, **45**, 54–61.
[12] Garcia A., Baquedano F.J., Navarro P. and Castillo F.J.: *Oxidative stress induced by copper in sunflower plants,* Free Radic. Res. 1999, **31**, 45-50.
[13] Tripathi B.N. and Gaur J.P.: *Relationship between copper- and zinc-induced oxidative stress and proline accumulation in Scenedesmus sp,* Planta 2004, **219,** 397–404.

WPŁYW JONÓW MIEDZI NA FIZJOLOGICZNE I BIOCHEMICZNE ZMIANY W MATERIALE ROŚLINNYM REGENEROWANYM Z ZARODKÓW ANDROGENNYCH MARCHWI

Katedra Ekofizjologii i Rozwoju Roślin, Uniwersytet Łódzki
Pracownia Biotechnologii Zakładu Genetyki, Hodowli i Biotechnologii, Instytut Warzywnictwa w Skierniewicach
Instytut Botaniki Eksperymentalnej, Akademia Nauk, Praga, Republika Czeska

Abstrakt: Zarodki androgenne 3 różnych odmian marchwi hodowano na pożywce z dodatkiem Cu^{2+} w 4 stężeniach: 0,1 μM (kontr.), 1 μM, 10 μM, i 100 μM. Wrażliwość marchwi na Cu^{2+} oceniano na podstawie zahamowania wzrostu, zmian w utlenianiu lipidów błonowych oraz zawartości wolnej proliny. Hodowla na pożywce wzbogaconej w Cu^{2+} spowodowała stopniowe zahamowanie wzrostu oraz zdolności regeneracyjnych zarodków marchwi odm. Narbonne, podczas gdy ilość prawidłowych rozet genotypu 1014 traktowanych 10 μM Cu^{2+} była największa. Badany metal w tym samym stężeniu znacząco zwiększył zawartość wolnej proliny w rozetach genotypu 1014, co było skorelowane ze stosunkowo niskim poziomem TBARS. Natomiast, w przypadku genotypu Feria, wzrost zawartości proliny skojarzonej z dużymi zdolnościami regeneracyjnymi zarodków zaobserwowano w rozetach traktowanych Cu^{2+} o stężeniu 100 μM.

Słowa kluczowe: jony miedzi, kultury pylnikowe, peroksydacja lipidów, prolina

ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Andrzej TATUR[1], Ewa KICIŃSKA[2], Agnieszka WASIŁOWSKA[1]
and Piotr GROMADKA[2]

POLYCYCLIC AROMATIC HYDROCARBONS IN HOUSE DUST FROM WARSAW

WIELOPIERŚCIENIOWE WĘGLOWODORY AROMATYCZNE W KURZU MIESZKAŃ WARSZAWSKICH

Abstract: The content of polycyclic aromatic hydrocarbons (PAHs) in the dust from Warsaw apartments were studied. Samples were collected from 48 flats, from a few places in each flat: living room (floor, shelves), bedroom (bed), kitchen, lavatory. At the same time a survey by questionnaire was carried out, in which outside and internal factors were described. PAHs concentration in samples were determined by reversed-phase high performance liquid chromatography (HPLC). The levels of PAHs concentration varied between 8 and 173 mg/kg. The content of PAHs in dust from most of Warsaw apartments was high (from 5 to 50 ppm), and in more than ten was extremely high (> 50 ppm). It could be one of the sources of health hazards in home environment. In the greater part of analyzed samples, very high levels of the following hydrocarbons have been found: benzo[b]fluoranthene, fluoranthene, pyrene, fluorene and phenanthrene. Among microenvironments, selected for the study, the sum of PAHs in kitchen and cellars has been two-three times higher as compared with that in other places. The results show a clear trend to an increased concentration of PAHs in apartment of tobacco smokers.

Keywords: house dust, Warsaw, polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are one of the most hazardous groups of xenobiotics that are polluting natural environment as a result of human activity [1–3]. They are present in commonly used raw materials, such as coal tar, coal-tar pitch, mineral oils, gas pitch, asphalts, soot, or cresol oil. Emission sources of these substances are various processes, [4, 5], for example:

– combustion of solid, liquid or gaseous fuels in cars, power stations, steelworks, and stoves;

– manufacturing of coke, aluminum, coal tar processing and other industrial processes;

[1] Department of Antarctic Biology Polish Academy of Sciences, ul. Ustrzycka 10/12, 02-141 Warszawa, Poland, phone: 22 846 33 83, fax 22 846 19 12, email: professor@dab.waw.pl

[2] Center for Ecological Research Polish Academy of Sciences, ul. M. Konopnickiej 1, Dziekanów Leśny, 05-092 Łomianki, Poland, phone: 22 751 30 46, fax 22 751 31 00

– food processing (barbecue or frying over open fire);
– smoking of cigarettes, cigars, or pipe tobacco;
– wearing of tires and abrasion of asphalt surfaces;
– naturally occurring processes such as forest or steppe fires or emission of volcanic gases and dust.

PAHs, formed in the aforementioned processes are emitted to the atmosphere, where they settle on the surface of aerosol particles. Hence, they could be transferred over long distances form their source. Depending on weather conditions, the pollutant could travel several thousands kilometers. They settle on roof surfaces, leaves, rocks, etc. from where they are washed down with rains and pollute soil and groundwaters. They can settle also in apartments as constituents of common dust where they become a direct hazard to the inhabitants [6]. The amount of PAHs in home dust depends on many factors such as the level of particulate contamination of surrounding air, as well as activity of inhabitants, methods of preparing food, presence of tobacco-smokers, frequency of ventilation, etc.

Numerous toxicological and epidemiologic studies indicate a strong correlation between exposure to those compounds and the increased risk of developing cancer [7]. Metabolites of those compounds (quinones, diols, phenols and epoxy derivatives) could add to DNA and RNA molecules, thus generating neoplastic transformations and genetic alterations. The compounds show systemic toxicity, causing damages of adrenal glands, the lymphatic, circulatory and respiratory systems [8, 9].

Considering the above information, it becomes obvious that monitoring of human environment for the level of PAHs in home dust is the essential part of environmental studies. Levels of PAHs are usually determined according to guidelines of US Environmental Protection Agency (US EPA) [10, 11] that recommends assaying 16 most representative PAHs: naphthalene (Nap), acenanaphthylene (Acy), acenaphtene (Ace), fluorene (Fle), phenanthrene (Ph), anthracene (An), fluorantene (Fla), pyrene (Py), benzo[a]anthracene (BaA), chryzene (Chr), benzo[b]fluorantene (BbF), benzo[k]fluorantene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DahA), benzo[g,h,i]perylene (BghiP), and indeno[1,2,3-c,d]pyren (Ind) in environmental samples. World Health Organization (WHO) and International Agency for Research on Cancer (IARC) have considered the compounds, in particular benzo[a]pyrene as extremely hazardous due to their extreme carcinogenicity and mutagenicity.

The purpose of this study was to determine according to EPA guidelines the content of PAHs in samples of home dust drawn from Warsaw apartments.

Materials and methods

Methods of sample collection

Samples of house dust were collected to cellulose bags from 48 flats (45 of them were situated within the town, 3 outside of the town) using Kärcher vacuum cleaner, in autumn 2003 and spring 2004. Samples were taken from a few places in the house: the living room (floor, shelves), bedroom (beds), kitchen, lavatory. At the same time a survey by questionnaire was carried out, in which some parameters referring to house such as the traffic density, age of the building, surrounding greenery, and to homeowner behaviour as smoking, frequency of housework and air filtration, presence of animals etc. were rated on a gradual scale.

Analytical methods

The aforementioned aromatic hydrocarbons have been assayed by reversed-phase high performance liquid chromatography (HPLC).

The samples were kept at 4 °C in glass containers protected against light. Before the analysis, the samples were sifted to obtain a fraction of particle size < 150 μm. The samples were then extracted by sonication [12] in dichloromethane that has proved to be the most effective among three solvents that had been tried in the study: petroleum ether, hexane and dichloromethane. The extracts were filtered through Nylon filters 25 mm of pore size 0.45 μm, and then evaporated *in vacuo* under nitrogen. The dry extracts were dissolved in acetonitrile and again filtered through PTFE syringe filters 4 mm of pore size 0.2 μm directly before the analysis.

Concentrations of PAHs have been determined using a Dionex liquid chromatograph provided with a UV/VIS diode array detector at the wavelength $\lambda = 254$ nm. The chromatographic system was controlled by Dionex's Chromeleon computer software. A highly selective chromatographic column Bakebound PAH 16-PLUS was used to separate PAHs. The water and acetonitrile in a gradient system has been used to elute PAHs from the column. Test conditions have been set up using EPA-610 reference standard that contains all 16 PAHs listed above and the certified reference material ERM-CC013, having similar matrix composition that has been used in quantitative analysis as the external standard. The studies on assaying PAHs in samples of home dust [13] have been used to optimize test conditions.

Results and discussion

The levels of PAHs found in samples of home dust vary between 8 and 173 mg/kg (Fig.1). According to the standard SBM-2003 [14], the content of PAHs in home dust (Table 1) found in our study is high (in 77 % of the samples very high, in 23 % of samples – extremely high) and it could be one of the sources of health hazards in home environment. The highest level of PAHs has been found in an outpatient clinic just after completed refurbishing. Literature references inform about similar, very high PAHs levels in home dust (up to 500 mg/kg) in Columbus, Ohio at the time of repairing an asphalt road 500 m from the sampling site [15, 16], or in New York City (200–300 mg/kg) after collapse of World Trade Centre [17].

Table 1

Ranges of PAHs concentration in house dust according to the standard SBM-2003 [14]

	Normal	High	Very high	Extremely high
PAHs concentration in house dust ($mg \cdot kg^{-1}$)	< 0.5	0.5–5	5–50	> 50

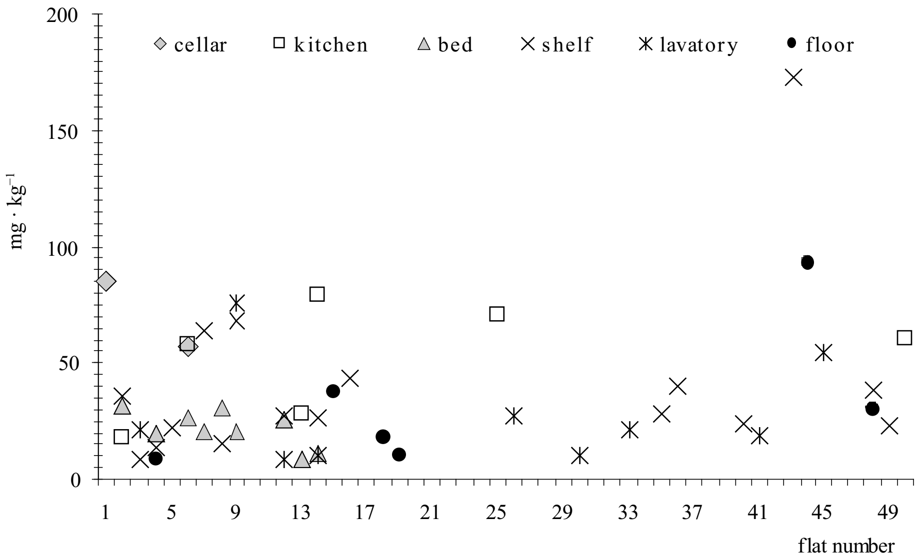

Fig. 1. Concentration of PAHs (the sum of the sixteen PAHs) in house dust from Warsaw

In majority of analyzed samples, extremely high levels of the following hydrocarbons have been found: benzo[b]fluoranthene (BbF), fluoranthene (Fla), pyrene (Py), fluorene (Fle) and phenanthrene (Ph) (Table 2). They constituted about 80 % of the

Table 2

Descriptive statistics referring to PAHs concentration in house dust from Warsaw

PAH	N	Sum	Mean	SD	Median	Max	Min
Nap	48	62.11	1.29	1.77	0.73	7.96	0.06
Acy	48	54.41	1.73	4.68	0.47	31.91	0.01
Ace	48	20.05	0.42	0.40	0.35	2.28	0.01
Fle	48	101.53	2.12	1.29	2.19	5.81	0.05
Ph	48	292.51	6.09	4.27	4.82	18.66	0.80
An	48	39.38	0.82	0.56	0.71	2.12	0.11
Fla	48	324.46	6.76	11.90	2.46	71.75	0.16
Py	48	184.23	3.84	6.98	2.17	38.99	0.13
BaA	48	68.37	1.42	1.94	0.54	8.05	0.02
Chr	48	13.39	0.28	0.36	0.16	1.64	0.00
BbF	48	420.45	8.76	10.27	5.28	49.07	1.51
BkF	48	36.46	0.76	1.74	0.17	10.65	0.01
BaP	48	33.67	0.70	1.78	0.18	11.29	0.01
DahA	48	24.73	0.52	1.00	0.14	4.86	0.01
BghiP	48	20.37	0.42	0.90	0.16	5.66	0.01
Ind	48	18.51	0.39	0.64	0.18	3.98	0.03

16 assayed PAHs (Fig. 2). The results confirm data obtained by other authors, and presented by Maertens [6], where it has been stated that the presence of these hydrocarbons could be a result of burning variety of fuels. A relatively high content of BbF in an urban agglomeration is not surprising as the main sources of this hydrocarbon as well as of Fla and Py are exhaust gases from gasoline and Diesel engines. According to Mukerjee [18], Ph, Fla, and Py hydrocarbons are being emitted from barbecuing and frying processes while Ph and Py are associated with the tobacco burning process. Pyrene and fluoranthene have been suggested as potential source markers for incineration, wood burning and oil combustion [19], with the ratio of fluoranthene to pyrene providing information about a PAH source. If the fluoranthene/pyrene ratio is greater than 1, the PAHs are considered to have been generated by pyrolytic processes, whereas if the ratio is less than 1, they are considered to be petrogenic in origin [20]. In our study, in 62 % of tested samples the ratio is greater than 1, which proves that the hydrocarbons come predominantly from combustion processes.

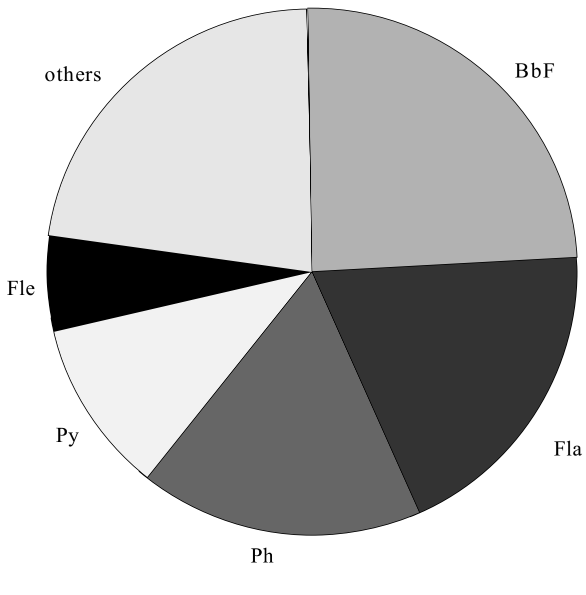

Fig. 2. Proportions [in %] of five PAHs (BbF, Fla, Ph, Py, Fle) in house dust from Warsaw

Among microenvironments, selected for the study, the sum of PAHs in kitchen and cellars has been from two- to three-fold higher as compared with that in other places (beds, shelves, bathrooms, and floors) (Fig. 3). The differences, assessed by the Mann-Whitney test were statistically significant at the level $p < 0.05$. The high content of PAHs in kitchens confirms the suggestion of Mukerjee [18] that gas cooking is significantly increasing the level of PAHs. On the other hand, the high level of PAHs in cellars is most likely associated with a long lasting sedimentation of dust due to infrequent cleaning.

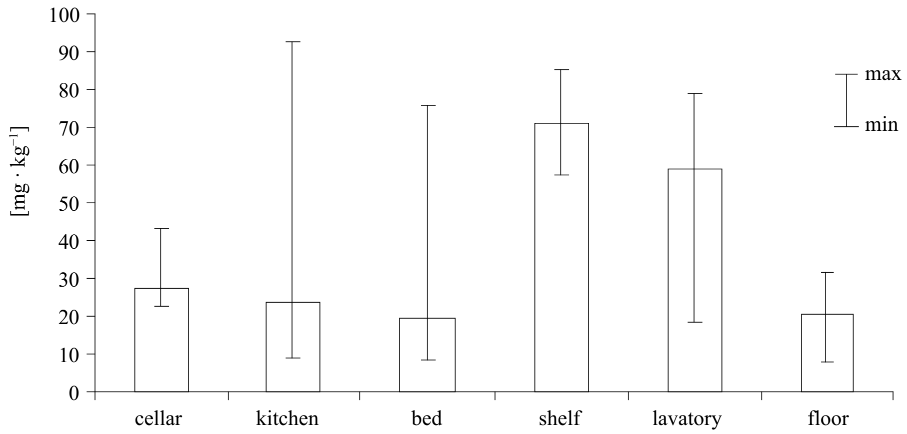

Fig. 3. Median values of PAHs concentration (the sum of the sixteen PAHs) in dust from different mikroenvironments

The high level of PAHs in dust samples is presumably related to numerous factors that have impact on concentration of these substances. In our studies, we have analyzed data from questionnaires concerning tobacco smoking, frequency of cooking, mobility of inhabitants and ventilation and their impact on the content of PAHs in dust samples. The results show a clear trend to an increased concentration of PAHs in apartment of tobacco smokers (Fig.4). A similar trend has also been found by Lewis [21] and Mukerjee [18].

A comparison of variability coefficients for the hydrocarbons that constitute about 80 % of all analyzed PAHs (BaP, Fla, BbF, BaA, Ph and Py) have proven that inter-

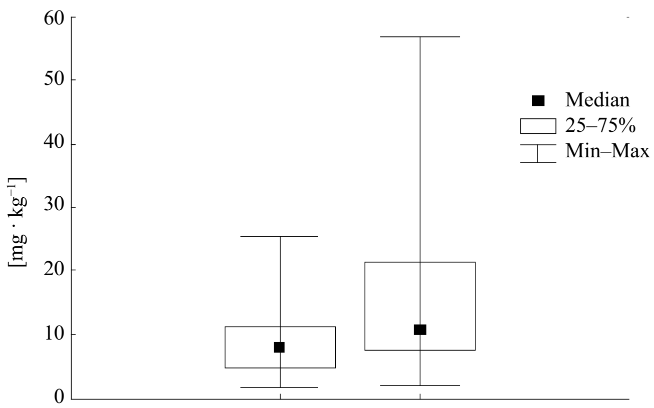

Fig. 4. Comparision of concentration of four PAHs (Ph, BaP, Chr and Fla) in house dust from nonsmokers (1) and smokers (2) apartments

apartment variability of the levels of these hydrocarbons is larger than that observed for various microenvironments within the same apartment. However, high PAHs variability coefficients observed within the apartments (although slightly lower than those inter-apartment ones), show an impact of environmental factors within an apartment on the level of PAHs. This observation confirms the above discussed trends associated with tobacco smoking or gas cooking. Inflow of hydrocarbons into the apartments along with the air aerosols that contain the compounds originating in industrial burning processes, asphalt abrasion and exhaust gases from combustion engines is the key issue. In this aspect, the impact of weather conditions that favor formation of smog and accumulation of pollutants, or strong winds that would counteract these conditions and disperse the pollutants accumulated in the air aerosol around wide area. The authors of all studies on PAHs emphasize that small impact of tobacco smoking, location, frequency of ventilation and other factors suggests that the content of PAHs in dust depends much on the sum of all environmental factors.

No significant differences have been found in the content of PAHs in samples drawn in Warsaw and in those drawn in Dziekanów Leśny near Warsaw and in Famułki Królewskie, a village approximately 50 km from Warsaw, in the middle of Kampinos Forest (*Puszcza Kampinosk*a), away from any industry, where one could expect a lower pollution level. The most probable explanation of this observation is that the sample has been drawn in a forester's lodge, where large amount of wood is burned, in particular pinewood. Pinewood contains large amounts of resin that emits large amounts of PAHs upon combustion.

One should emphasize that in the natural environment aromatic hydrocarbons could undergo decomposition caused by such factors as sunlight, oxygen, ozone, increased temperature. The impact of these factors in home environment is smaller, and on the other hand, home environment favors accumulation of the hydrocarbons and their absorption by deposited dust. It is confirmed by high levels of PAHs found in cellars. Considering the above facts, and because more and more time is being spent indoor these days, one should avoid accumulation of dust in his/her nearest environment. It is particularly important for little children that by putting their hands and dusty objects in their mouth can swallow from 50 to 100 mg PAHs per day [6]. This route of entry for dust and the noxious substances accumulated on it could be a reason of increased incidence of diseases (allergies and similar diseases) in preschool children.

References

[1] Lee M.L., Novotny M.V. and Bartle D.: Analytical chemistry of polycyclic aromatic compounds. Academic Press, Tunbridge, Wells. 1997, England, pp. 46.

[2] US EPA: Raport for PAH Matter. 1984, Durham.

[3] Maliszewska-Kordybach B.: *Sources, concentrations, fate and effects of PAHs in the environment. Part A PAHs in air.* Polish J. Environ. Stud., 1999, **8**(3), 131–136.

[4] Zakrzewski S. F.: Podstawy toksykologii środowiska. 1995 PWN, Warszawa.

[5] Dridi S., Driss M.R., Sabbah S. and Bouguerra M.L.: *Determination of aromatic hydrocarbons in airborne Diesel exhaust particulates by HPLC with UV detection and wavelength programming*. J. Liquid. Chrom. & Relet. Technol., 1998, **21**(4), 475–489.

[6] Maertens R.M. et al.: *The mutagenic hazards of settled house dust: a review*. Mutat. Res., 2004, **567**, 401–425.

[7] *Substancje rakotwórcze w środowisku pracy – wielopierścieniowe węglowodory aromatyczne*. t. 2, Instytut Medycyny Pracy, Łódź 1987.
[8] IARC Monographs: The Evaluation of the Carcinogenic Risk of Chemicals to Humans. Polynuclear Aromatic Compounds, **32**, Part II, Lyon 1983.
[9] IARC Monographs: The evaluation of carcinogenic risks to humans. **46**, 41–185. Lyon 1989.
[10] US EPA: *Method 610 Polynuclear Aromatic Hydrocarbons*. Federal Register, 1984, **49**(209), 112.
[11] Warzecha L., Luks-Betlej K. and Bodzek D.: *Determination of polynuclear aromatic hydrocarbons in aerosol by Solid-Phase Extraction and Gas Chromatography-Mass Spectrum*. Chem. Anal., **33**, 1988.
[12] Kicinski H., G. Adamek S. and Kettrup A.: *Trace enrichment and HPLC analysis of polycyclic aromatic hydrocarbons in environmental samples, using Solid Phase Extraction in connection with UV/VIS Diode-Array and Fluorescence Detection*. Chromatographia, 1989, **28**(3/4), 203–208.
[13] Butte W. and Heinzow B.: *Pollutants in house dust as indicators of indoor contamination.* [In:] Reviews of Environmental Contamination and Toxicology, (Ed: Ware C.). Springer-Verlag, New York, Heidelberg, 2002, **175**, 1–46.
[14] SBM: Standard of baubiologie methods of testing. 2003 – www.baubiologie-ibn.de.
[15] Chuang J.: *Monitoring methods for polycyclic aromatic hydrocarbons and distribution in house dust and truck-in soil*. Environ. Sci. Technol., 1995, **29**, 494–500.
[16] Chuang J.C. at al.: *Analysis of soil and house dust for polycyclic aromatic hydrocarbons.* EPA/600/SR-96/060, 1996.
[17] Lioj P.J. at al.: *Characterization of the dust/smoke aerosol that settled East of the World Trade Center (WTC) in Lower Manhattan after the collapse of the WTC 11 September 2001*. Environ. Health Perspect., 2002, **110**(7), 703–714.
[18] Mukerjee S., Ellenson W.D., Lewis R.G., Stevens R.K., Somerville M.C., Shadwick D.S. and Willis R.D.: *An environmental scooping study in the Lower Rio Grande Valley of Texas: III. Residential microenvironmental monitoring for air, house dust, and soil*. Environ. Int., 1997, **23**(5), 657–673.
[19] Harrison R.M., Smith D.J.T. and Luhana L.: *Source apportionment of atmospheric polycyclic aromatic hydrocarbons collected from an urban location in Birmingham*. UK. Environ. Sci. Technol, 1996, **30**, 825–832.
[20] Baumard P., Budzinski H. and Garrigues P.: *Polycyclic aromatic hydrocarbons in sediment and mussels of the Western Mediterranean Sea.* Environ. Toxicol. Chem., 1998, **17**, 765–776.
[21] Lewis R.G. at al.: *Distribution of pesticides and polycyclic aromatic hydrocarbons in house dust as a function of particle size*. Environ. Health Perspect., 1999, **107**(9), 721–726.

WIELOPIERŚCIENIOWE WĘGLOWODORY AROMATYCZNE W KURZU MIESZKAŃ WARSZAWSKICH

Zakład Biologii Antarktyki PAN, Warszawa
Ceentrum Badań Ekologicznych PAN, Dziekanów Leśny

Abstrakt: Badano poziom zanieczyszczenia wielopierścieniowymi węglowodorami aromatycznymi (WWA[PAHs]) kurzu z mieszkań warszawskich. Próbki pobrano z 48 mieszkań, z kilku miejsc w każdym mieszkaniu: pokój dzienny (podłoga, półki), sypialnia (łóżko), kuchnia, łazienka. Równocześnie przeprowadzano ankietę, w której w kilku stopniowej skali oceniano różne czynniki zewnętrzne i wewnętrzne. Stężenie WWA w próbkach oznaczono metodą chromatografii cieczowej (HPLC). Uzyskane zakresy stężeń dla sumy szesnastu WWA, wahają się od 8 do 173 mg/kg. Wartości stężeń sumy WWA w większości próbek były duże, w granicach od 5 do 50 ppm, co może stanowić duże zagrożenie dla zdrowia. W kilkunastu próbkach stężenia WWA osiągały wartości bardzo duże. W większości próbek stwierdzono wyjątkowo duże stężenia takich węglowodorów, jak: benzo[b]fluoranten, fluoranten, piren, fluoren i fenantren. W wytypowanych do badań mikro-środowiskach stwierdzono dwu-trzykrotnie większe stężenie sumy szesnastu WWA w kuchniach i piwnicach niż w pozostałych miejscach. Porównanie zawartości węglowodorów w kurzu ze zwaloryzowanymi danymi z ankiety wskazuje na wyraźną tendencję większego stężenia WWA w mieszkaniach osób palących.

Słowa kluczowe: kurz domowy, Warszawa, węglowodory aromatyczne

Vol. 16, No. 7 2009

Maciej WALCZAK[1] and Maria SWIONTEK BRZEZINSKA[1]

INFLUENCE OF REACTIVE PHOSPHORUS (RP) CONCENTRATIONS ON OCCURRENCE OF HETEROTROPHIC BACTERIA CAPABLE OF MATTER TRANSFORMATION, INCLUDING PHOSPHORUS IN WATER ENVIRONMENT

WPŁYW STĘŻEŃ REAKTYWNYCH FORM FOSFORU NA WYSTĘPOWANIE W ŚRODOWISKU WODNYM BAKTERII HETEROTROFICZNYCH ZDOLNYCH DO PRZEMIAN MATERII ZAWIERAJĄCEJ FOSFOR

Abstract: This survey has been aimed at estimation of the Vistula water number of heterotrophic bacteria, capable of decomposition of various phosphorus compounds, both organic and inorganic ones, as well as determination of bacteria participation in biogeochemical phosphorus cycle. The studies were conducted from spring 2000 to spring 2001. The water for analyses was sampled from three current sites of the Włocławek Reservoir along former bed of the Vistula River. In this studies has been estimated number of heterotrophic bacteria, capable to release mineral phosphorus from organic and inorganic matter. The result of the conducted study demonstrated that the key role in recovery of biologically available phosphorus amounts is the one of bacteriological phosphatases.

Keywords: phosphorus in water, release of mineral phosphorus from matter, heterotrophic bacteria

Phosphorus is one of the most important elements for both autotrophic and heterotrophic organisms. Phosphoric acid radicals are comprised in ADP and ATP, as well as oxidoreductases NADP, which take part in transfer of energy and phosphoric acid radicals into intermediate products of photosynthesis or respiration. This element is contained in nucleic acids (RNA and DNA).

Environmental shortage of phosphate limits the ecosystem productivity while its excess in water environment leads to eutrophication [1, 2]. Environmental phosphorus can be divided into two major groups of compounds: organic and mineral. Organic phosphorus compounds emerging in water origin mainly from floral and animal deritus, micro-

[1] Department of Environmental Microbiology and Biotechnology; Nicolaus Copernicus University, ul. J. Gagarina 9, 87-100 Toruń, Poland, email: walczak@umk.pl

biological synthesis products, and possibly sewage [3]. These substances influenced by microorganisms undergo active dephosphorylation with phosphates abstraction. Enzymatic phosphatase play special role in this process [4]. Heterotrophic bacteria and other microorganisms produce alkaline and acidic phosphatases as well as 5'-nucleotidase, *ie* enzymes which release mineral phosphorus from such compounds as nucleotides, phosphosaccharides and phospholipids, contributing to decomposition of organic phosphorus forms [5]. Released phosphate is partially reassimilated by microorganisms, which cause degradation within processes of biochemical decompositions. Plants absorb large part of it. However, in water environments some amount of released phosphate is likely to bind cations of calcium, magnesium, aluminium or iron and produce sparingly soluble phosphates, which sink as deep as to bottom sediments [3]. Microorganisms biochemical activity triggers transformations of inorganic phosphates. This is brought about by acids produced by microorganisms [6]. In case of heterotrophic microorganisms, phosphate transformation is triggered by the impact of produced organic acids or complex-creative factors. Autotrophs are also capable of transforming insoluble phosphates through production of sulphuric and nitric acids.

This survey has been aimed at estimation of the Vistula water number of heterotrophic bacteria, capable of decomposition of various phosphorus compounds, both organic and inorganic ones, as well as determination of bacteria participation in biogeochemical phosphorus cycle.

Material and methods

Samples were taken from spring 2000 to spring 2001. The water for the purpose of physicochemical and microbiological analyses was sampled from three current sites of the Włocławek Reservoir along former bed of the Vistula River.

Site I "Płock" – situated by the road bridge in Płock (632 km of the river reaches). It is typical river site with average flow velocity of ca 0.1 m $\cdot$ s^{-1}.

Site II "Dobiegniewo" – in the vicinity of Dobrzyn nad Wisla (660 km of the river reaches). It is a middle section of the reservoir with a vast overflow area. This site has not a typical river nature. The average water flow velocity amounts to ca 0.1 m $\cdot$ s^{-1}.

Site III "Włocławek dam" – the samples were taken around 300–400 m before the dam (675 km of the river reaches) from the surface layer as well as the bottom water (site IV) at a depth of ca 11 m, *ie* 0.5 m above the bottom sediment. The average water flow velocity amounts to ca 0.1–0.4 m $\cdot$ s^{-1}.

The water for the purpose of analyses was received by means of Patalas' sampler and poured into sterile glass containers. Collected water samples were carried to the laboratory in sealed container at the temperature of ca + 7 °C.

The number of heterotrophic bacteria (CFU) the analysed samples contained was estimated according to grated screening method with application of yeast extract medium. 0.1 cm^3 accordingly dissolved water was delivered to the medium surface and spread with a sterile glass device. The incubation lasted 7 days at 20 °C. The emerged colonies were counted and received numbers were converted into number of heterotrophic bacteria in investigated water considering the material dissolution degree.

Isolation of bacterial strains. Representative strains collection was detached from the cultures that were a base of heterotrophic bacteria number estimation. Each site was represented by ca 20 strains, which were transported into the yeast extract medium bevels. After microbiological material has been generated the bevels were stored at 4 °C yeast for the purpose of further research. Bacterial strains were transferred into fresh yeast extract media every two months.

Concentrations of reactive phosphorus (RP) were estimated according to Hermanowicz [7]

Production of alkaline phosphatase by bacterial strains was analysed by culturing them on the medium containing:

KNO_3 – 0.5 g; $MgSO_4 \cdot 7H_2O$ – 0.4 g; $CaCl_2 \cdot 2H_2O$ – 0.2 g; NaCl – 0.1 g; Fe Cl_3 – 0.01 g; casein hydrolysate – 4.0 g; H_2O (d) – 1000 cm^3; pH – 7.0–7.2.

The medium was distributed among 5 cm^3 test tubes and sterilized at 117 °C for 20 min. Afterwards the medium was populated with investigated strains and incubated at 26 °C for 24 hours. Next step involved addition of 1 cm^3 sterile colourless substrate solution *p*-nitrophenyl phosphate (Sigma) in 1 $mg \cdot cm^{-3}$ concentration, prepared in Tris-HCl buffer (pH 9.0). After further 24 hours of incubation in darkness, the colour of the culture was observed. Yellow colour would have proved a hydrolysis of the substrate into a coloured product as a result of exogenous phosphatases.

Capability of DNA hydrolyse by surveyed strains was tested with application of the medium DNase TEST AGAR (DIFCO). The sterile medium on pans was inoculated with the surveyed strains applying an inoculation loop. The incubation took place at 20 °C for 24–72 hours. 1 M HCl was used for reading off the results. A positive result was deemed emerging of brighter zone around the colony – an evidence for DNA decomposition.

Capability of lecithin decomposition was conducted using test medium [7]

The sterile medium on the Petri dishes was inoculated with the surveyed strains using inoculation loop. The results were read after 2 and 4 days of incubation at 26 °C. A positive result was indicated by cloudiness zones around the colony.

Capability of mineral phosphorus compounds decomposition by bacterial strains was surveyed by culture them on the medium with a content of $Ca_3(PO4)_2$ according to Rodina [8]. The sterile medium on the Petri dishes was inoculated with the surveyed strains using an inoculation loop. The incubation was conducted at 20 °C for 7 days. Starting from 2 days of incubation, the results were read every 2 days. Emerging of brighter zones around the colony indicated a positive result.

Results and discussion

Phosphorus plays a key part in water ecosystems functioning. It is the most important element determining water environment fertility. Therefore the research on the phosphorus biogeochemical cycle are rated among the most essential activities concerning element circulation.

Bacteria constitute a key and dominant factor of mineral compounds and organic matter circulation processes in any environment. Water microorganisms metabolic processes activity and rate determine circulation of series of elements, both in specific ecosystems and on a global scale [9–12]. Heterotrophic bacteria are particularly significant, since they are a vital component of trophy network of natural water environments. These microorganisms are responsible for processes of degradation, mineralization, and disposal of organic matter. Heterotrophic bacteria enable energy flow as well as biogene circulation in water ecosystems [13].

Conducted studies confirmed that the heterotrophic bacteria number in the investigated length of the Lower Vistula within the whole research period ranged from $3.5 \cdot 10^3$ to $145.5 \cdot 10^3$ (Table 1). Such substantial differences of bacteria numbers in the investigated water are results of specific nature of the Vistula. Since the Vistula is the biggest Polish river, it collects waters from a vast area. Furthermore, the water levels in the river as well as the amounts of suspended solids significantly vary depending on the season and precipitation volumes. The organic suspension is a nutritional source of supply for the heterotrophic bacteria, while according to Donderski [14] it is the most vital factor affecting bacteria growth in water bodies. Large numbers of bacteria in surveyed water were observed in the spring 2001. Seasonal increase is connected with water body refill with a fresh nutritional substance carried with waters from spring melt.

Table 1

Numbers of heterotrophic bacteria in investigated samples of water (cells $\cdot$ 10^3 $\cdot$ cm^{-3})

Date of sampling	Site I	Site II	Site III	Site IV
May 2000	21.50	130.00	3.50	45.00
August 2000	49.50	10.50	6.00	7.50
November 2000	78.00	55.00	13.00	42.00
March 2001	108.50	145.50	71.50	32.50
Average	64.38	85.25	23.50	31.75

Apart from estimation of heterotrophic bacteria number, which constituted a background for further investigations, this study included estimation of number of bacteria capable of decomposition of various compounds containing phosphorus.

Many investigations confirmed that heterotrophic bacteria and other microorganisms secrete series of hydrolytic enzymes that able decompose organic matter. At least three primary groups of phosphohydrolytic enzymes commonly produced by water organisms take part in the processes of organic phosphorus derivatives decomposition and disposal. These inculde phosphoesterases, that are alkaline and acidic phosphatases, nucleotidases (mainly 5'-nucleotidase), and nucleases (endo- and exonucleases): RNAse and DNAse that decompose nucleic acids RNA and DNA [4]. Most of them are exoenzymes. Only some of them are likely to occur in the environment in forms of free enzymes or enzymes adsorbed on seston or on mineral particles [15].

A has been demonstrated by former research, on average: 52.42 % (site I); 32.90 % (site II); 45.88 % (site III); 26.66 % (site IV) of bacterial strains produced exogenous phosphatases (Table 2).

Table 2

Occurrence of bacteria capable of various phosphorus forms transformations (in %)

Date of sapling	Production of alkaline phosphatase	DNA hydrolyse	Lecithin decomposition	Mineral phosphorus decomposition
Site I				
May 2000	68.00	28.00	0.00	4.00
August 2000	56.67	26.67	10.00	3.33
November 2000	20.00	44.00	8.00	12.00
March 2001	65.00	55.00	5.00	0.00
Average	52.42	38.42	5.75	4.83
Site II				
May 2000	57.14	46.67	16.67	3.33
August 2000	13.33	9.52	9.52	0.00
November 2000	11.11	44.44	0.00	0.00
March 2001	50.00	25.00	0.00	0.00
Average	32.90	31.41	6.55	0.83
Site III				
May 2000	87.50	75.00	12.50	0.00
August 2000	25.00	0.00	0.00	0.00
November 2000	4.35	30.43	8.70	17.39
March 2001	66.67	20.00	5.00	0.00
Average	45.88	31.36	6.55	4.35
Site IV				
May 2000	22.22	11.11	11.11	0.00
August 2000	25.00	15.00	0.00	0.00
November 2000	29.41	47.06	0.00	0.00
March 2001	30.00	15.00	0.00	0.00
Average	26.66	22.04	2.78	0.00

Production of phosphatases by bacteria is affected by RP concentration in water. RP concentration levels are presented in Table 2. Siuda [15] proved that alkaline phosphatases content varies depending on phosphorus availability in the environment. A rapid increase of alkaline phosphatases activity was observed in the cells of algae and bacteria, which were deprived of phosphorus sources, on more than one occasion. In conditions of excessive phosphorus amounts there were minimum levels of these enzymes. In the presented study we noted, that in March when the concentrations of RP were very low (Table 3), the percentages of bacteria capable of production of alkaline phosphatase were high. In May, the concentrations of RP were also very high, but in late spring, phosphorus is frequently released microbiologically from the sediments into the water depths [2].

Table 3

The concentrations of RP in investigated samples of water [mg · cm^{-3}]

Date of sampling	Site I	Site II	Site III	Site IV
May 2000	0.085	0.810	0.085	0.092
August 2000	0.044	0.112	0.061	0.092
November 2000	0.059	0.056	0.058	0.086
March 2001	0.029	0.036	0.032	0.032

This study included also decomposition of: DNA, lecithin, and mineral phosphorus compounds. According to Rodina [8] many microorganisms that have an enzyme phosphatases conduct hydrolysis of organic compounds with content of phosphorus. It is assumed that there are 15–20 enzymes of that group, which vary with respect to chemical nature of hydrolysed substrates. Phosphatases affect nucleic acid and lecithin beside other enzymes, eventually causing an abstraction of phosphates by series of transition compounds.

The data concerning DNA decomposition analysis confirms that mean contents of those bacteria in waters (sites I-IV) amounted to: 38.42; 31.41; 31.36; and 22.04%, respectively (Table 2).

In the study pertaining to the lake Jeziorak Mały, Donderski et al [16] obtained results, which proved that 27 % of total isolated bacteria were capable of DNA hydrolysis. Present study results concerning contents of bacteria capable of DNA decomposition are very similar.

The data concerning lecithin confirm that this compound was decomposed to a much lower degree by bacteria inhabiting investigated waters. Mean percentages of bacteria capable of lecithin mineralization on sites I–IV waters amounted to 5.75; 6.55; 6.55, and 2.78 %, respectively (Table 2). In the studies, lecithin decomposing bacteria conducted in the same Vistula length Piasecka [17] received following results: November 2000 as much as 77.14 % strains decomposed lecithin; March 2001 – 65.22 %; May 2001 – 66.67 %; July 2001 – 40 %. The differences between results of both studies pertaining to lecithin decomposition abilities can corroborated by justified with various results concerning phosphatases production, which in Piasecka's study amounted to 100 %, whereas in present study were 39.6 %. Results obtained by Wu Gen Fu and Zhoe Xue-Ping [6] proved that only small number of bacterial strains was capable of lecithin decomposition. They made up as little as ca 13 in 1 cm^3.

Analysis of occurrence of bacterial strains capable of mineral phosphorus compounds decomposition suggests that there were few of such strains. In water on individual sites they constituted a minor percentage from 0 to 4.83 % (Table 2). Hence, the role those bacteria play in the Vistula water enrichment with mineral phosphorus forms does not seem significant. Bacterial strains capable of RP release are abundantly represented in bottom sediments – their numbers can reach level of $5 \cdot 10^4$ cells/cm^{-3} [6]. Mentioned authors claim that those strains numbers in water is insignificant – ca. 100 cells/cm^{-3}.

Summing, for capability of transforming various phosphorus forms, both organic and inorganic ones, heterotrophic bacteria are immensely important elements of trophy network within water ecosystems. This study confirms results of Hermandez et al [4] that the key role in recovery of biologically available phosphorus amounts is the one played by bacteriological phosphatases.

References

[1] Mohamed M.N., Lawrence J.R. and Robarts R.D.: Microb. Ecol. 1998, **36**, 121–130.
[2] Mainstone C.P. and Parr W.: Sci. Total Environ. 2002, **282–283**, 25–47.
[3] Neal C., Howarth S.M., Whitehead P.G., Williams R.J., Neal M. and Wickham H.: Sci. Total Environ. 2000, **251–252**, 477–495.
[4] Hermandez I., Perez-Pastor A. and Llorens J.L.: Aquatic Ecol. 2000, **34**, 107–117.
[5] Ammerman J.W. and Azam F.: Limnol. Oceanogr. 1991, **36**, 1427–1436.
[6] Gen-Fu W. and Xue-Ping Z.: Water Res. 2005, **39**, 4623–4632.
[7] Hermanowicz W.: Fizyczno-Chemiczne Badanie Wody i Ścieków, Arkady, Warszawa 1976.
[8] Rodina A.: Mikrobiologiczne metody badania wód. PWRiL, Warszawa, 1968.
[9] Hobbie J.E. and Melillo J.M.: Current perspectives in micriobial ecology, American Society for Microbiology, Washington, 1983.
[10] Krumbein W.E.: Micriobial geochemistry. Blackwell, Oxford 1983.
[11] Cho B.C. and Azam F.: Mar. Ecol. Prog. Ser. 1990, **63**, 253–259.
[12] Azam F., Smith D.C., Steward G.F. and Hagström E.: Microb. Ecol. 1995, **28**, 167–179.
[13] Gajewski A.J. and Chróst R.J.: Biotechnologia 1994, **26**, 12–22.
[14] Donderski W.: Tlenowe bakterie heterotroficzne jezior o różnej trofii. Rozprawy UMK, Toruń 1983.
[15] Siuda W.: Post. Mikrobiol. 2001, **40**, 187–217.
[16] Donderski W., Walczak M., Mudryk Z. and Skórczewski P.: Polish J. Environ. Stud. 1998, **7**, 125–129.
[17] Piasecka K.: Identyfikacja bakterii heterotroficznych występujących w toni wodnej Dolnej Wisły na odcinku Wyszogród–Toruń. Praca magisterska, UMK, Toruń 2002.
[18] Nitsch B. and Kutzner H.J.: Experientia 1969, **25**, 220–221.

WPŁYW STĘŻEŃ REAKTYWNYCH FORM FOSFORU NA WYSTĘPOWANIE W ŚRODOWISKU WODNYM BAKTERII HETEROTROFICZNYCH ZDOLNYCH DO PRZEMIAN MATERII ZAWIERAJĄCEJ FOSFOR

Zakład Mikrobiologii Środowiskowej i Biotechnologii
Uniwersytet Mikołaja Kopernika

Abstrakt: Celem pracy było oznaczenie liczebności bakterii heterotroficznych w wodzie Wisły, zdolnych do rozkładu różnych związków fosforu zarówno organicznych, jak i nieorganicznych oraz określenie udziału bakterii w cyklu biogeochemicznym fosforu. Badania prowadzono od wiosny 2000 r. do wiosny 2001 r. Próbki wody do badań pobierano z trzech stanowisk nurtowych Zbiornika Włocławskiego wzdłuż dawnego koryta rzeki Wisły. W badaniach oznaczano liczebność bakterii heterotroficznych zdolnych do uwalniania mineralnego fosforu z materii organicznej i nieorganicznej. Wyniki przeprowadzonych badań wskazują, że najważniejszą rolę w odbudowie dostępnej biologicznie puli mineralnego fosforu odgrywają bakteryjne fosfatazy.

Słowa kluczowe: fosfor w wodzie, uwalnianie z materii mineralnego fosforu, bakterie heterotroficzne

Varia

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 of 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),
email: chemanal@pg.gda.pl
homepage: http://www.pg.gda.pl/chem/ichmet/

INVITATION FOR ECOPOLE '09 CONFERENCE

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 18th annual Central European Conference ECOpole '09, which will be held in **14–17 X 2009** (Thursday–Saturday) on Wilhelms Hill at Uroczysko in Piechowice, PL.

The Conference Programme includes oral presentations and posters and will be divided into four sections - SI, SII, SIII and SIV:

– **SI – Chemical Pollution of Natural Environment and its Monitoring;**
– **SII – Environment Friendly Production and Use of Energy;**
– **SIII – Forum of Young Scientists and Environmental Education;**
– **SIV – 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 presentations (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: **01.10.2009**. 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
University of Opole,
email: Maria.Waclawek@uni.opole.pl
and mrajfur@o2.pl
tel. +48/77/ 401 60 42
fax/tel. +48/77/ 455 91 49

Conference series

1. 1992 Monitoring ’92 Opole
2. 1993 Monitoring ’93 Turawa
3. 1994 Monitoring ’94 Pokrzywna
4. 1995 EKO-Opole ’95 Turawa
5. 1996 EKO-Opole ’96 Kędzierzyn-Koźle
6. 1997 EKO-Opole ’97 Duszniki Zdrój
7. 1998 CEC ECOpole ’98 Kędzierzyn-Koźle
8. 1999 CEC ECOpole ’99 Duszniki Zdrój
9. 2000 CEC ECOpole 2000 Duszniki Zdrój
10. 2001 CEC ECOpole ’01 Duszniki Zdrój
11. 2002 CEC ECOpole ’02 Duszniki Zdrój
12. 2003 CEC ECOpole ’03 Duszniki Zdrój
13. 2004 CEC ECOpole ’04 Duszniki Zdrój
14. 2005 CEC ECOpole ’05 Duszniki Zdrój
15. 2006 CEC ECOpole ’06 Duszniki Zdrój
16. 2007 CEC ECOpole ’07 Duszniki Zdrój
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		

ACCOMODATION

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 czterech Sekcjach SI–SIV:

– **SI – Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring**,

– **SII – Odnawialne źródła energii i jej oszczędne pozyskiwanie oraz użytkowanie**,

– **SIII – Forum Młodych (FM) i Edukacja prośrodowiskowa**,

– **SIV – 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: miesięczniku lub kwartalniku *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 Konfe-rencji 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 2008 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. 77 401 60 42
tel. 77 455 91 49
fax 77 401 60 51

Kalendarium

1. 1992 Monitoring ’92 Opole
2. 1993 Monitoring ’93 Turawa
3. 1994 Monitoring ’94 Pokrzywna
4. 1995 EKO-Opole ’95 Turawa
5. 1996 EKO-Opole ’96 Kędzierzyn-Koźle
6. 1997 EKO-Opole ’97 Duszniki Zdrój
7. 1998 ŚEK ECOpole ’98 Kędzierzyn-Koźle
8. 1999 ŚEK ECOpole ’99 Duszniki Zdrój
9. 2000 ŚEK ECOpole 2000 Duszniki Zdrój
10. 2001 ŚEK ECOpole ’01 Duszniki Zdrój
11. 2002 ŚEK ECOpole ’02 Duszniki Zdrój
12. 2003 ŚEK ECOpole ’03 Duszniki Zdrój
13. 2004 ŚEK ECOpole ’04 Duszniki Zdrój
14. 2005 ŚEK ECOpole ’05 Duszniki Zdrój
15. 2006 ŚEK ECOpole ’06 Duszniki Zdrój
16. 2007 ŚEK ECOpole ’07 Duszniki Zdrój
17. 2008 ŚEK ECOpole ‘08 Piechowice

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

Nazwisko i imię . . . .

Tytuł (stopień) naukowy/stanowisko . . . .

Miejsce pracy . . . .

Adres . . . .

Tel./Fax . . . . email . . . .

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

. . . .

. . . .

. . . .

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			—

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 A*
(Ecol. Chem. Eng.)
Uniwersytet Opolski
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 or mail 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 recent 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, eg Corel-Draw, Grapher for Windows or at least in a bitmap format (TIF, PCX, 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:

[1] 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, pp. 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. 77 401 60 42, fax 77 455 91 49
email: waclawek@uni.opole.pl

w postaci cyfrowej w formacie Microsoft Word (ver. 7.0 dla Windows) emailem (mrajfur@o2.pl) lub na dyskietce.

Redakcja przyjmuje, że przesyłając artykuł do druku autor w ten sposób oświadcza, że jest upoważniony do tego oraz zapewnia, że artykuł ten jest oryginalny i nie był wcześniej drukowany gdzie indziej i nie jest wysłany do druku gdzie indziej oraz, że po jego wydrukowaniu copyright do tego artykułu uzyskuje Towarzystwo Chemii i Inżynierii Ekologicznej.

W przygotowaniu manuskryptu należy przede wszystkim wzorować się na postaci najnowszych artykułów opublikowanych w Ecological Chemistry and Engineering, na przykład zamieszczanych na stronie webowej Towarzystwa: http://tchie.uni.opole.pl/tchie/index.php?option=content&pcontent=1&task=view&id=49&Itemid=76

Prace przesyłane do publikacji winny być napisane w języku angielskim oraz zaopatrzone w streszczenia oraz słowa kluczowe w języku angielskim oraz polskim.

Zalecamy, ażeby artykuł zawierał adresy i emaile oraz numery telefonów i faksów wszystkich autorów danej pracy, szczególnie głównego autora, którego nazwisko wyróżniamy gwiazdką.

Usilnie prosimy o stosowanie układu jednostek SI. Zwracamy uwagę, że osie wykresów oraz główki tabel powinny bezwzględnie zawierać jednostki stosownej wielkości. Polecamy symbolikę zalecaną przez PTChem (Symbole i terminologia wielkości i jednostek stosowanych w chemii fizycznej, Ossolineum, Wrocław 1989; Pure Appl. Chem. 1979, **51**, 1–41). Materiał graficzny (rysunki, wykresy), obok wersji na papierze, powinien również być dostarczony w postaci cyfrowych plików wektorowych, np. za pomocą programu: CorelDraw wersja 3.0–8.0, Grafer dla Windows lub przynajmniej bitowe (TIF, PCX, BMP). W przypadku trudności z wypełnieniem tego warunku Redakcja zapewnia odpłatne wykonanie materiału graficznego na podstawie dostarczonego szkicu. Bliższe informacje można uzyskać telefonicznie 77 401 60 42.

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.

Tytuły czasopism należy skracać zgodnie z zasadami przyjętymi przez amerykańską Chemical Abstracts Service. Autor może, jeżeli uważa to za wskazane, podawać też tytuł cytowanych artykułów z czasopism, który będzie składany kursywą oraz numer zeszytu danego woluminu (w nawiasie, po numerze woluminu).

Redakcja potwierdza emailem otrzymanie artykułu do druku. W przypadku braku potwierdzenia prosimy o interwencję: emailem, faksem, listem lub telefonicznie.

REDAKTOR TECHNICZNY
Halina Szczegot

SKŁAD KOMPUTEROWY
Henryk Kobiela

PROJEKT OKŁADKI
Marian Wojewoda

Druk: „Drukarnia Smolarski", Józef Smolarski, 45-326 Opole, ul. Sandomierska 1. Objętość: ark. wyd. 15,25, ark. druk. 12,75.