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VARIA

SPIS TREŚCI

VARIA

Mariusz DUDZIAK[1]

CHANGE OF TOXICITY OF WATER CONTAINING BISPHENOL A DURING ITS TREATMENT BY COMPLEX OXIDATION PROCESS

ZMIANY TOKSYCZNOŚCI WODY ZAWIERAJĄCEJ BISFENOL A W TRAKCIE JEJ UZDATNIANIA W ZŁOŻONYM PROCESIE UTLENIAJĄCYM

Abstract: Water containing bisphenol A was UV irradiated (medium pressure immersion lamp with the electric power of 150 W) with and without the addition of H_2O_2 (6–12 mg/dm^3 dose). To control of the water quality Microtox® biotest was used. Bioluminescent bacteria *Vibrio fisheri* was involved. Decomposition of bisphenol A was depended on the UV irradiation time and dose of the H_2O_2. The observations connected with the bioluminescence value in the examined solutions were surprised. Decomposition of the compound did not cause of decrement the bioluminescence inhibition value characterizing solution indicating the formation of toxic intermediates products. However, the combined use of H_2O_2 with UV radiation improves the rate of decomposition of bisphenol A, but also causes an increase in bioluminescence inhibition of the solutions. For this reason selection of the most favorable conditions for the oxidative process have to be proceed based on both agents: effectiveness of the compounds decomposition and the toxicity of the solution after process.

Keywords: bisphenol A, water treatment, toxicity, Microtox® biotest

Modern technologies of water and waste water treatment are increasingly using various chemical oxidation processes. Oxidation in the water treatment may be used for different purposes, mostly for the oxidation of Fe(II), Mn(II) and other reduced inorganic substances, oxidation of organic substances of natural and anthropogenic origin and for disinfection [1]. Chemical oxidants may be added to the treated water at different stages of the treatment system, thus preliminary, intermediate and final steps of disinfection can be distinguished. In the case of waste water treatment oxidation is used as a key step, for example, in industrial waste water treatment [2], or as a treatment method for the waste water containing biologically active organic compounds [3–6]. Apart from oxygen the oxidants used in water and waste water treatment include

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chlorine, chlorine dioxide, ozone, potassium permanganate and hydrogen peroxide [1–2]. Moreover, the oxidation processes in which the generated hydroxyl radical is used (or another peroxide radical) belong to the group of advanced oxidation process (AOPs).

AOPs more and more frequently employ synergistic combination of different oxidants (ozone, hydrogen peroxide and others) and UV radiation, which increases the efficiency and rate of organic compounds degradation [2, 7–11]. The authors of [7] compared the effectiveness of single oxidation processes, this is, photolysis (UV) and ozonation (O_3) with a coupled system combining both of these processes, in terms of the elimination of selected antibiotics from aqueous stream. Based on the obtained results it was determined that the process of photolysis proved to be completely ineffective for the elimination of the tested antibiotics. On the other hand, the efficiency of the ozonation process depended on the contact time of ozone with the treated solution. Satisfactory results of antibiotics degradation were observed only after 30 minutes of ozonation. In contrast, when both UV and O_3 were used the removal efficiency of antibiotics was over 87 % after 10 minutes. On the other hand, according to other authors [8–10] the use of UV and H_2O_2 significantly increases the removal efficiency of micropollutants, compared to the use of the UV process itself. UV radiation causes direct photolysis of hydrogen peroxide molecules and as a result hydroxyl radicals are generated. In the case of the research conducted by Esplugass et al [11] the addition of a solution of 10 mg/dm^3 H_2O_2 caused an increase of the removal efficiency of psychotropic drug carbamazepine, which is practically non-UV-degradable, to a level of 99 %. These studies confirmed that a combination of both of these processes causes a synergistic effect.

However, as shown by previous studies, none of the chemical oxidants that are available and used in practice are neutral towards the quality of purified water or waste water [1–2]. All the strong oxidizing agents cause, to a various extent, the formation of oxidation by-products, which are often of unknown biological activity. This problem also applies to AOPs, however, in this case the information available in the literature is limited.

The above-mentioned issues were taken into account in the present study, which assessed the toxicity changes in water containing bisphenol A (as a selected xenobiotic) during the treatment using a combined oxidation process UV-H_2O_2.

Materials and methods

Bisphenol A, which was selected for the study, is an organic compound belonging to a group of phenols used, among others, for the production of plastics [12]. The subject of the study was model solutions prepared using deionized water and an analytical standard of the studied xenobiotic at a concentration of 0.5 to 5.0 mg/dm^3. The analytical standard of bisphenol A was purchased from Sigma-Aldrich (Poznan, Poland). The pH of the solution was adjusted to pH 7 with 0.1 mol/dm^3 HCl solution or 0.2 mol/dm^3 NaOH. The compound was determined by solid phase extraction (SPE) method and liquid chromatography analysis (HPLC). Supelclean^{TM} ENVI-18 cartridges (volume 6 cm^3, 1.0 g solid phase) from Supelco (Poznan, Poland) were used for the extraction. The filling of the cartridges prior to the extraction was conditioned with methanol (5 cm^3) and acetonitrile (5 cm^3), and then washed with deionized water (5 cm^3). The analyte was eluted with a 1 cm^3 mixture of acetonitrile and methanol (60:40, v/v). The qualitative and quantitative analysis of the xenobiotic in the eluent was performed using HPLC with a UV detector (λ = 218 nm) from Varian (Warsaw, Poland). The eluents were previously concentrated in a gentle stream of nitrogen. The chromatographic column used for the analysis was Microsorb 100 C18 with a length of 25 cm, a diameter of 4.6 mm and a pore size of 5 μm. A mixture of acetonitrile and water (85:15, v/v) was used as the mobile phase. Organic solvents of analytical grade purchased from the Avantor Performance Materials International Company(Gliwice, Poland) were used in this study.

The applied analytical procedure allows the determination of bisphenol A in water at low concentrations, this is, 0.3 μg/dm^3. The extraction yield exceeded 61 % for the concentration of the compound in deionized water equal to 0.5 mg/dm^3 and 74 % for the concentration of 5 mg/dm^3. The obtained analysis results did not differ by more than 10 %.

At the preliminary stage of the study, the bioluminescence inhibition was assessed in the model solutions with varying bisphenol A concentrations (Fig. 1). The analysis was carried out using the MICROTOX® bioassay system in the Microtox Model 500 analyser from Tigret Ltd. (Warszawa, Poland) in accordance with the *Screening Test* procedure of the MicrotoxOmni system. This analyser serves both as an incubator and a photometer. Percent bioluminescence inhibition against the control sample (bacteria not treated with the potential toxicant) was measured after 5 minutes of exposure.

It was observed that the increasing concentration of xenobiotic in water was accompanied by simultaneous increase of the bioluminescence inhibition. The presented

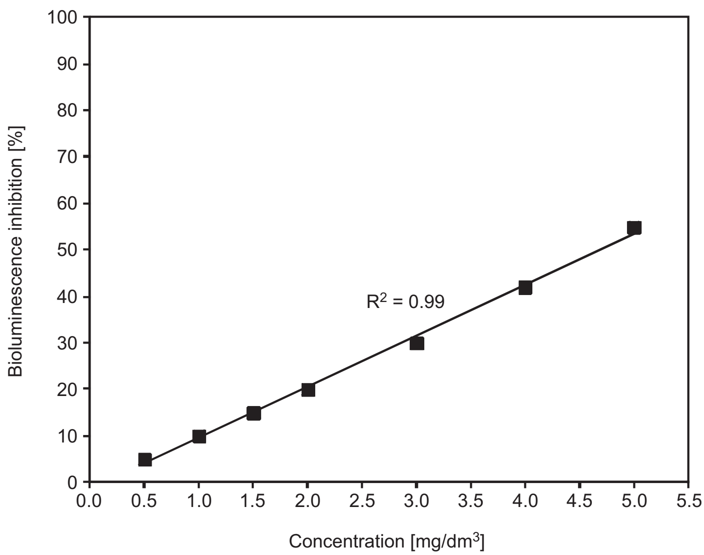

Fig. 1. Impact of bisphenol A concentration on the bioluminescence inhibition value

graphical relationship between the concentration of xenobiotic and the bioluminescence inhibition shows linear correlation between the two parameters (R^2 = 0.99), confirming that the toxicity of water depends on the concentration of the xenobiotic. Based on this observation, it can be hypothesized that the effective elimination of the xenobiotic from water using a variety of physical and chemical processes should be accompanied with a reduction of the toxic effect. Any exception to this rule may prove the occurrence of other dangerous phenomena accompanying the implementation of these processes.

The UV irradiation of the model solutions was performed at 20 ^{o}C in a reactor from the Heraeus Company (Warsaw, Poland) with a medium-pressure immersion lamp with the power of 150 W for 45 min (Fig. 2). The irradiation was carried out with and without the addition of hydrogen peroxide (H_2O_2) for comparison. The analysed H_2O_2 doses were in the range of 6 to 12 mg/dm^3. Analytical grade 30 % hydrogen peroxide purchased from the Stanlab Company (Gliwice, Poland) was used in this study. It was diluted by 10-fold prior to its use. The samples for the analysis were collected at different times of the process, this is 5, 10, 15, 20, 30 and 45 min. The degree of decomposition of the xenobiotic was assessed by the chromatographic analysis, and the bioluminescence inhibition was assessed by the Microtox® bioassay, which allowed determination of the toxicity of the solution.

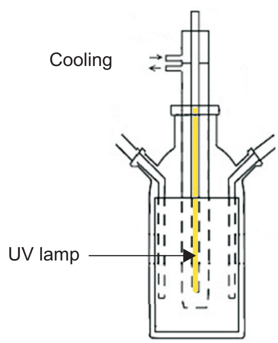

Fig. 2. The scheme of the laboratory UV reactor Heraeus

Results and discussion

The degree of decomposition of the xenobiotic and the change of the bioluminescence inhibition occurring in the solutions during the UV irradiation with and without the addition of hydrogen peroxide H_2O_2 (dose of 9 mg/dm^3) depending on the duration of the process is shown in Figure 3. During the irradiation of water with UV the studied xenobiotic was undergoing decomposition (Fig. 3a). The effectiveness of decomposition of bisphenol A in water increased with time of exposure to UV radiation. For example, after 5 minutes of the process the decomposition of bisphenol A was approx. 38 %, and after 45 minutes approx. 72 %. The observations related to the bioluminescence inhibition in the test solutions were somewhat surprising. The decomposition of bisphenol A did not cause reduction of the bioluminescence inhibition of the solution.

Regardless of the UV irradiation time the bioluminescence inhibition in the test solutions was greater than that specified in the solution before the process. The value of the bioluminescence inhibition depended clearly on the duration of the process. The highest value of this parameter was observed in the sample taken after 45 minutes of the ongoing process (approx. 66 %), in which, paradoxically, the decomposition of

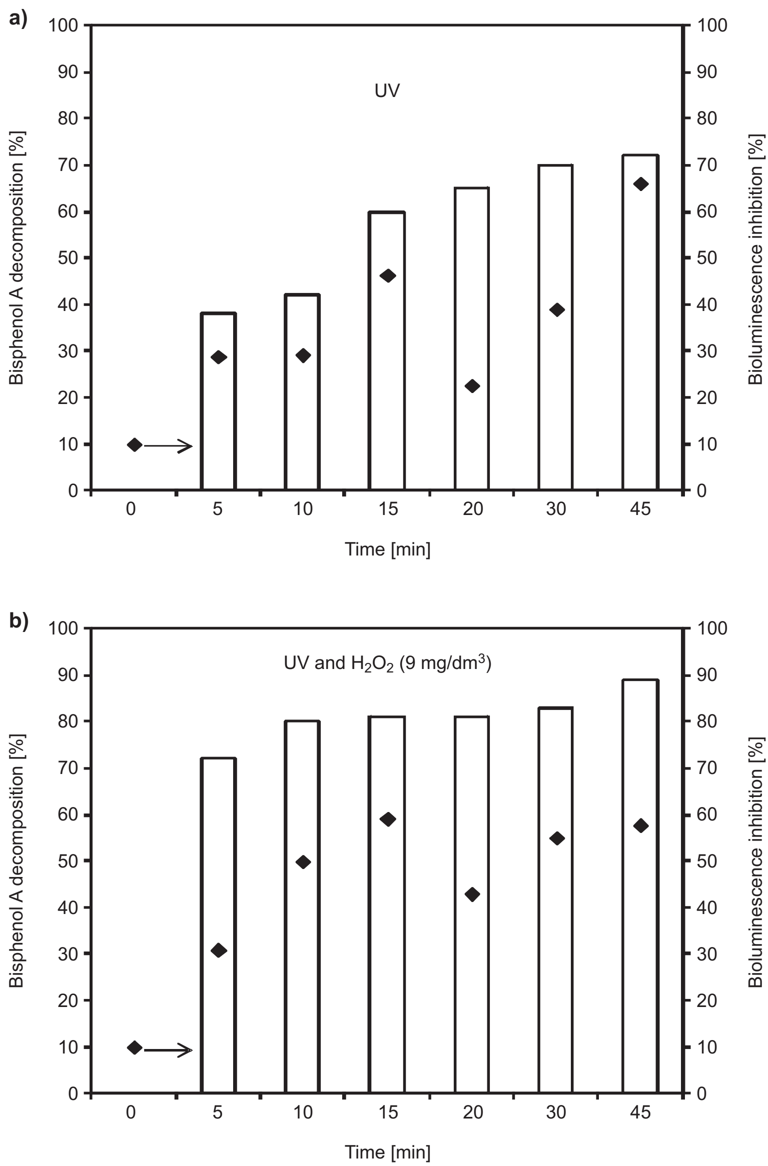

Fig. 3. Decomposition of bisphenol A and change of the inhibition of bioluminescence in solutions irradiated with and without the addition of H_2O_2

bisphenol A was the highest. The difference between the values of the bioluminescence inhibition of the solutions containing the studied compound was already observed in the initial studies (Fig. 1). In contrast, the use of hydrogen peroxide along with UV radiation significantly improved the degree of degradation of the studied xenobiotic, but also resulted in a significant increase in the bioluminescence inhibition of the solutions (Fig. 3b). The increased intensity of xenobiotic degradation was probably the result of the formation of a larger amount of hydroxyl radicals (OH) in the presence of the oxidant. The observed increase in the bioluminescence inhibition during the UV irradiation with and without the addition of hydrogen peroxide H_2O_2 implies the formation of toxic intermediate products of decomposition of the compound, whereas the intensity of this phenomenon was greater when the complex oxidation process with UV and H_2O_2 was applied.

In their earlier work in this field [13] the authors of this paper were testing the feasibility of removing bisphenol A from water using the UV and UV/O_3 process. In this case it was also shown that the decomposition of bisphenol A was greater in the process of coupling irradiation of water and ozonation (ozone dose was 3 mg/dm^3), but the toxicity of the water after the addition of ozone significantly increased. Moreover, in [14] the treated water was containing zearalenone (mycotoxin of estrogenic properties produced by certain *Fusarium* fungi). The paper presented comparatively water ozonation (ozone dose of 1 mg/dm^3, contact time 1 min) and photocatalysis (dose 100 mgTiO$_2$ catalyst/dm^3, time 5 min). It was shown that both ozonation and photocatalysis allow efficient decomposition of zearalenone in water, but both processes induce formation of by-products during the oxidation of the studied mycotoxin, which was evaluated using GC-MS technique. In terms of the amount of oxidation by-products

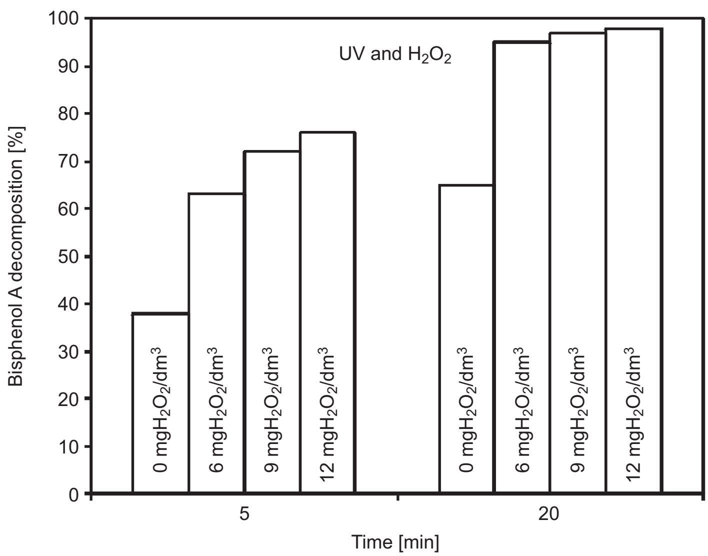

Fig. 4. The dependence of decomposition of bisphenol A in the UV irradiation process of the solution in the function of a dose of H_2O_2 (process time of 5 and 20 min)

water ozonation proved to be more disadvantageous than photocatalysis. However, regardless of the oxidation process and the intensity of the formation of oxidation by-products, the treated water showed no toxic effect.

The observations from previous studies and the results shown in the present study indicate a significant impact of both the oxidation process itself (or a combination of different processes) as well as the type of the removed contaminant on the water toxicity.

Further steps of the present study focussed on assessing the effect of hydrogen peroxide doses on the decomposition of bisphenol A (Fig. 4) and the value of the bioluminescence inhibition of the solutions (Fig. 5a), which was studied at two UV irradiation times, this is 5 and 20 min. Additionally, a toxicity class of the solutions was determined (Fig. 5b).

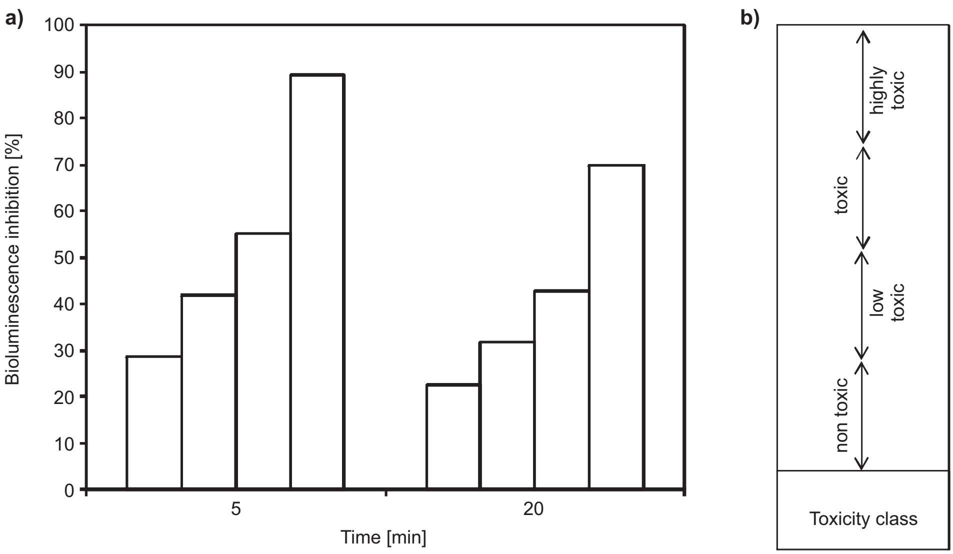

Fig. 5. Changing of the bioluminescence inhibition of the solutions containing bisphenol A in the UV irradiated water in the function of the H_2O_2 dose (a) and their toxicity class (b): time of the process equal to 5 min and 20 min

The increase of the dose of H_2O_2 caused an increase of both the degree of degradation of bisphenol A and the bioluminescence inhibition of the solutions (Fig. 4, Fig. 5a). Comparing the values of the studied parameters for two selected process durations, this is, 5 and 20 min, it can be seen that a longer duration causes the reduction of the toxicity of the solutions (Fig. 5b). However, regardless of the dose of hydrogen peroxide the solutions subjected to the irradiation showed inhibition of vital functions of the bacteria *Vibrio fischeri* at a level of higher than 25 %, even at the longer duration of the process, which classifies them outside the class of non-toxic solutions. Only the solution subjected to the UV irradiation without H_2O_2 for 20 min was found to be non-toxic. Taking the above-mentioned observation into account, it can be concluded that the selection of the process conditions should be based not only on its effectiveness, but also on the possibility of the occurrence of adverse phenomena.

The applied Microtox® bioassay using the bioluminescent bacteria *Vibrio fisheri* was successfully used for toxicological evaluation of the quality of water containing bisphenol A during the treatment with the coupled oxidation process UV-H_2O_2. Currently, an increasing use of the Microtox® bioassay can be observed in the studies regarding elimination of toxic organic substances from water or waste water [15–18]. Its popularity can be attributed, amongst others, to the fact that this bioassay can be carried out in a very short time. It is a considerable advantage compared to other toxicological bioassays currently used in environmental research.

Conclusions

Based on the results of the present study the following specific conclusions regarding the assessment of the quality of water containing bisphenol A during the UV irradiation with and without the addition of H_2O_2 can be drawn:

– the degree of decomposition of the xenobiotic depended on the time of the UV irradiation,

– the decomposition of the compound did not reduce the bioluminescence inhibition of a given solution, indicating the formation of toxic by-products,

– the combined use of hydrogen peroxide and UV radiation increased the decomposition efficiency of the xenobiotic, but also caused an increase in the bioluminescence inhibition of the solutions,

– the selection of the most favorable conditions for the oxidation process cannot be merely based on the effectiveness of the decomposition of the compound but should also consider the toxicity of the resulting solution.

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ZMIANY TOKSYCZNOŚCI WODY ZAWIERAJĄCEJ BISFENOL A W TRAKCIE JEJ UZDATNIANIA W ZŁOŻONYM PROCESIE UTLENIAJĄCYM

Instytut Inżynierii Wody i Ścieków
Politechnika Śląska w Gliwicach

Abstrakt: Wodę zawierającą bisfenol A poddano napromieniowaniu UV (zanurzeniowa lampa średniociśnieniowa o mocy elektrycznej 150 W) bez i z dodatkiem H_2O_2 (dawka 6–12 mg/dm^3). Do kontroli jakości wody zastosowano biotest Microtox® wykorzystujący bakterie bioluminescencyjne *Vibrio fisheri*. Określono, że rozkład bisfenolu A zależał od czasu napromieniowania UV jak i dawki H_2O_2. Zaskakujące z kolei były obserwacje związane z wartością inhibicji bioluminescencji w badanych roztworach. Rozkład związku nie powodował obniżenia wartości inhibicji bioluminescencji charakteryzującej roztwór, co wskazuje na powstawanie toksycznych produktów pośrednich. Z kolei łączne zastosowanie nadtlenku wodoru z promieniowaniem UV poprawia stopień rozkładu bisfenolu A, ale jednocześnie powoduje wzrost wartości inhibicji bioluminescencji roztworów. Z tego względu w doborze najkorzystniejszych warunków prowadzenia procesu utleniającego nie można opierać się wyłącznie na skuteczności rozkładu związków, lecz należy również rozważyć toksyczność roztworu poprocesowego.

Słowa kluczowe: bisfenol A, uzdatnianie wody, toksyczność, biotest Microtox®

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Angela POTASZNIK[1*], Ilona ŚWITAJSKA[1]
and Sławomir SZYMCZYK[1]

THE IMPACT OF THE CULTIVATION OF *Salix viminalis* L. ON CONCENTRATION OF COMPONENTS IN GROUNDWATER

WPŁYW UPRAWY *Salix viminalis* L. NA ZAWARTOŚĆ SKŁADNIKÓW W WODACH GRUNTOWYCH

Abstract: The influence of the cultivation of the common osier on sodium, calcium, potassium and magnesium concentrations in groundwater was evaluated between January 2011 and December 2012 in north-eastern Poland. The analyzed site is situated in Samławki on the premises of the Educational and Research Station in Łężany administered by the University of Warmia and Mazury in Olsztyn. Groundwater samples for chemical analyses were collected once a month from seven piezometers. Four piezometers were installed in a willow plantation: one on a hilltop, one on a slope and two varied-depth piezometers (904A – depth of 1.62 m, 904B – depth of 2.65 m) in a surface depression. The remaining three piezometers were installed for comparative purposes on arable land, in a forest on a hilltop and in a surface depression. Magnesium, calcium, sodium and potassium concentrations were determined in water samples by standard methods. The highest groundwater levels were noted in arable land (110.8 ± 53.7 cm below ground level), and the lowest levels – on the forest hilltop (572.8 ± 27.0 cm below ground level). In the willow plantation, the highest groundwater table was noted in a surface depression (272.0 ± 25.4 cm below ground level). Growing common osiers for energy significantly influenced magnesium and calcium concentrations in groundwater, which were highest on the slope of the plantation (15.1 ± 3.8 $mgMg \cdot dm^{-3}$ and 88.8 ± 26.4 $mgCa \cdot dm^{-3}$) and on the hilltop (13.6 ± 4.5 $mgMg \cdot dm^{-3}$ and 109.1 ± 22.3 $mgCa \cdot dm^{-3}$). The highest sodium levels in groundwater were noted on the plantation hilltop (10.2 ± 1.6 $mgNa \cdot dm^{-3}$) and in arable land (11.5 ± 2.0 $mgNa \cdot dm^{-3}$). Potassium concentrations in groundwater were determined by the height of the groundwater table, and they were highest on hilltops in the willow plantation (5.2 ± 4.4 $mgK \cdot dm^{-3}$) and in the forest (4.3 ± 2.7 $mgK \cdot dm^{-3}$).

Keywords: common osier *Salix viminalis* L., groundwater, magnesium, calcium, sodium, potassium

The production of biomass for energy, including in willow and osier plantations, is becoming an important part of modern agricultural systems. Energy willows can be grown throughout Poland, and the main factors that limit their cultivation are water

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deficit and nutrient deficiency, mainly low supply of nitrogen as a yield-forming factor. Willows are a highly versatile tree species that may be used for the manufacture of bioenergy [1]. Conventional agricultural practices cause soil salinification, which reduces crop yield due to the accumulation of soluble inorganic salts in soil. The above leads to a rise in magnesium, calcium, sodium and potassium concentrations in the soil solution that exceed the assimilative capacity of plants. Salinification also reduces the availability of water and nutrients in soil [2]. Plants resistant to pollution are introduced to arable land to prevent excessive salinification. The common osier (*Salix viminalis* L.), a species of willow, is tolerant to pollution, and it is used as a pioneer species in biological purification projects. The cultivation of energy crops can significantly influence mineral concentrations in groundwater. Poland is characterized by low quality and quantity of water resources, and energy willow plantations contribute to the protection of the country's meager water reserves. Low precipitation, high surface evaporation and uneven distribution of precipitation and evaporation contribute to a further reduction in water resources. Human activities also exert a negative impact on the availability of fresh water [3].

Materials and methods

The objective of this study was to determine the influence of the cultivation of the common osier (*Salix viminalis* L.), a species of willow, on sodium, calcium, magnesium and potassium concentrations in groundwater. The experiment was carried out between January 2011 and December 2012 in Samławki, Region of Warmia and Mazury, approximately 80 km from the city of Olsztyn, on the premises of the Educational and Research Station of the University of Warmia and Mazury in Olsztyn. Piezometers were installed in the willow plantation at points that represented different land relief features characteristic of lakelands. One piezometer was installed on the hilltop (902), one on the slope (903), and two in a surface depression (904A – depth of 1.62, 904B – depth of 2.65 m). Three piezometers were installed for comparative purposes: one in arable land in the direct vicinity of the willow plantation (905), one on the hilltop in the forest (906) and one in a surface depression in the forest (907). Water samples for chemical analysis were collected once a month with the use of the GIGANT submersible pump. Magnesium, calcium, sodium and potassium concentrations in water samples were determined in the laboratory of the Department of Land Reclamation and Environmental Management (Mg using method of yellows titanium – visual method and Ca, Na and K using Atomic Absorption Spectrometry). The Ca:Mg ratio was calculated as a ratio of concentration of calcium and magnesium. The height of the groundwater table was determined with a whistle unit.

The results of chemical analyzes of groundwater was respondents statistical analyzes in *STATISTICA PL 10.0*, was determined position measurement (mean, median) and dispersion (variance, variations, and skewness). Was using parametric tests (Shapiro Wilk test) and nonparametric (U Manna-Whitney'a test) at the significance level $\alpha = 0.05$ was significanced of differences was examined properties of the tested groundwater and verified the significance of the differences in values and normality in relation to the growing and nongrowing season and catchment management (willow plantation and points of comparison).

Results and discussion

The mineral content of groundwater is determined by the movement of minerals into the soil profile. The rate of infiltration is influenced by the physicochemical properties of soil, land-use type, location and plant cover [4]. Soil moisture content is determined by rainfall intensity and distribution, surface evaporation, groundwater levels, which can be influenced by land improvement measures (drainage, irrigation), and land-use type. Moisture content affects the movement of minerals into the soil profile and their distribution in groundwater [5]. In the present study (observations from January 2011 to December 2012), the highest groundwater levels in the willow plantation were noted in a surface depression (904A – 133 m below ground level; 904B – 124 cm below ground level in the winter half-year to 130 cm below ground level in the summer half-year on average), and the lowest groundwater table was reported on the hilltop (from 372 cm below ground level in the summer half-year to 334 cm below ground level in the winter half-year on average), regardless of season (Fig. 1).

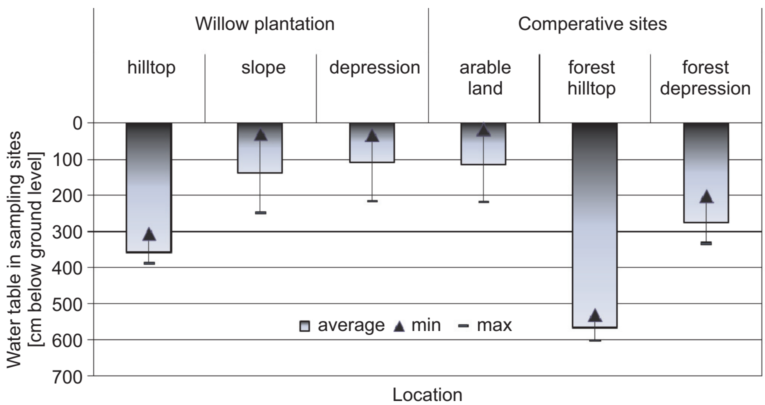

Fig. 1. Groundwater table in sampling sites
Key: Willow plantation: 1 – hilltop; 2 – slope; 3 and 4 – surface depression. Comparative sites: 5 – arable land; 6 – hilltop in the forest; 7 – surface depression in the forest.

The lowest groundwater table in the willow plantation and in comparative sites was observed on the hilltop in the forest (from 574 cm below ground level in summer to 568 cm below ground level in winter on average). Groundwater levels were highest in arable land (116–105 cm below ground level). The height of the water table influenced soil moisture levels.

The highest water table (572.8 m below ground level) was determined in sampling site 906 on the hilltop in the forest. Ground water levels in the above location differed significantly from the remaining sampling sites, excluding that on the hilltop of the willow plantation (363.54 m below ground level), which could be attributed to similarities in land relief (Fig. 2).

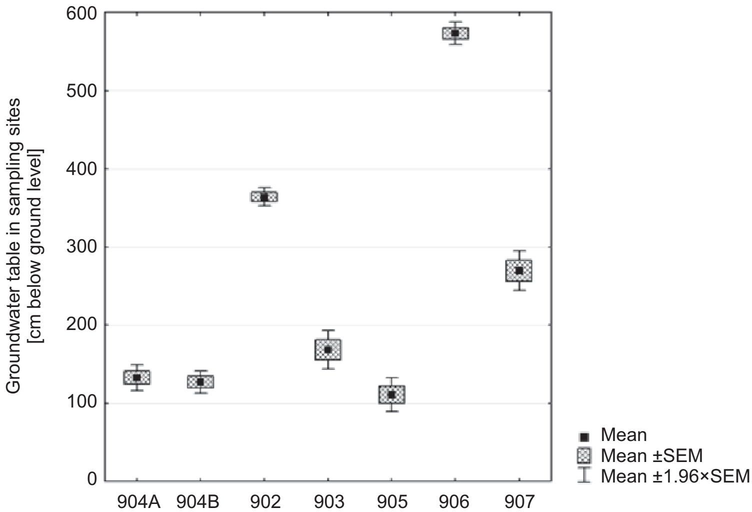

Fig. 2. Groundwater levels determined in 2011–2012 in sampling sites
Key: Willow plantation: 902 – hilltop; 903 – slope; 904A and 904B – surface depression. Comparative sites: 905 – arable land; 906 – hilltop in the forest; 907 – surface depression in the forest.

The highest fluctuations in soil moisture content are noted in arable land and forests characterized by shallow subsoil water. The variations in soil moisture content are minimized with an increase in groundwater table depth. Groundwater levels significantly influence the active soil layer, the processes that take place inside that layer and, consequently, the development of plant root systems [3].

Calcium and magnesium are essential for the growth and development of plants, and those macronutrients are taken up by plants in large quantities. Magnesium is a component of plant chlorophyll, and it activates various enzymatic reactions, can mitigate the effects of phytotoxicity [6]. Calcium controls water intake and metabolic processes, and it also regulates the accumulation of soil colloids Sodium and potassium influence vital life processes in plants. Potassium is responsible for synthesis and respiration processes, and it regulates the water balance in plant tissues.

The magnesium content of the analyzed groundwater samples was determined mainly by the type of land use in the catchment, land relief features and the levels of plant-available magnesium, which varied on a seasonal basis. The lowest magnesium concentrations (3.6 mg $\cdot$ dm^{-3} on average) were noted in a surface depression in the forest, and they differed significantly from the remaining sampling sites (Fig. 3). Magnesium concentrations in groundwater on the hilltop in the forest (9.2 mg $\cdot$ dm^{-3} on average) were similar to those reported in a surface depression in the willow plantation (9.3–9.5 mg $\cdot$ dm^{-3}) (Fig. 3).

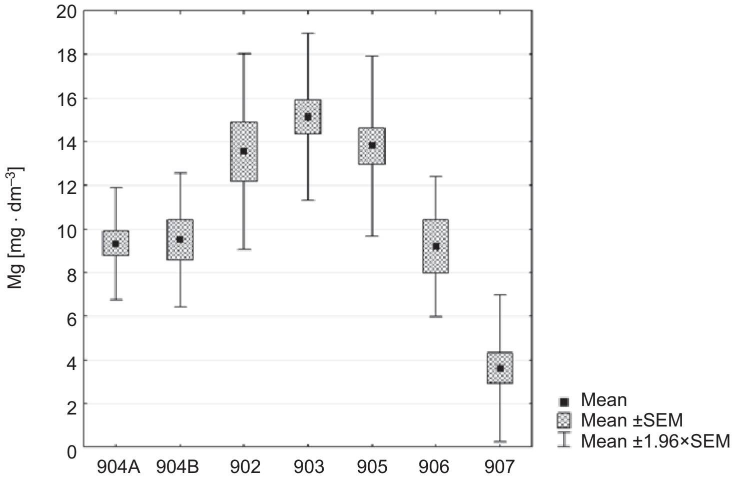

Fig. 3. Magnesium concentrations in groundwater determined in 2011–2012 in sampling sites
Key: Willow plantation: 902 – hilltop; 903 – slope; 904A and 904B – surface depression. Comparative sites: 905 – arable land; 906 – hilltop in the forest; 907 – surface depression in the forest.

The highest magnesium concentrations were noted in groundwater samples collected on the slope of the willow plantation (15.1 mg $\cdot$ dm^{-3} on average), and they were 1.3 mg $\cdot$ dm^{-3} higher than those reported in arable land. The lowest magnesium content (1.2 mg $\cdot$ dm^{-3}) was observed in groundwater sampled from the plantation hilltop. In both the summer and winter half-years and in both years of the study on average, similar magnesium levels were noted in groundwater samples from the hilltop and the slope of the willow plantation and from arable land. Magnesium concentrations noted in a surface depression in the plantation were similar to those reported on the hilltop in the forest. In comparison with the willow plantation, much greater differences in the magnesium content of groundwater were observed in the forest between sampling sites on the hilltop and in a surface depression. Magnesium levels on the hilltop were three-fold higher than in the surface depression, which could be attributed to higher assimilation of magnesium by rapidly growing forest plants in the depression. Variations were also reported in the willow plantation where magnesium concentrations differed by 42–48 % between sampling sites. Magnesium levels in groundwater were higher in the winter half-year when this mineral was not accumulated or when its uptake was limited. A high water table limits the thickness of the active soil layer and the root zone, whereas low groundwater levels support soil aeration and the formation of a healthy root system [3]. The greatest variations in the magnesium content of groundwater between the seasons were observed in the forest and in arable land, where magnesium concentrations were approximately 30 % and 20 % higher in winter, respectively. According to Burzyńska [7], the average magnesium content of subsoil water in grasslands reached 11.80 mg Mg $\cdot$ dm^{-3}. In the cited study, the rate of magnesium uptake by plants was determined mainly by the species composition of meadow vegetation, soil pH and the ratio of magnesium to the accompanying cations.

Soil pH significantly affects the magnesium content of subsoil water. Soil acidity decreases when agricultural production is terminated, which supports the migration of magnesium to shallow subsoil water. Similar trends were reported by Koc et al [4] regardless of the type of land use. Soil acidification processes (acid rain, nitrification) can contribute to the leaching of magnesium from soil to groundwater.

During both years of the study (2011 and 2012), the highest calcium concentrations (109.5 mg · dm^{-3} on average) were observed in groundwater sampled from the hilltop and slope (88.8 mg · dm^{-3}) of the willow plantation and the lowest – in a surface depression in the forest (12.8 mg · dm^{-3}). Calcium levels in the above location differed significantly from those noted in the remaining sampling sites (Fig. 4). The average calcium concentration values were similarly distributed. Similarly to magnesium, the concentrations of calcium were much lower in groundwater sampled from a surface depression due to limited water availability. Greater variations in groundwater levels were noted in arable land than in the forest [8].

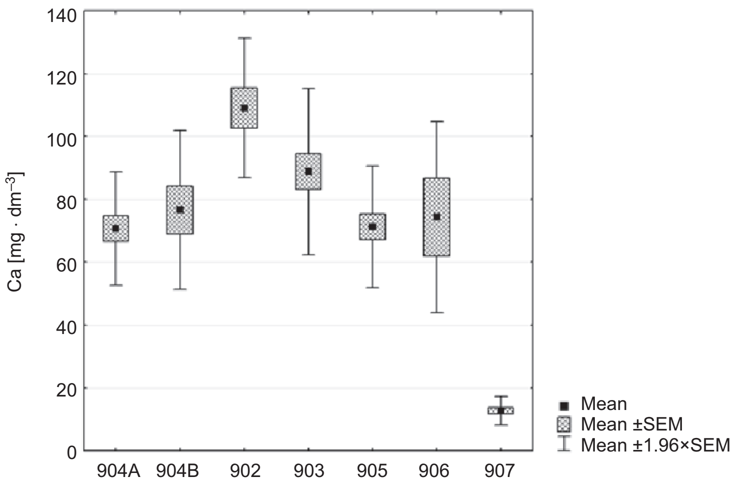

Fig. 4. Calcium concentrations in groundwater determined in 2011–2012 in sampling sites
Key: Willow plantation: 902 – hilltop; 903 – slope; 904A and 904B – surface depression. Comparative sites: 905 – arable land; 906 – hilltop in the forest; 907 – surface depression in the forest.

In groundwater samples collected from the willow plantation and arable land, calcium concentrations were lower in winter, whereas significantly higher levels of calcium were noted in the forest in summer.

The Ca:Mg ratio was similar in groundwater sampled from the willow plantation and the forest (7.25 and 7.14, respectively), but it was somewhat narrower (5.2) in arable land. According to Orzepowski and Pulikowski [9], the Ca:Mg ratio of groundwater in arable land ranges from 2.7 to 6.9.

In this study, the highest (11.5 and 10.2 mg · dm^{-3}) were noted in arable land and hilltop of willow plantation. The lowest (3.6 mg · dm^{-3}) sodium concentrations were noted in surface of depression in the forest. The sodium content of groundwater in the willow plantation ranged from 5.6 mg · dm^{-3} in surface depression 904A, 8.5 mg · dm^{-3} on the slope of plantation, to 13.2 mg · dm^{-3} on the hilltop. The average sodium content of groundwater in a surface depression in the willow plantation was significantly lower in comparison with the remaining sampling sites (Fig. 5), which could be attributed to differences in land-use type and the rate of sodium uptake by forest plants.

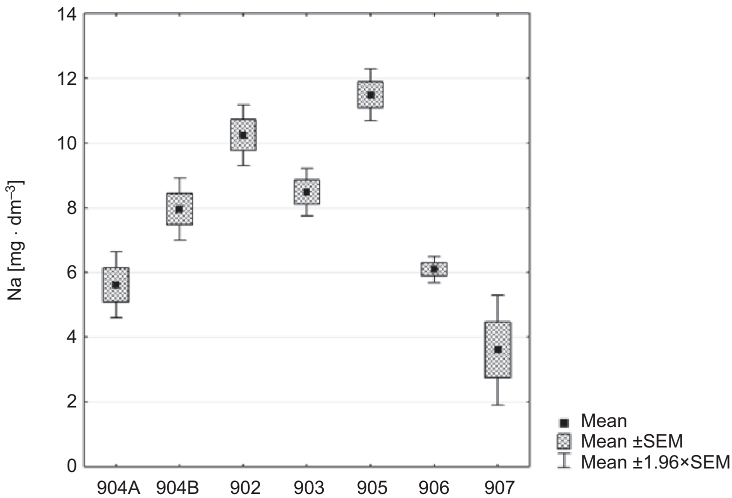

Fig. 5. Sodium concentrations in groundwater determined in 2011–2012 in sampling sites
Key: Willow plantation: 902 – hilltop; 903 – slope; 904A and 904B – surface depression. Comparative sites: 905 – arable land; 906 – hilltop in the forest; 907 – surface depression in the forest.

In both years of the study, the average sodium content of the analyzed groundwater samples were somewhat higher in arable land than in the willow plantation (regardless of land relief) and two- to three-fold higher than in the forest. The above could point to progressing salinification of groundwater in arable land, which could be attributed to intensive farming (loose topsoil layer) and mineral fertilization. In view of the average summer and winter values, the highest average sodium concentrations in groundwater were reported in arable land (from 11.3 mg · dm^{-3} in the summer half-year to 11.6 mg · dm^{-3} in the winter half-year). Relatively high sodium levels were also observed on the hilltop in the willow plantation (8.6–10.8 mg · dm^{-3}), and higher values were noted in summer. In the plantation, the lowest sodium concentrations in groundwater (5.6 mg · dm^{-3} on average) were determined in a surface depression (904A), and the lowest sodium levels in the entire analyzed area (3.6 mg · dm^{-3}) were noted in a surface depression in the forest.

According to Orzepowski and Pulikowski [9], mineral concentrations in groundwater are determined by soil type, and they are increased by the inflow of pollutants from farmland. In the cited study, the highest sodium concentrations (109.7 mgNa · dm^{-3}) were not noted on arable land (black/clayey soil and silty clay), but in an area where farm effluents constituted a point source of pollution.

Cymes and Szymczyk [5] reported lower concentrations of magnesium, calcium and sodium in groundwater in arable land than in grasslands. The analyzed minerals are more readily assimilated by plants in arable land due to higher soil aeration and more supportive air-water relationships. The mineral content of groundwater is also significantly influenced by land relief, which affects the movement of elements in soil and their uptake by plants. Water-soluble minerals migrate towards the outflow of the water source, which contributes to nutrient deficiency in soils and groundwater on hilltops. Groundwater resources on slopes and in surface depressions are more abundant in minerals. In areas with better access to water, nutrients are more available for plants, whereas water deficits, which are more frequently noted on slopes than in surface depressions, can lower the rate of mineral accumulation and increase nutrient concentrations in groundwater [5].

Potassium levels in the analyzed groundwater samples ranged from 1.8 ± 0,8 mg · dm^{-3} in surface depressions in the willow plantation and in arable land to 5.2 ± 4.4 mg · dm^{-3} on the hilltop in the plantation. Potassium concentrations in the latter location differed significantly from the remaining sampling sites, excluding the forest hilltop (Fig. 6). The lowest potassium content was determined in groundwater sampled from piezometers installed in a ground depression in the willow plantation (1.8 ± 0.8 mg · dm^{-3} and 2.0 ± 0.7 mg · dm^{-3}, on average). The above can probably be attributed to the migration of potassium into the soil profile and outside the root zone, which significantly impaired potassium accumulation, in particular in water-deficient sites.

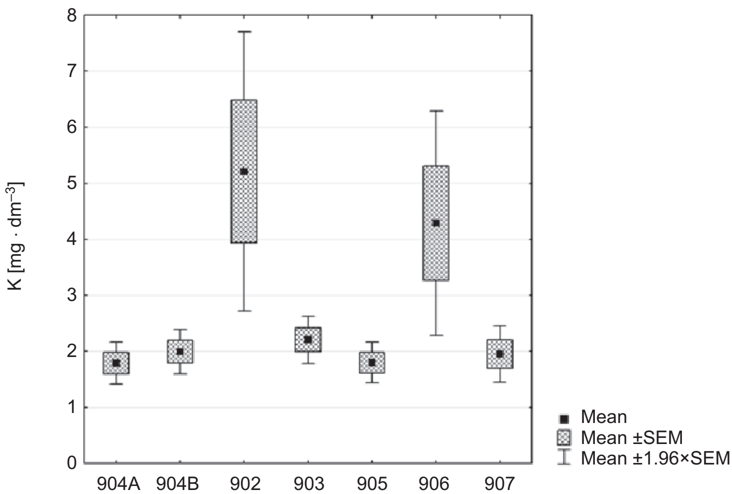

Fig. 6. Potassium concentrations in groundwater determined in 2011–2012 in sampling sites
Key: Willow plantation: 902 – hilltop; 903 – slope; 904A and 904B – surface depression. Comparative sites: 905 – arable land; 906 – hilltop in the forest; 907 – surface depression in the forest.

The correlations between potassium concentrations vs. land relief and land-use type were somewhat different when analyzed across seasons. Potassium levels on the hilltop and on the slope in the willow plantation and in arable land were significantly higher in the summer half-year. A reverse trend was noted in the forest and in a surface depression in the plantation.

Based on the Shapiro-Wilk test, it was found that all the indicators that have been identified in the examined surface waters were characterized by asymmetric compared to the average, $p < 0.05$ (course inconsistent with the Gaussian curve – normal distribution, Table 1).

Table 1

Statistical analyzes of chemical indicator in groundwater

Parameter	Level [cm]	Mg [mg · dm^{-3}]	Ca [mg · dm^{-3}]	Na [mg · dm^{-3}]	K [mg · dm^{-3}]
Mean ± SEM	238.6 ± 152.3	10.8 ± 5.6	67.8 ± 32.3	7.7 ± 3.7	2.5 ± 2.1
Variance	23199.4	30.9	1314.4	13.6	4.4
Median	203.0	11.1	72.4	8.2	1.8
Varibility	63.8	51.6	53.5	47.6	84.7
Skewness	1.0	–0.1	–0.2	0.00	4.0
Normal distribution – probability p-test					
Shapiro-Wilk test	0.000	0.004	0.000	0.004	0.000
Determine significance of difference between means					
U Manna Whitneya (1)*	0.066	0.494	0.121	0.311	0.926
U Manna Whitneya (2)*	<u>0.038</u>	<u>0.001</u>	<u>0.000</u>	<u>0.421</u>	<u>0.038</u>

* (1) – U Manna Whitney test used to analyzes to difference between means in growing and no growing seasons; (2) – U Manna Whitney test used to analyzes to difference between means In groundwater on willow plantation and comparative points.

During the statistical analysis model statistics, which was equivalent to the non-parametric Student's t-test, which was the U Manna-Whitney test was formulated hypothesis for H_0: means values of the tested ingredients contained groundwater during the growing season and non growing aren't different significantly (m1 = m2) (1), as well as: the average values of the studied ingredients contained in the groundwater located under the willow plantation and comparison points aren't different significantly (m1 = m2) (2) to the alternative hypothesis (H1): the average values of the studied ingredients contained in groundwater in growing and non growing season aren't equal (m1 ≠ m2) (1) and the average values of the studied ingredients contained in the groundwater beneath the willow plantation and benchmarks aren't equal (m1 ≠ m2) (2).

On this basis, it was found that the differences between the concentrations of the components under consideration in all periods studied groundwater did not different significantly, in turn, was significantly statistical difference between the mean values of the analyzed components in the groundwater under the plantation of willow and comparative points at the significance level $\alpha = 0, 05$ (Table 1). This demonstrates of the significant impact of catchment management, including willow cultivation for energy purposes in the development of the components of magnesium, calcium, sodium, potassium in groundwater.

The mineral content of groundwater was significantly influenced by the type of land use in the catchment and fertilization. According to Burzynska [7], nitrogen fertilization (calcium and ammonium nitrate) affected potassium levels in soil and groundwater. Czajkowska [10] demonstrated correlations between the potassium content of groundwater and season, where potassium concentrations were higher in fall (13.8 mg $\cdot$ dm^{-3} in October and 11.7 mg $\cdot$ dm^{-3} in summer) regardless of soil porosity and aeration. The above could result from the fact that potassium fertilizers are applied in late summer and early spring and that potassium ions migrate easily in agricultural catchments.

The mineral content and, consequently, the quality of groundwater is determined by a combination of natural processes and factors, many of which are human-induced. The chemical composition of groundwater varies in space and time [11].

Conclusions

1. Magnesium, calcium, sodium and potassium concentrations in groundwater were determined by land-use type, land relief and weather conditions that influenced mineral accumulation and the movement of water and minerals into the soil profile.

2. The mineral, calcium, sodium and potassium content of groundwater sampled from a willow plantation was determined by the availability of water in the growing season. Periodic water deficits on the hilltop and on the slope lower nutrient availability for plants, which increases magnesium, calcium, sodium and potassium concentrations in groundwater.

3. Under similar water availability to plants in arable land and in the willow plantation (surface depression), the cultivation of the common osier decreases magnesium, calcium, sodium and potassium concentrations in groundwater.

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WPŁYW UPRAWY *Salix viminalis* L. NA ZAWARTOŚĆ SKŁADNIKÓW W WODACH GRUNTOWYCH

Katedra Melioracji i Kształtowana Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

Abstrakt: Badania nad wpływem uprawy wierzby krzewiastej na jakość wód gruntowych pod względem zawartości sodu, wapnia, potasu i magnezu realizowano od stycznia 2011 roku do grudnia 2012 roku na terenie Polski północno-wschodniej. Obiekt badawczy znajduje się na gruntach należących do Stacji Dydaktyczno-Badawczej Uniwersytetu Warmińsko-Mazurskiego w Olsztynie z siedzibą w Łężanach – obiekt Samławki. W celu analiz chemicznych wody gruntowe pobierano systematycznie raz w miesiącu z zainstalowanych 7 piezometrów. Cztery z nich zostały zlokalizowane na plantacji wierzby: po jednym na wierzchowinie, stoku oraz dwa o zróżnicowanej głębokości (904A – 1,62 m głębokości i 904B – 2,65 m głębokości) w obniżeniu terenu. Trzy pozostałe punkty stanowiły obiekty porównawcze i umieszczono je: na gruncie ornym oraz w lesie na wierzchowinie i w obniżeniu terenu). W pobranych wodach oznaczono standardowymi metodami stężenia magnezu, wapnia, sodu i potasu. Na podstawie przeprowadzonych obserwacji można stwierdzić, że najwyższy poziom zalegania wód gruntowych stwierdzono na gruntach ornych (110,8 ± 53,7 cm p.p.t.), z kolei najniższy występował na wierzchowinie w lesie (572,8 ± 27,0 cm p.p.t.). Na terenie plantacji najwyższe stany wód były charakterystyczne dla punktu w obniżeniu terenu (272,0 ± 25,4 cm p.p.t.). Uprawa wierzby wiciowej na cele energetyczne w istotny sposób wpłynęła na zawartość w wodach gruntowych magnezu i wapnia, które występowały w największych stężeniach w wodzie gruntowej na stoku plantacji (15,1 ± 3,8 $mgMg \cdot dm^{-3}$ oraz 88,8 ± 26,4 $mgCa \cdot dm^{-3}$) oraz na wierzchowinie (13,6 ± 4,5 $mgMg \cdot dm^{-3}$ i 109,1 ± 22,3 $mgCa \cdot dm^{-3}$). Największe koncentracje sodu również występowały w wodzie gruntowej wierzchowinie plantacji (10,2 ± 1,6 $mgNa \cdot dm^{-3}$) oraz na gruntach ornych (11,5 ± 2,0 $mgNa \cdot dm^{-3}$). Stężenia potasu w wodach gruntowych uzależnione były od poziomu zalegania, co potwierdzają najwyższe koncentracje w punktach zlokalizowanych na wierzchowinie zarówno na plantacji wierzby (5,2 ± 4,4 $mgK \cdot dm^{-3}$), jak i w lesie (4,3 ± 2,7 $mgK \cdot dm^{-3}$).

Słowa kluczowe: wierzba krzewiasta, *Salix viminalis* L., wody gruntowe

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and Dariusz ROPEK[2]

MICROBIOLOGICAL PROPERTIES OF SOIL IN THE AREA OF THE MUNICIPAL WASTE DUMP FOR THE KRAKOW AGGLOMERATION

WŁAŚCIWOŚCI MIKROBIOLOGICZNE GLEBY W STREFIE ODDZIAŁYWANIA SKŁADOWISKA ODPADÓW KOMUNALNYCH AGLOMERACJI KRAKOWSKIEJ

Abstract: The main aim of the research was the evaluation of the municipal waste dump impact on the quantity of bacteria and fungi in the soil environment in different distances from the collected waste. The field analyses were carried out for 12 months, from June to May, during 2 years. Analytical data show that 1 g of the soil's dry mass, collected 1230 meters from the municipal waste dump Barycz contains: 27 630 to 4 223 860 cfu of vegetative forms of bacteria and 4580 to 1 806 110 cfu of the bacterial spores. Ratio of bacterial spores and vegetative cells was between 1.3 and 98.6 %. *Microscopic fungi – Micromycetes* (yeasts and filamentous fungi) occurred in quantities between 1180 and 229 630 cfu/g of the soil's dry mass.

Keywords: soil, microorganisms, municipal waste dump

Among the natural environment, soil is the specific element, because of different types of collected pollutants, including microbiological ones, originating from the municipal waste dump. Moreover, there are many small, illegal objects in the area of the municipal waste dump, which very often increase the soil pollution [1–4]. Therefore, in most cases the waste dumps became the potential sources of long-term, various pollutions of the ground. Huge diversity of the waste dumps' pollutants evidences the fact that they are very dangerous source of the soil contamination. These potential pollutants are: organic compounds including petrochemicals, inorganic compounds including heavy metals and large amounts of microorganisms (including pathogens) [5–9].

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Having in mind the above mentioned issues, the aim of the research was evaluation of the influence of the municipal waste dump on the quantity of bacteria and fungi in the soil environment in different distances from the collected waste.

Material and methods

The soil's microbiological analyses were carried out nearby the municipal waste dump Barycz in Krakow. The object is placed about 3,5 kilometers from Wieliczka in the area of the foredeep which originated in the exploited aquifiers of the Bogucice sands, in the valley of the small Malinowka stream. The waste dump lies on the Southwestern-Northeastern axis and its coordinates are: 250 m AMSL to the North-East direction and 270 m AMSL to the South-West direction. The municipal waste dump Barycz in Krakow is the biggest and the longest exploited object in the Lesser Poland Voivodeship and one of the biggest in Poland. It started running in December 1974, its total area is 37 ha. It is estimated that in one year the Barycz waste dump collects about 175 thousand tones of the municipal waste. Alkaline and acidic brown soils are predominant in this area. These are mainly light and average loess, sometimes heavy and loam-loess soils.

For the analyses' purposes the soil samples were taken from June until May, monthly, from 10 analytical points within and in some distance from the municipal waste dump Barycz in Krakow. In order to do this, before starting the analyses, the area was evaluated and the representative analytical points were set for sampling. The analytical points were located in the following places:

POINT NO.:	POINT DESCRIPTION
1	In front of the III section, nearby the fence, surrounding the waste dump, to the South-West direction.
2	Nearby the III section, at its middle part level, to the South-West direction.
3	By the entrance, to the North direction (the entrance is between the II and III section)
4	Inside the II section – the active zone, until February.
5	Nearby the Malinowka stream, by the unused collector for the soaking water, next to the incoming road, to the North-East direction.
6	By the piezometer P-3, to the North-East direction.
7	By the Malinowka stream, on the fallow land, covered with grass and scrub, to the North direction from the borders of I section of the waste dump.
8	By the piezometer P-6, nearby the buildings, to the North direction from the borders of the II section of the waste dump.
9	By the piezometer P-8, by the Malinowka stream in the reed bed, to the North from the borders of the II section of the waste dump.
10	By the piezometer G, 1230 m to the North from the borders of the II section of the waste dump.

It needs to be stressed that the 8 to 10 analytical points were outside of the protective area – 500 meters from the waste dump's borders. The samples were transported to the laboratory of Department of Microbiology, University of Agriculture in Krakow, where the humidity and pH tests were carried out, together with the microbiological analyses, using the serial dilution method. The following media were used for the purpose of the quantitative analyses: nutritious agar for the vegetative cells and spores of bacteria and Malt Extract Agar (MEA) for microscopic fungi. After the incubation period, colony forming units (cfu) were counted and then calculated for 1 gram of the soil's dry mass.

Results and discussion

Apart from the naturally existing microorganisms, the foreign microbes can be found in the soil environment, which migrate from the municipal objects. Microbiological contamination of soils nearby the municipal waste dumps is mostly caused by the bioaerosols and microorganisms' spreading by the wild animals, rodents, flies and other insects. Soils may also become polluted during the delivery and uloading waste, incorrect waste dump exploitation and incorrect water export from the object [2, 10–13].

Analytical data presented in the Table 1 shows that in 1 g of the soil dry mass within and nearby the municipal waste dump Barycz in Krakow (up to 1230 m North from the borders of the section II) from 27 630 to 4 223 860 cfu of vegetative forms and from 4580 to 1 806 110 cfu of the bacterial spores can be found. The percent ratio of the spores to the general quantity of bacteria – vegetative forms, was from 1.3 to 98.6 %. Maximum amount of the bacterial vegetative cells was found in June by the entrance to the waste dump and similar amount was found in the same period inside the II section. These places were profusely inhabited by these microorganisms in different periods of the soil sampling. The lowest amount of the vegetative forms was found in January above the III section – about 570 m from the gate to the waste dump (analytical point No. 1). The highest amount of the bacterial spores was found in December inside the II section of the waste dump (analytical point No. 4) and in June in the sample from the 5 analytical point – by the unused soaking water collector and by the P-3 piezometer, point no. 6 (Table 2). Low amounts of the general spores' number were observed in June and January in the soil taken from the point no. 7, located between piezometers P-3 and P-6 (about 300m from the borders of the I section) and in November and December in the point no. 10 (1230 m from the borders of the II section) nearby the G piezometer. It needs to be noted that a large amount of the bacterial spores (over 90 %) were found in relation to the vegetative cells nearby the waste dump's entrance and around the old soaking water collector and the incoming road (Krzemieniecka Street) in the different time periods. Difficult environmental conditions for the development of bacteria were found in the area of the municipal waste dump, therefore very often spore formation was observed. It may be the result of the fact that microorganisms change their metabolism and the microbiocenotic composition as the response for numerous stress and stimulating factors, which can occur in the soil environment [2, 14].

The Table 3 presents the results from the whole analytical period about the average number of bacteria in the soil samples from the analytical points. They show that the average quantity of the vegetative cells of bacteria was from 344 550 to 961 657 cfu/g of the soil dry mass. The highest number was found in the soil samples taken by the waste dump's entrance – about 10 m from the gateway and slightly lower number was found inside the III section. In the mentioned points, the average number of vegetative bacteria cfu was over 800 000 (by the III section), and even over 900 000 (entrance, section II) in 1 gram of the soil dry mass. On the basis of the gained results it was ascertained that the highest average number of the bacterial spores (314 983 cfu/g of the soil's dry mass) and their highest percentage (62.6 % in comparison to the vegetative cells) were found in the soil by the old soaking water collector (point No. 5). The average number of spores in this analytical point was over 2 times higher than in the point No. 1. In the other tested soil samples the average quantity was from 89 594 to 241 393 cfu/g of the soil dry mass, which counted for 19.9 to 41.1 % in relation to the vegetative forms of the bacteria isolated from the tested field. The quantitative analyses of the bacteria occurrence in 10 analytical points showed that their quantity was various, however it was still higher in the soil samples by the waste dump's entrance, in the II active section and near the middle of the III section (it was being built during the research) and by the P-3 piezometer nearby the old, unused soaking water collector. The carried out research revealed that the occurrence of the vegetative forms of bacteria as well as their spores is strongly influenced by the location of the analytical point, time of sampling, direction of the wind, soil reaction and the tested soils' humidity. It is usual that the bacterial contamination and suspensions are being transported to relatively short distances from the waste dumps. The range of their infiltration depends on their survival rates in the aquifier layer and the distance of the ground water flow. It is assumed that they can reach from several to 100 meters from the waste dump. While investigating the seasonal changes, the maximum quantity of vegetative forms of bacteria was found in most points in August and minimal quantity was found in the period from November until February (Table 1). However, large numbers of bacterial spores were found in June, November and in January, which was related to high or low air temperatures (Table 2).

Soil biomass consists mostly of fungi, which are very common in this environment. In the neutral conditions, with high concentration of the hydrogen ions, especially with high content of organic matter, fungi are much more profuse than bacteria. Thanks to their individual biochemical properties, *eg* Ability to produce slime, water accumulation, production of organic acids and releasing from the soil minerals such nutrients as: potassium, phosphorus *etc* their role in pedogenesis and plant nutrition is very important [1, 15, 16].

On the basis of the mycological research, it was ascertained that the microscopic fungi – *Micromycetes* (yests and filamentous fungi) occurred in quantities from 1180 to 229 630 cfu/g of the tested soil dry mass (Table 4). The lowest number was noted in September in the soil sample taken from the bank of the Malinowka stream in the reed

Table 1

Relationship between the bacterial vegetative forms [cfu in 1 g of the soil d.m.] and the sampling period and the sampling point in the area of the municipal waste dump Barycz in Krakow

Point	Sapmpling dates											
	June	July	August	September	October	November	December	January	February	March	April	May
1	212 540	525 240	630 150	667 600	125 640	480 400	180 680	27 630	390 130	84 690	422 210	766 850
2	2 082 840	2 222 710	2 105 550	57 670	309 290	352 850	224 500	449 730	96 760	252 910	37 100	1 494 340
3	4 223 860	48 430	424 000	1 607 700	518 970	1 141 740	311 780	915 040	250 560	478 000	903 000	716 800
4	4 114 350	264 710	298 440	2 872 760	306 320	527 310	489 000	451 350	678 870	569 850	236 870	136 460
5	2 337 610	100 400	262 020	458 070	489 410	133 340	390 120	584 000	294 000	509 600	234 000	243 500
6	2 300 280	198 260	1 008 150	266 840	404 710	260 330	318 410	242 260	202 660	367 890	153 230	727 500
7	149 800	802 000	706 340	571 330	508 640	216 430	74 530	91 730	123 600	421 700	743 200	198 470
8	436 930	520 170	1 925 680	944 880	433 850	45 230	65 830	537 640	390 860	746 700	654 300	412 400
9	843 880	561 450	363 860	110 240	278 650	251 720	118 570	226 370	309 700	164 300	413 560	492 300
10	515 640	401 660	420 450	483 950	1 360 770	44 730	38 320	1 273 300	891 300	352 000	397 830	431 000

Table 2

Relationship between the bacterial spores [cfu in 1 g of the soil d.m.] and the sampling period and the sampling point in the area of the municipal waste dump Barycz in Krakow

Point	Sapmpling dates											
	June	July	August	September	October	November	December	January	February	March	April	May
1	106 270	292 000	52 050	371 000	46 650	239 190	68 900	22 600	85 300	69 810	66 380	164 440
2	539 150	64 600	90 140	38 840	232 850	315 210	64 800	7 480	86 500	175 790	29 730	278 340
3	130 490	47 260	399 500	220 040	35 410	684 150	124 300	112 460	164 000	196 000	81 500	97 000
4	118 190	99 120	264 250	1 179 170	171 150	231 130	198 000	84 960	236 000	134 700	103 580	76 460
5	1 806 110	90 590	167 610	407 840	91 070	125 200	85 200	336 830	246 870	152 840	135 900	133 730
6	1 352 190	116 220	195 060	205 740	5 080	83 500	114 320	72 920	174 320	83 400	93 400	196 350
7	4 580	173 440	41 330	371 180	13 130	56 380	43 700	6 620	45 280	81 360	153 800	84 330
8	85 090	127 090	509 380	289 200	348 980	30 150	43 220	423 940	87 200	153 200	111 400	63 200
9	831 940	375 670	16 840	60 950	16 910	11 930	22 350	31 870	96 500	84 200	65 980	84 600
10	226 450	42 180	405 060	160 170	468 080	4 710	7 400	1 038 160	59 600	37 000	131 430	189 000

Table 3

Average number of microorganisms [cfu in 1 g of the soil d.m.] in the area of the municipal waste dump Barycz in Krakow

Tested microorganisms	Sampling points									
	1	2	3	4	5	6	7	8	9	10
Bacterial vegetative forms	376 147	807 188	961 657	912 190	503 006	537 543	383 980	592 873	344 550	550 913
Bacterial spores	132 050	160 286	191 010	241 393	314 983	224 375	89 594	189 340	141 650	230 770
Fungi	33 613	75 623	35 844	26 380	60 915	39 618	17 495	33 350	27 173	28 430

bed in the wetland (point no. 9) and the highest number was found in the same period in the unused soaking water collector (point No. 5). The amount of microscopic fungi was found to be influenced by the area and period of sampling as well as by the soil reaction and the environment's humidity. The often noticed acidic reaction and high soil humidity created very good conditions for the growth and development of *Micromycetes*. Comparison of the average amount of fungi, presented in the Table 3 showed the significant domination of the microscopic fungi (75 623 cfu/g of the soil dry mass) in the soil nearby the III section of the waste dump, which was being built at that time and nearby the old soaking water collector (60 915 cfu/g of the soil dry mass). The increased air movement is known to promote the transport and falling of the fungi spores. According to Kulig [17] the dangerous objects cause negative effects to the environment mostly during their exploitation. During their building and after closing them, the municipal objects become smaller threat to the environment. In the other places, average number of the tested microorganisms was on the level from 17 495 cfu to 39 618 cfu/g of the soil's dry mass. The highest average was found in the analytical point no. 6 (by the P-3 piezometer about 70 m from the unused soaking water collector) and the lowest was found in the point No. 7 (above the P-3 piezometer, 300 meters from the borders of the II section – the oldest one and long time ago recultivated). According to Drzał et al [11] and Kulig [17] the microbiological contamination of the soils is observer nearby different waste dumps in various distances and with different intensity.

The Pearson correlation coefficient and its statistical significance was calculated to evaluate the influence of pH and humidity on the quantity of the tested soil microorganisms (Table 5). In most cases this correlation was negative and not significant for the soil samples from the municipal waste dump Barycz in Krakow. Only in the analytical point No. 10, the number of vegetative forms of bacteria was highly positively correlated with the soil humidity. In the other points no significant correlation was found between the soil humidity and the number of the tested microorganisms. On the other hand, the occurrence of the vegetative cells of bacteria and fungi was negatively correlated with the soil's pH in the points no. 3, 5 and 10.

The presented research as well as the results gained by the other authors confirm the differentiation of the microorganisms' occurrence in the soil in the area of the municipal objects as well as various environment contamination in that area [14, 18–20].

Conclusions

1. On the basis of the research it may be ascertained that the municipal waste dump Barycz in Krakow causes negative effects on the soil environment, especially during the exploitation, but also during the building period.

2. After comparing all the results, it may be concluded that the waste dump does not have negative influence on the general bacteriological and mycological state of the environment only in the distance longer than 300 meters North from the borders of the waste dump's sections.

Table 4

Relationship between the fungi [cfu in 1 g of the soil d.m.] and the sampling period and the sampling point in the area of the municipal waste dump Barycz in Krakow

Point	Sampling dates											
	June	July	August	September	October	November	December	January	February	March	April	May
1	38 190	49 850	58 700	69 560	9 950	12 050	12 800	10 900	16 850	39 300	37 200	48 000
2	101 810	111 740	24 000	32 370	106 110	96 600	42 600	47 100	68 350	31 600	98 300	146 890
3	54 050	60 680	10 000	75 120	25 540	31 460	34 300	19 800	27 700	12 680	24 200	54 600
4	260	8 210	28 000	62 260	56 800	70 030	2 400	40	1 600	12 800	34 500	39 680
5	92 350	4 620	72 400	229 630	29 740	22 600	34 800	23 800	36 400	56 680	72 700	55 260
6	63 270	32 320	26 500	23 580	16 510	98 000	54 300	13 400	20 260	31 260	43 720	52 300
7	16 770	27 940	29 000	26 980	9 070	1 190	3 680	5 000	18 460	36 870	15 330	19 650
8	27 760	18 330	31 400	83 200	41 550	11 800	35 400	9 800	12 300	23 000	67 300	38 400
9	9 250	8 900	6 500	1 180	18 680	57 860	36 450	26 410	39 780	40 670	67 800	12 600
10	47 800	10 260	9 400	71 860	59 000	14 120	12 430	18 380	22 100	17 300	18 340	40 200

Table 5

Correlation between the number of microorganisms and the soil humidity and pH

Number of microorganisms	Sampling points									
	1	2	3	4	5	6	7	8	9	10
				Humidity						
Bacterial vegetative forms	–0.35	–0.46	0.35	0.56	–0.01	–0.19	–0.39	0.06	–0.44	0.57
Bacterial spores	–0.22	–0.14	0.11	–0.01	–0.05	–0.37	–0.32	0.40	–0.34	0.79*
Fungi	–0.57	–0.06	–0.42	–0.51	–0.19	–0.43	–0.47	–0.40	0.16	–0.15
				pH						
Bacterial vegetative forms	–0.22	0.48	–0.65*	0.30	–0.17	–0.03	0.02	0.14	0.18	–0.17
Bacterial spores	–0.16	0.09	–0.16	0.32	–0.15	–0.06	–0.39	0.16	0.06	–0.15
Fungi	–0.13	–1.12	–0.32	–0.57	–0.73*	0.21	–0.05	–0.45	0.12	–0.62*

3. The gained results indicate the need to carry out the long-term analyses of the microbiocenotic content of the soils nearby the waste dumps. Only this can improve the knowledge about the dynamics and the range of the biological contamination and it can create the effective environment protection strategy.

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WŁAŚCIWOŚCI MIKROBIOLOGICZNE GLEBY W STREFIE ODDZIAŁYWANIA SKŁADOWISKA ODPADÓW KOMUNALNYCH AGLOMERACJI KRAKOWSKIEJ

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Abstrakt: Głównym celem przeprowadzonych badań była ocena wpływu składowiska odpadów komunalnych na kształtowanie się liczebności bakterii i grzybów w środowisku glebowym w różnej odległości od składowanych odpadów. Badania terenowe związane z tematem pracy prowadzono przez 12 kolejnych miesięcy, od czerwca do maja w okresie 2 lat. Z danych analitycznych wynika, że w 1 g suchej masy gleby na terenie i w odległości do 1230 m od granic składowiska odpadów komunalnych Barycz w Krakowie występuje; od 27 630 do 4 223 860 jtk/g suchej masy gleby form wegetatywnych i od 4580 do 1 806 110 jtk form przetrwalnych bakterii. Procentowy stosunek przetrwalników do liczby bakterii form wegetatywnych wynosił od 1,3 do 98,6 %. Natomiast grzyby mikroskopowe – *Micromycetes* (drożdże i grzyby strzępkowe) występowały w ilościach od 1180 do 229 630 jtk/g s.m. badanych gleb.

Słowa kluczowe: gleba, mikroorganizmy, składowisko odpadów komunalnych

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EFFECT OF SOIL FACTORS ON SULPHUR CHANGES AND AVAILABILITY FOR PLANTS

WPŁYW CZYNNIKÓW GLEBOWYCH NA PRZEMIANY I DOSTĘPNOŚĆ SIARKI DLA ROŚLIN

Abstract: The study presents the analysis of the effect of soil factors such as oxidation, reduction, immobilization, mineralization, leaching, ion competition, and water management on sulfur metabolism in soil and its availability to the crops. Sulfur is one of the nutrients that largely affects the quantity and quality of crop yields, and is also a component of many compounds, the lack of which causes some disturbances to the development of plants and diseases in humans and animals. Despite such a great importance, it was not subject to agricultural research until the early 80s, and was not taken into consideration when determining the fertilizing needs of plants. However, the situation has changed in recent years, when problems associated with sulfur deficiency in crop production began more and more to be emphasized. The absence of this component is to be expected especially at the lighter, usually acidic mineral soils, located away from industrial centers. Therefore, the transformation of sulfur in the soil and its availability to the crop becomes an important issue.

Keywords: sulfur forms, sulfur conversion in soil, sulfur migration, sulfur availability

Besides nitrogen, phosphorus, and potassium, sulfur belongs to a group of nutrients that play an important role in the metabolism of crops. Plants absorb sulfur mainly through their roots in the form of SO_4^{2-} ions. A certain amount of this element may also be uptaken through the leaves in the form of SO_2 [1, 2].

Sulfur absorbed by plants is relatively quickly built into organic compounds, in which it occurs in the reduced form. Cysteine, that is a product of this process, is the precursor of another sulfur-containing amino acid – methionine. Two cysteine molecules (R – SH) may also react with each other to form a disulfide bridge between them, thereby forming cystine. Disulfide bonds play an important role in reinforcing the structure of proteins, especially enzymes, and the ability of sulfur to make such bindings is one of the most important functions of this element [1, 3].

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Other significant sulfur compounds are also: lipoic acid, coenzyme A, biotin, and thiamin. In some plants, sulfur can be additionally present in the form of volatile compounds, an example of which may be allyl oil and its glycoside derivatives called mustard oils [4, 5].

Despite the importance of sulfur for plant growth and development, as well as the quality of harvested plant material, it has not been paid too much attention until recently. It was not the subject of interest in agricultural research, and was not taken into consideration when determining the fertilizing needs of crops. This was due to the fact that in most parts of the European continent, the sulfur balance was positive. On the positive balance of this component is largely influenced by SO_2 emitted into the atmosphere during the combustion of fuels, especially coal, brown coal and crude oil. The sulfur in this period was also introduced into the soil in large quantities with certain mineral fertilizers [6].

In recent years, various countries, including the Polish increasingly reach but some symptoms of sulfur deficiency in plant production have been recently observed in various countries, including Poland (Table 1) [7].

Table 1

Areas with sulfur deficiency in agricultural production [7]

Continent	Country
Africa	Burkina Faso, Cameroon, Central African Republic, Chad, Egypt, Ghana, Guinea, Côte d'Ivoire, Kenya, Mali, Malawi, Mozambique, Nigeria, Senegal, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe
North and South Americas	USA, Canada, Latin America, Argentina, Brazil, Chile, Colombia, Costa Rica, Dominican Republic, Salvador, Honduras, Mexico, Nicaragua, Panama, Puerto Rico, Venezuela, Windward Islands
Asia	Bangladesh, Burma, China, India, Indonesia, Lebanon, Malaysia, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand
Europa	Belgium, Bulgaria, Denmark, Finland, France, Germany, Island, Ireland, Italy, Netherlands, Norway, Poland, Spain, Sweden, Great Britain, former USSR, former Yugoslavia
Oceania	Australia, Fiji, New Guinea, New Zealand, Solomon Islands

It resulted from a significant reduction in sulfur dioxide (IV) emissions into the atmosphere, decline of organic fertilization, and reduction in the consumption of mineral fertilizers containing sulfur [8, 9]. On the other hand, high demands of certain crops for this component, and remarkable leaching of sulfates make that in many cases the sulfur balance is negative [9, 10]. The absence of this component is to be expected especially on lighter, usually acidic, mineral soils located far away from industrial centers [3, 11]. Hence, the purpose of this paper based on the literature research, was to discussion of the soil factors affecting the metabolism and availability of sulfur for crops.

Sulfur forms in soils

Sulfur makes up about 0.05 % of the Earth's crust and in terms of element prevalence, it ranks at the fifteenth place. Its content in the soil depends primarily on a bedrock, the quantity and quality of organic matter, soil acidity, water and climate conditions, as well as air pollution [9, 12, 13].

A constant lack of oxygen in swampy soils and wetlands precludes the possibility to form and accumulate sulfates. Therefore, the inorganic sulfur is found mainly in the form of sulfides and elemental sulfur. On the other hand, under conditions of water excess and oxygen deficit, accumulation of organic matter takes place, due to which the organic soil is clearly richer in sulfur than mineral soils [14].

In turn, in the moderately moist soils, in which aerobic processes of leaching over water evaporation dominate, there are conditions favoring the oxidation of sulfur compounds to sulfate forms that easily move to deeper soil layers, and even into the groundwater. Therefore, organic sulfur contained in the concentrated organic matter is a basic form of sulfur presence in the humus layers of humid and well ventilated soils, in which mineral sulfur compounds are not very stable [13, 15].

Sulfates, that often form a well-developed levels, such as gypsum levels in saline soils occurring in a steppe and semi-desert climate, predominate in dry climate soils, where evaporation much exceeds atmospheric precipitation [12].

Total sulfur content in Polish mineral soils ranges from 0.05 to 0.4 $gS \cdot kg^{-1}$ of soil, while in organic ones it may be up to 10 times higher (up to 4.5 $gS \cdot kg^{-1}$ of soil). It must be stressed that about 90–95 % of that amount of sulfur present in various organic combinations and only 5–10 % are minerals, which plants can directly use [11, 16].

Sulfur sources in arable soils

Undoubtedly minerals are the primary source of sulfur in the soil. The most important among them are: gypsum ($CaSO_4 \cdot 2H_2O$), ferrous sulfides (FeS and FeS_2), hydrotroilite ($FeS \cdot nH_2O$). Also sphalerite (ZnS), chalcopyrite ($CuFeS_2$), and cobaltite (CoAsS) are found in soils at lower quantities. Under dry climate conditions, there can be found readily soluble sulfates, whereas in very dry – sodium alun ($NaAl(SO_4)_2 \cdot 12H_2O$) and tamarugite ($NaAl(SO_4)_2 \cdot 6H_2O$). The arable soils often contain sulfates of iron ($FeSO_4$), potassium (K_2SO_4), sodium (Na_2SO_4), magnesium ($MgSO_4$), as well as compounds with lower oxidation number such as sulfites, thiosulfates, pentathionates, and elemental sulfur [4, 12].

As it was previously indicated, an overwhelming amount of sulfur in the soil is present in organic forms. Much of the sulfur forms are part of the humus. Other organic compounds get into the soil along with the plant and animal remains, and microorganisms. The most important are: amino acids (methionine and cysteine), peptides (glutathione), proteins, sulfolipids, and vitamins (thiamin and biotin) [3].

Considering agriculture, sulfur is also introduced into the soils along with some mineral fertilizers. The largest quantities of the element are transported with: ammonia sulfate (240 $kgS \cdot Mg^{-1}$ fertilizer), gypsum or phosphogypsum (180–190 $kgS \cdot Mg^{-1}$

fertilizer), magnesium sulfate (130 kgS · Mg^{-1} fertilizer), kieserite (220 kgS · Mg^{-1} fertilizer), and elemental sulfur [6].

A valuable source of this nutrient are also natural organic fertilizers, especially manure in which sulfur content oscillates around 0.3–0.6 kgS · Mg^{-1} fertilizer [19].

Sulfur can also penetrate the soils from the atmosphere. Sulfur oxide(IV), the main component of air pollution, can be absorbed on the soil surface in gaseous form and then dissolved and oxidized in the soil solution. It is referred to as dry deposition. Sulfur oxides can be also oxidized in the atmosphere to sulfuric acid and reach the soil with rain or other precipitation. This is wet subsidence that produces acid rains [2].

Sulfur conversions in soils

Primary sulfur compounds and those that get into the soil from above described sources undergo a number of changes. Basic sulfur conversions in the soil can be divided into four phases: oxidation, reduction, immobilization, and mineralization. They are a combination of mutually dependent processes, the intensity of which is determined by a deficiency or complete lack of oxygen, soil pH, and contents of energy and nutritive components that affect the development of various groups of microorganisms [18, 19].

Oxidation. The processes of soil sulfur compounds oxidation can be carried out by soil microorganisms or occur during abiotic reactions. Sulfur bacteria oxidizing most of inorganic sulfur bindings in soils are the autotrophic organisms. They use the exothermic oxidation reactions of hydrogen sulfide, elemental sulfur, and other incompletely oxidized sulfur compounds as a source of energy required for the assimilation of carbon dioxide(IV). Heterotrophic organisms can also participate in the oxidation process. Although they do not carry out the full oxidation of reduced sulfur to sulfates, however they take part in particular stages of the process [13, 20].

The *Thiobacillus* genus bacteria are responsible for most of the reactions during oxidation of various mineral bindings of sulfur. They belong to the absolute aerobes with the exception of *T. denitryficans*, that is capable of oxidizing sulfur forms in anaerobic environment. Other species require appropriate soil pH and oxygen access for their development. Under low pH conditions *T. thiooxidans* and *T. ferrooxidans* prevail, while *T. thioparus* well develops near neutral pH values [13, 18, 20].

Compounds at different oxidation levels are a substrate for sulfur bacteria development, while sulfates are the final product. In the case of *T. thiooxidans*, elemental sulfur oxidation processes are as follows:

$$S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$$

Each of these processes is a separate energy reaction, and the final product of one reaction is the starting point for the next one (depending on the environment) carried out by the same or other bacteria [4].

The sulfur compounds present at a lower oxidation state may also be oxidized by abiotic factors, which are often intertwined with reactions caused by microorganisms.

The abiotic reactions occur most often with iron participation and they tend to produce sulfates, basic ferrous sulfates, and sulfuric acid. These reactions are accompanied by a significant decrease in soil pH [18].

Reduction. Under anaerobic conditions, sulfates are reduced becoming acceptors of a hydrogen derived from microbial degradation of simple organic compounds. These reactions are usually carried out with the participation of numerous microorganisms of *Desulfovibrio* and *Spiryllum* genera. A common feature of these bacteria is the tolerance to high concentrations of salts and hydrogen sulfide, and that the optimal development of the majority of them occurs in an environment having a pH 5.5–9 range. In environments below pH 5, disappearance of these microorganisms activity is recorded [4].

The sulfate reduction process is carried out through a number of intermediate steps that are not separate reactions, as in the case of oxidation, and hydrogen sulfide is the only final product [18].

Iron is often involved in the processes of sulfate reduction; it substitute the alkaline cation combined with the sulfate ion thus causing its release and deposition in soil in the form of a suitable carbonate. Decaying organic matter is the source of carbon dioxide(IV) that is necessary to produce carbonate. In this type of soils, some increase in pH and a color change to dark brown or black, can be observed [21].

Immobilization and mineralization. Plants uptake sulfur in the form of sulfate ions and certain simple organic combinations. The same compounds are also a source of sulfur for most of heterotrophic microorganisms responsible for the organic substances degradation. Hence, biological immobilization prevents easily soluble sulfur bindings from leaching by rainwater and maintaining a supply of this nutrient in the soil. Under natural conditions, mineralization of organic residues and oxidation of mineral sulfur compounds to sulfates gradually launch the supply, enabling further development of microorganisms and plants. Thus, there is a dynamical balance between mineralization and biological immobilization [4, 14, 19, 22].

Human activities often affect the equilibrium of these processes. Irreversible removal of forage crops along with yields is one of the causes of the impoverishment in the soil sulfur reserves. The use of natural fertilizers poor in sulfur also can lead to competition between microorganisms and higher plants in relation to this nutrient. The degree of sulfur mineralization depends on carbon to sulfur ratio. If C:S ratio is less than 200, part of mineral sulfur compounds released is absorbed by microorganisms, while the remainder is available for higher plants. If the ratio C:S is greater than 400, immobilization of mineral sulfur bindings by a fast-growing heterotrophic organisms takes place. Thus, symptoms of sulfur deficit are very frequently observed at plants after adding a large amount of organic substances with low sulfur content, such as cereal straw, to the soil [4, 15, 17].

The process of sulfur compounds mineralization can exert a significant impact on the nitrogen to sulfur ratio in decaying material. It is assumed that if N:S ratio in decaying plants amounts to about 14.2:1, sulfates immobilization occurs only to a small extent under such conditions. Introducing organic residues into the soil with a high value of N:S ratio may cause substantially greater sulfur than nitrogen immobilization [23].

Migration of sulfur compounds within the soil profile

The amount of sulfur in the form directly accessible to plants is determined by movement of the component into the deeper soil layers. Migration of sulfur compounds within the soil profile depends on several factors such as chemical properties and pH value of the environment, nature of the component bindings, redox conditions, presence of organic matter, and soil microflora composition [24–26]. The fact that sulfates are highly mobile in the substrate and are not absorbed at above pH 6 indicates that their contents in soil are closely related to water management. The water management, as a factor affecting the amount of sulfates available for plant, was paid too little attention until recently. The model calculations conducted by Schnug [27] suggest that rapeseed can cover up to 50 % of its sulfur demands from this source

Sulfates are very readily leached out of the soil profile. Annual sulfur loss due to this process can reach from 13 up to 141 $kgS \cdot ha^{-1}$ [3].

Sulfate leaching beyond the soil profile is partially mitigated by non-specific sorption of anions. Hydrated aluminum and iron oxides, as well as kaolinite present in the soil profile plays an important role. There is also a strong correlation between pH and sulfate sorption. The maximum sorption takes place at a pH in the range from 2 to 4, which is consistent with properties of the main sorbents, *ie* hydrated oxides of aluminum and iron. It needs to be mentioned that hydrated iron oxides retain significantly less sulfates than aluminum ones under the same conditions [28, 29].

Influence of different factors on sulfur availability for plants

The content of sulfur in the soil and its availability to plants is much influenced by other ions present in the environment, in particular phosphates, molybdates, arsenates, thiocyanates, and oxalates. Considering inorganic ions, phosphates have the greatest reducing effect on sulfate absorption, while oxalates can be quoted as organic ions. Additionally, OH^- and HCO_3^- ions present in the soil solution, like phosphates, make the sulfate sorption is diminished [29, 30].

The influence of various anions on sulfate sorption results most probably from the following reasons:

– competition for “sites of exchange”;

– ability of anions to form chelates with iron and aluminum; these compounds durably block the active centers within the sorption complex of the soil;

– precipitation reactions [1].

Transformations of sulfur have a large impact on the content of available forms of this nutrient in the soil. These forms are primarily sulfates, the content of which is constantly changing. Differentiation amounts of sulfates in soil results from the soil pH, microbial processes, atmospheric deposit, discharge of sulfur along with crops, soil fertilization, and changes in water management [12].

Influence of sulfur on the properties of arable soils

The oxidation of sulfur compounds in the soil, can contribute to an increase in acidification of soil, providing at the same time disadvantageous conditions are often quite toxic to most plants. Sulphated soil degradation factor is not only acidic but also an accompanying nutrient deficiency and excess phytotoxic elements such as aluminum and heavy metals [12, 27].

Under the influence of long-term contamination of the soil with sulfur are also subject to adverse changes in their sorption properties is reduced because the sum of the content of alkaline cations, mainly due to loss of exchangeable calcium and magnesium [12].

Sulphation is attributed to the significant impact on the mineralogical composition of soils. This is due to the fact that in an acidic environment there is a higher rate of weathering of rocks and minerals. In these processes there is not only the release of substantial amounts of aluminum and other elements, but also to changes in the mineral composition by decomposition and transformation of minerals such as carbonates in that dolomite. Contamination of soil sulfur so they deteriorate the basic parameters of fertility, including a reduction in the amount of soil humus and causes changes in the content of digestible components, in particular phosphorus, potassium, magnesium and molybdenum. Generally, states that as a result of acidification of soil sulfur compounds content of available forms of these elements is reduced [3, 12].

Excess sulfur in the soil environment also creates unfavorable conditions for the life of many organisms. The soils contaminated with sulfur followed by a decrease in the number of bacteria and actinomycetes, and at pH 3.0 or below these micro-organisms are no longer present. In such conditions, only the presence of fungi found [12].

Summary and conclusions

It has been well known since years that sulfur plays an important role in the growth and development of plants. Given the quantitative requirements of plants for this component, it is usually ranked at fourth place after nitrogen, potassium, and phosphorus.

Sulfur plays a specific role in the metabolism of plants. It is a component of many important compounds, the lack of which causes interference with plant growth and disease in humans and animals.

Despite its importance, until the early 80s of this century sulfur in European countries not received much attention. It was not the subject of interest in agricultural research, and it was not taken into account in determining fertilizer needs of plants. This was due to the fact that in most parts of the continent sulfur balance was positive. On the positive balance of this component in Poland is largely influenced by SO_2 emitted into the atmosphere during the combustion of fuels, especially coal, brown coal and crude oil (Table 2). The sulfur in the 80s was also introduced into the soil in large quantities with certain mineral fertilizers.

Table 2

Total emission of sulphur dioxide in Poland in years of 1975–2006 [3]

Years	SO_2 [kg · ha^{-1}]	Years	SO_2 [kg · ha^{-1}]
1975	99.8	1997	69.9
1980	132.1	1998	60.8
1985	127.2	1999	55.1
1990	102.6	2000	48.4
1991	95.8	2001	50.1
1992	90.2	2002	46.7
1993	87.1	2003	44.1
1994	84.6	2004	39.8
1995	74.9	2005	39.2
1996	75.7	2006	38.3

Lowering the sulfur deposit from the atmosphere and decrease the amount of input of mineral fertilizers led to a deficiency of this nutrient. The deficit of sulfur in the environment plant growth takes place mainly in the countries of Western and Northern Europe. However, many facts indicate that in some parts of Polish, the sulfur balance in the soil can be negative. The absence of this component is expected mainly on lighter, usually acidic, mineral soils located far away from industrial centers. Hence, to identify the factors affecting the metabolism of sulfur in the soil and its availability to plants becomes an important issue.

Sulfur is in fact a nutrient that has a big impact on the amount and quality of the yield of crops [31]. Deficiency of this component is limited primarily protein synthesis, causing further disturbances in the metabolism of sugars, in the case of oilseeds, the lack of sulfur leads to a reduced fat content. A suitable degree of sulfur supply of plants is also important for cereals. Sulfur deficiency decreases because the quality of wheat flour by the deterioration of its baking value. The beneficial effects of sulfur on the quality of the yield was also observed in the case of vegetables, where it increased carotene content and contribute to improving the flavor of onions and garlic. Bearing in mind these elements primarily must ensure is that the sulfur in the soil was in the forms and quantities to satisfy the nutritional needs of crops.

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WPŁYW CZYNNIKÓW GLEBOWYCH NA PRZEMIANY I DOSTĘPNOŚĆ SIARKI DLA ROŚLIN

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Abstrakt: W pracy przeanalizowano wpływ czynników glebowych, takich jak utlenianie, redukcja, immobilizacja, mineralizacja, wymywanie, konkurencja jonowa oraz gospodarka wodna na przemiany siarki w glebie i jej dostępność dla roślin uprawnych. Siarka jest jednym ze składników pokarmowych, który w dużej mierze wpływa na wielkość i jakość plonów roślin uprawnych, jest również składnikiem wielu związków, których brak powoduje zakłócenia w rozwoju roślin oraz schorzenia u ludzi i zwierzą. Pomimo tak dużego znaczenia aż do początku lat 80. nie była ona przedmiotem badań rolniczych, a także nie brano jej pod uwagę przy ustalaniu potrzeb nawozowych roślin. Sytuacja jednak zmieniła się w ciągu ostatnich lat, kiedy to coraz częściej zaczęto podkreślać problemy związane z deficytem siarki w produkcji roślinnej. Braku tego składnika należy się spodziewać przede wszystkim na lżejszych, zwykle zakwaszonych glebach mineralnych, usytuowanych z daleka od ośrodków przemysłowych. Stąd ważnym zagadnieniem stają się przemiany siarki w glebie i jej dostępność dla roślin uprawnych.

Słowa kluczowe: formy siarki, przemiany siarki w glebie, migracja siarki, dostępność siarki

Edyta KOSTERNA[1]

NUTRITIVE VALUE OF TOMATO GROWN UNDER COVERS WITH SOIL MULCHING

WARTOŚĆ ODŻYWCZA POMIDORA UPRAWIANEGO POD OSŁONAMI NA GLEBIE MULCZOWANEJ

Abstract: The experiment was carried out between 2010 and 2012 at the Experimental Station of Siedlce University of Natural Sciences and Humanities. The field experiment was established as a split-block design with three replicates. The effect of plant covering (without cover, under polypropylene non-woven and soil mulching with different kind of straw (rye, corn, rape, buckwheat) on the changes of selected nutrient components in 'Polfast F_1' tomato was investigated. The effect of straw was compared to a control plot without mulch. The content of dry matter, ascorbic acid, total sugars and flash acidity of tomato depended to a higher degree on weather conditions. The most favourable for accumulation of these components was warm and moderately wet 2011. The application of polypropylene non-woven at beginning of growing period of plant did not cause changes in chemical composition of fruits. The influence of straw mulch on the nutritive value of tomato was differentiated. The most of dry matter and ascorbic acid contained tomato cultivated on the rye and buckwheat straw. A higher content of total sugars and monosaccharides was found in fruits from plant cultivated on the corn and rye straw, respectively. Tomato cultivated on the mulch with buckwheat straw was characterized by higher flesh acidity of fruits.

Keywords: dry matter, ascorbic acid, sugars, flesh acidity, polypropylene non-woven, straw

Introduction

The chemical composition of vegetables is genetically determined as well as being modified by factors affecting the plant during growth, and particularly climatic conditions and agro-technology practices [1, 2]. Tomato, as a warm-season vegetable, requires relatively high temperatures for the proper growth and development. Low temperatures constitute the main cause of the large variability of tomato yield in field cultivation and contributing to worsen the quality of the fruits [3]. The application of plastic covers can have an influence on the tomato yield. Covers are used to modify plants natural environment in order to optimize plants growth, increases yields and improve their quality. In the study by Moreno et al [4] an application of polypropylene non-woven contributed to increased dry matter and ascorbic acid content in the edible

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parts of Chinese cabbage. Rekowska and Skupień [5] demonstrated that winter garlic plants grown under covers had significantly higher dry weight content in bulbs and total sugars in leaves. On the contrary, in the study by Majkowska-Gadomska [6] covers with polypropylene non-woven caused a decrease of dry matter, total sugars and disaccharides content in melon fruit.

Soil mulching is one way of soil water protection and also helps maintain a constant soil temperature within the root system of crops [7, 8]. It is particularly recommended for thermophilous vegetables, because mulch, by maintaining proper moisture and decreasing soil warming in summer months, improves soil conditions for plant growth and development. Mulches also suppress weed growth and contribute to reducing the use of herbicides in vegetable production [9]. A favourable effect of soil mulching on the chemical composition of vegetables was found by Samaila et al [10] and Majkowska-Gadomska et al [11] in tomato, Parmar et al [12] and Kosterna et al [13] in melon, Najafabadi et al [14] in garlic.

The study aimed to determine the effect of plant covering with polypropylene non-woven and soil mulching with organic mulch on the chemical composition of tomato fruit.

Material and methods

The experiment was carried out between 2010 and 2012 at the Experimental Station of Siedlce University of Natural Sciences and Humanities, which is located in central-eastern Poland (52°03′N, 22°33′E), 115 km east of Warsaw. According to the international system of FAO classification, the soil was classified as a Luvisol (LV) [15].

The experiment was established as a split-block design with three replicates. The effect of plant covering (without cover, under polypropylene non-woven and soil mulching with different kind of straw (rye, corn, rape, buckwheat) on the changes of selected nutrient components in ‘Polfast F_1’ tomato was investigated. The effect of straw was compared to a control plot without mulch.

The forecrop was triticale. In the autumn each year, preceding tomato cultivation, ploughing was performed. At the same time, farmyard manure was incorporated at a rate of 30 $Mg \cdot ha^{-1}$. In the spring, two weeks before the seedlings were planted, disc harrowing was applied to loosen the upper soil layer and prepare it for planting. After that, mineral fertilisers were applied in the amount of supplementary content to the optimal level for tomato: 85 kgN, 104 P_2O_5, 234 K_2O per 1 ha. Mineral fertilisers were applied in the form of ammonium nitrate, triple superphosphate and 60 % potassium salt.

Directly before seedling planting, the particular kind of straw was applied at a dose of 10 $Mg \cdot ha^{-1}$. The thickness of the mulch layer depended on the kind of straw. In the case of rye and rape straw mulch thickness amounted to 7–8 cm on average; in the case of corn straw, the mulch layer amounted to about 5 cm; however, for buckwheat straw the average was 8–10 cm.

Tomato seedlings were grown in a heated greenhouse. The seeds were sown at the rate of 8 g to seedling containers with peat substrate on 18 March in 2010 and 2011 and 28 March in 2012. After cotyledon formed and at the beginning of first leaf emergence, the seedlings were bedded into pots with a diameter of 8–10 cm (1 April in 2010 and 2011 and 11 April in 2012). Prior to transplanting the seedlings were hardened off and then moved permanently outdoors. Plants were planted in the successive study years on 20, 16 and 14 May, at a spacing of 60 × 40 cm.

Directly after seedlings planted suitable combinations were covered with polypropylene non-woven Pegas Agro 17UV. The cover removed after 3 weeks. After that, 50 kgN · ha^{-1} in the form of ammonium nitrate was applied (top dressing), each year. During the growing season, systematic tomato plant protection against fungal diseases was carried out. At 10-days intervals, the following plant protection sprays were applied: Infinito 687.5 SC, Amistar 250 SC and Ridomil Gold ® MZ Pepite 67.8 WG.

Tomato fruit harvesting was performed several times as the fruit ripened. The beginning of harvest occurred in the last 10 days of July and ended in the first 10 days of September. From each plot a fruit sample (20 fruits) was taken to perform chemical analyses. Tomato fruits intended for analysis were taken during full fruiting (mid-August). The following was determined:

– dry mass – by drying to a constant weight at 105 ^{o}C (Polish Standard PN-90/A-75101/03) [16],

– total sugars and monosaccharides – using the Luff-Schoorl method (Polish Standard PN-90/A-75101/07) [17],

– L-ascorbic acid – using the Tillmans method (Polish Standard PN-A-04019) [18].

The flesh acidity of tomato (g · 100 g^{-1} counted on lemon acid) was also estimated.

The results of the experiment were statistically analysed by means of the analysis of variance following the mathematical model for the split-block design. Significance of differences was determined by the Tukey test at the significance level of $p = 0.05$.

Weather conditions in the years of the study were varied (Fig. 1).

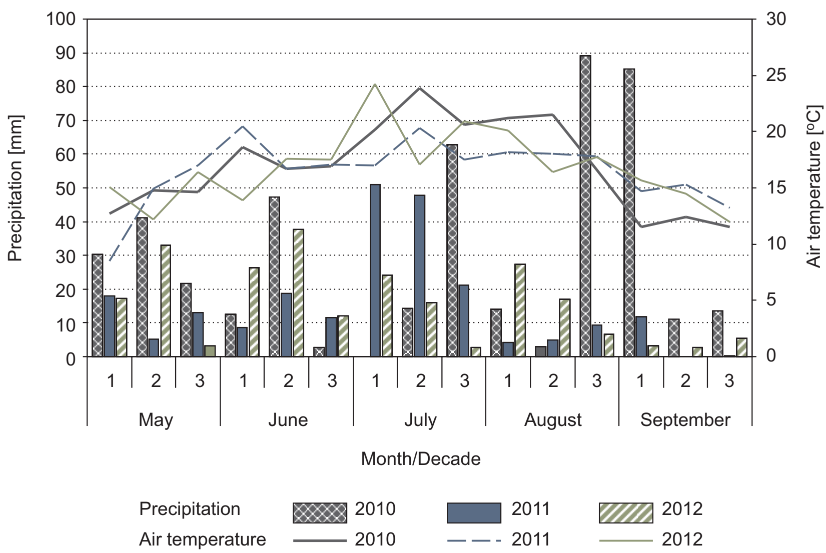

Fig. 1. Weather conditions in the vegetation period of tomato

Year 2010 was characterised by rather high air temperature but was also more moisture compared to the following years of the study, in particular during the final period of plant growth. Year 2011 was characterised by high and similar air temperature from the planting time to the beginning of harvest. Insufficiency of water in June, especially in the first 10-days, was compensated for by heavy rainfall in July. A lower rainfall at the end of vegetation period was profitable to fruits maturing. Mean air temperatures in 2012 were high, with only a slight fall in the temperature in the first 10-days of June. The drought in the last 10-days of May had no influence on the growth of plants. June, July and August were characterised by quite regular rainfall distribution.

Results and discussion

The content of dry matter in tomato fruits amounted on average 6.54 % (Table 1). The most of this component was noted in tomato cultivated on the buckwheat straw (7.66 %) and the least in fruits from plots mulched with rape straw (5.45 %). Similar content of dry matter (on average 5.30 %) had tomatoes fruits in the study by Majkowska-Gadomska et al [11]. In the study by Winiarska and Kolota [19] the content of dry matter in fruits ranged from 5.22 to 6.42 %, and in the study by Jankauskienë et al [20] from 4.59 to 5.67 %.

Table 1

Dry matter content [%] in tomato depending on covering and kind of mulching straw in the years 2010–2012

Specification		Kind of straw					Mean
		control	rye	corn	rape	buckwheat	
Years	2010	6.38	6.45	6.34	5.45	6.39	6.20
	2011	7.00	7.20	6.82	7.35	7.66	7.21
	2012	6.33	6.30	6.28	6.16	5.98	6.21
Covering	no cover	6.69	6.68	6.71	6.47	6.65	6.64
	polypropylene non-woven	6.45	6.63	6.25	6.17	6.70	6.44
Mean		6.57	6.65	6.48	6.32	6.68	6.54
$LSD_{0.05}$ for: years = 0.65; covering = n.s.; kind of straw = 0.21; years × kind of straw = 0.36; covering × kind of straw = n.s.							

Climatic conditions, mainly air temperature and precipitation have a decisive effect on vegetable yield and its quality [2, 21], what is confirmed in the present study. A higher content of dry matter in fruits was found in moderate wet 2011. The increase in dry matter amounted to 1.01 % compared to relatively wet 2010 and 1.00 % compared to 2012. The influence of weather conditions on the dry matter accumulation in tomato fruits was confirmed in the study by Skowera et al [22]. The amount of dry matter in the fruits of all tomato cultivars was the largest in 2009 (the most adequate to tomato water needs) and fluctuated between 4.7 and 5.4 %. According to Mitchell et al

[23] an optimal distribution of precipitation, especially in July and August (period of fruit setting), confirm not only higher yield but also the physical and chemical properties of fruits.

The study results did not indicate the effect of plants covering on the dry matter content in fruits. However, it was observed slightly and did not confirm tendency to higher accumulation of this component in fruit from non-covered cultivation. The slight increased of dry matter content in the fennel bulb in the cultivation without plant covering was confirmed in the study by Błażewicz-Woźniak [24]. In turn, in the study by Moreno et al [4] an application of polypropylene non-woven contributed to increased dry matter content in the leaves of Chinese cabbage on average by 18 % compared to non-covered control.

A significant influence on the dry matter content had the kind of organic mulch application (Table 1). A higher dry matter content was found in fruits from plots mulched with rye and buckwheat straw as well as in non-mulched control compared to fruits from object mulched with rape straw. The differences ranged from 0.25 to 0.36 %. The effect of the kind of straw on the dry matter content depended to a higher degree on the weather conditions in the study years. In 2010 a significantly the lowest content of dry matter accumulated fruits from plants cultivated on the mulch with rape straw. In 2011 more dry matter was found in fruits from plants cultivated on the buckwheat straw compared to rye and corn straw and control plot without mulch. The tomato grown on the rape straw was characterized by higher content of this component than cultivated on the corn straw. In 2012 the difference was small and was not statistically confirmed. In the studies by Majkowska-Gadomska et al [11] and Samaila et al [10], soil mulching contributed to a significant increase in dry matter content in tomato fruits compared to the control without mulch. In the study by Olfati et al [25], organic mulch applied in carrot cultivation did not have any influence on the dry matter content in roots. It was not found a significant influence an interaction between investigated factors on the dry matter content in tomato fruit.

The biological value of the edible parts of vegetables is determined by ascorbic acid content. The content of vitamin C in vegetables can be influenced by various factors such as genotypic differences, climatic conditions and cultural practices, maturity and harvesting methods. The higher the intensity of light during the growing season, the greater is vitamin C content in plant tissues [2]. In the present study, the ascorbic acid content in tomato fruit ranged from 15.95 to 22.34 mg $\cdot$ 100 g^{-1} f.m. (Table 2). A similar content of this component in fruits was found by Winiarska and Kolota [19]. The content of vitamin C depending on cultivar ranged from 14.44 to 20.65 mg $\cdot$ 100 g^{-1} f.m. In the study by Majkowska-Gadomska et al [11] the content of ascorbic acid was higher and fluctuated between 27.7 and 32.9 mg $\cdot$ 100 g^{-1} f.m. In turn, in the study by Caliman et al [26] the content of ascorbic acid depending on the cultivation way amounted from 12.83 to 17.02 mg $\cdot$ 100 g^{-1} f.m.

Weather conditions in the years of the study significantly differentiated the content of ascorbic acid in tomato. A significantly more of ascorbic acid was found in tomato cultivated in moderately wet 2011 and 2012. The differences compared to 2010 amounted to 4.97 and 3.74 mg $\cdot$ 100 g^{-1} f.m., respectively. The content of L-ascorbic acid in tomato fruit in the experiment by Skowera et al [22] was significantly different in the years of the study, however authors did not confirm dependence between L-ascorbic acid content and the hydrothermal index (K).

Table 2

Ascorbic acid content [mg · 100 g^{-1} f.m.] in tomato depending on covering and kind of mulching straw in the years 2010–2012

Specification		Kind of straw					Mean
		control	rye	corn	rape	buckwheat	
Years	2010	17.50	15.95	16.00	16.36	17.60	16.68
	2011	21.49	22.34	21.13	21.17	22.15	21.65
	2012	20.33	20.53	20.42	20.49	20.31	20.42
Covering	no cover	19.90	18.89	20.00	18.89	20.00	19.54
	polypropylene non-woven	19.65	20.31	18.36	19.79	20.04	19.63
Mean		19.77	19.60	19.18	19.34	20.02	19.58
$LSD_{0.05}$ for: years = 1.60; covering = n.s.; kind of straw = 0.52; years × kind of straw = 0.90; covering × kind of straw = 1.33							

The effect of successive kinds of straw on the ascorbic acid accumulation depended on weather conditions. In 2010 a significantly more of ascorbic acid was obtained in fruits from objects mulched with buckwheat straw and non-mulched control compared to the remaining kinds of straw. In 2011 a significantly higher content of this component were characterized fruits from plant cultivated on the rye and buckwheat straw compared to the remaining kinds of straw. In 2012 the content of ascorbic acid was on the similar level. In the study by Majkowska-Gadomska et al [11] soil mulching in tomato cultivation had no influence on the ascorbic acid accumulation. It was only observed slight and did not statistically confirm tendency to increase of this component in plot without soil mulching. Similarly in the study by Sekhon et al [27] significantly higher content of ascorbic acid in sweet pepper fruits cultivated in a control plot without mulch, compared to the plots with organic mulch was observed.

The study results showed a significant influence of the interaction between the covering and the kind of straw applied to soil mulching on the ascorbic acid content in fruits. In the cultivation under polypropylene non-woven more of ascorbic acid was noted in fruits from plants grown on the rye, rape and buckwheat straw compared to corn straw. In the study by Majkowska-Gadomska [6] plant covering and soil mulching exerted a similar effect on the L-ascorbic acid content of the edible parts of melon fruit. A tendency towards higher L-ascorbic acid concentrations in melon plants grown in mulched soil was noted.

The flavor of tomato fruit is also dependent on the concentrations of sugars. The increase of sugars concentration improved the flavor of fruits. The content of total sugars in tomato fruit ranged from 2.40 to 2.86 % f.m. and monosaccharides from 0.93 to 1.06 % f.m. (Tables 3 and 4).

Table 3

Total sugars content [% f.m.] in tomato depending on covering and kind of mulching straw in the years 2010–2012

Specification		Kind of straw					Mean
		control	rye	corn	rape	buckwheat	
Years	2010	2.63	2.40	2.61	2.60	2.60	2.57
	2011	2.72	2.86	2.79	2.72	2.72	2.76
	2012	2.56	2.69	2.69	2.53	2.61	2.62
Covering	no cover	2.60	2.58	2.68	2.71	2.75	2.66
	polypropylene non-woven	2.68	2.72	2.71	2.52	2.54	2.63
Mean		2.64	2.65	2.70	2.61	2.65	2.65
$LSD_{0.05}$ for: years = 0.16; covering = n.s.; kind of straw = 0.06; years × kind of straw = 0.10; covering × kind of straw = 0.09							

Table 4

Monosaccharides content [% f.m.] in tomato depending on covering and kind of mulching straw in the years 2010–2012

Specification		Kind of straw					Mean
		control	rye	corn	rape	buckwheat	
Years	2010	0.99	1.06	1.00	1.06	0.95	1.01
	2011	0.96	1.05	1.02	0.99	0.95	0.99
	2012	0.99	0.97	0.95	1.00	0.96	0.97
Covering	no cover	0.97	1.01	1.00	1.02	0.97	1.00
	polypropylene non-woven	0.99	1.04	0.98	1.01	0.93	0.99
Mean		0.98	1.03	0.99	1.01	0.95	0.99
$LSD_{0.05}$ for: years = n.s.; covering = n.s.; kind of straw = 0.03; years × kind of straw = 0.04; covering × kind of straw = n.s.							

In the study by Winiarska and Kolota [19] the content of total sugars in fruits amounted on average 3.11 % f.m. and monosaccharides 2.84 % f.m. According to Majkowska-Gadomska et al [11] the content of total sugars in tomato amounted from 3.4 to 4.6 % f.m. In turn, in the study by Caliman et al [26] the content of monosaccharides fluctuated between 2.06 and 2.71 % f.m. According to Davies and Hobson [28], there may be a great variation in the content of reducing sugars (from 1.66 to 3.99 g 100 $\cdot$ g^{-1}) among genotypes, even when they are cultivated in the same environment.

Weather conditions in the study years had a significant influence on the total sugars contents in fruits but did cause any change in monosaccharides. A higher content of total sugars found in 2011, which was more favourable for tomato cultivation. The influence of weather conditions on the sugars content in melon fruits was confirmed in the study by Majkowska-Gadomska [6]. On the contrary, in the study by Skowera et al [22] was not found an influence of weather conditions on the sugars content in tomato fruits.

Weather conditions significantly differentiated total sugars and monosaccharides content on the successive kind of straw. The content of total sugars in 2010 significantly decreased soil mulching with rye straw. However, more monosaccharides compared to remaining kinds of straw and non-mulched control contained fruits from plots mulched with rye and rape straw. The cultivation on the corn straw also increased the content of monosaccharides compared to buckwheat straw. In turn in 2011, more favourable influence both on the total sugars and monosaccharides had cultivation on the mulch with rye straw. It was also observed an increased in monosaccharides content in tomato cultivated on the corn straw compared to buckwheat and control plot. A higher content of total sugars in 2012 was noted in fruits from plots mulched with rye and corn straw and monosaccharides from rape straw. In the study by Samaila et al [10], soil mulching with rice straw caused a significant increase of carbohydrates in tomato fruits compared to the control without mulch. Parmar et al [12] was found that soil mulching with straw and dry leaves in melon cultivation caused an increased of monosaccharides and total sugars contents compared to non-mulched control.

The interaction between investigated in the experiment factors significantly differentiated content of total sugars, however had no influence on the monosaccharides (Tables 3 and 4). In the non-covered plots a higher content of total sugars were characterized fruits from plants grown on the mulch with corn, rape and buckwheat straw. However, in the cultivation under polypropylene non-woven more of this component was found in fruits from plots mulched with rye and corn straw and non-mulched control compared to remaining kinds of straw. In the study by Majkowska-Gadomska [6] a higher content of total sugars was observed in simultaneously mulched and covered plots and monosaccharides in plots covered only. On the contrary, in the study by Caliman et al [26] tomatoes cultivated under cover was contained significantly less of monosaccharides than grown in the open field. According to Beckmann et al [29] the lower reducing sugar content of fruits produced in the protected environment may be related to lower light intensity, approximately 25 % lower than in the field. The greater sugar content in the fruits produced in the field may be due, in part, to the greater light intensity in this crop environment and greater photosynthetic plant activity. The influence of solar radiation on sugar content in cherry tomatoes also has been found in the study by Rosales et al [30].

According to Mahakun et al [31] the genetic factor is the major acid content determinant in tomato plant fruits. These authors reported variation in fruit acidity (% citric acid) for different accessions of *Lycopersicon esculentum*, from 0.40 % to 0.91 %. According to Davies and Hobson [28] during fruit development, acidity increases, reaching a maximum value at the first signs of yellow coloration and progressively decreasing with the appearance of the red color. In the present study flesh acidity of tomato amounted to average 0.41 $g \cdot 100\ g^{-1}$ (Table 5).

Table 5

Flesh acidity of tomato [$g \cdot 100\ g^{-1}$ counted on lemon acid] depending on covering and kind of mulching straw in the years 2010–2012

Specification		Kind of straw					Mean
		control	rye	corn	rape	buckwheat	
Years	2010	0.38	0.36	0.36	0.36	0.38	0.37
	2011	0.46	0.47	0.45	0.46	0.48	0.46
	2012	0.41	0.41	0.41	0.41	0.40	0.41
Covering	no cover	0.41	0.40	0.42	0.40	0.42	0.41
	polypropylene non-woven	0.42	0.42	0.39	0.41	0.42	0.41
Mean		0.42	0.41	0.41	0.41	0.42	0.41
$LSD_{0.05}$ for: years = 0.03; covering = n.s.; kind of straw = 0.01; years × kind of straw = 0.02; covering × kind of straw = 0.03							

The flash acidity in the study by Caliman et al [26] was relatively lower and amounted from 0.31 to 0.35 %. According to authors, the determination of fruit acidity at complete maturation, when acidity declines, is likely the reason for the low values.

The flesh acidity of the tomato fruit diversified in each year of the study (Table 5). The fruits were characterized by the highest acidity (0.45–0.48 $g \cdot 100\ g^{-1}$) in 2011, whereas the fruits from 2010 had the lowest (0.36–0.0.38 $g \cdot 100\ g^{-1}$). A similar dependence was observed in the study by Skowera et al [22]. The authors found that the highest flesh acidity had fruits in moderately wet 2008 and the lowest in 2010, which was characterized by the most unfavourable for tomato rainfall distribution.

The effect of the kind of straw on the flesh acidity depended on weather conditions. The higher flesh acidity in 2010 was characterized fruits from plants cultivated on the mulch with buckwheat straw and in control plot. In 2011 a higher acidity had fruits cultivated on the buckwheat straw. It was not found a difference between fruit acidity in 2012. In the study by Parmar et al [12] soil mulching with straw and dry leaves slightly reduced flesh acidity of melon fruit.

On the basis of the obtained results, a significant influence of the covering and the kind of straw applied to soil mulching on tomato flesh acidity was found. In the covered plots the fruits acidity increased cultivation on the mulch with rye and buckwheat straw and in non-mulched control.

Conclusions

1. Weather conditions in the years of the study had a higher degree of influence on the chemical composition of tomato fruits. The highest content of nutritive compounds was characterized fruits in warm and moderately wet 2011.

2. The application of polypropylene non-woven covers in tomato cultivation did not cause changes in the content of investigated compounds in fruits.

3. The investigated in the experiment organic mulches influences on the selected compounds of nutritive value without regular pattern.

4. Irrespective of covering, accumulation of dry matter and ascorbic acid favoured cultivation on the mulch with rye and buckwheat straw. A higher content of total sugars was found in fruits from plots mulched with corn straw and monosaccharides with rye straw. However, cultivation on the buckwheat straw contributed to increase flesh acidity of tomato fruits.

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WARTOŚĆ ODŻYWCZA POMIDORA UPRAWIANEGO POD OSŁONAMI NA GLEBIE MULCZOWANEJ

Katedra Warzywnictwa
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Abstrakt: Badania przeprowadzono w latach 2010–2012 w Stacji Doświadczalnej należącej do Uniwersytetu Przyrodniczo-Humanistycznego w Siedlcach. Doświadczenie polowe założono w układzie split-blok w trzech powtórzeniach. Badano wpływ osłaniania roślin (bez osłony, włóknina polipropylenowa) i mulczowania gleby różnymi rodzajami słomy (żytnia, kukurydziana, rzepakowa, gryczana) na zmiany zawartości wybranych składników odżywczych pomidora 'Polfast F_1'. Wpływ mulczu ze słomy porównano z obiektem kontrolnym bez słomy. Zawartość suchej masy, kwasu askorbinowego, cukrów ogółem oraz kwasowość miąższu pomidora zależały w dużym stopniu od warunków pogodowych. Najbardziej korzystne warunki do gromadzenia tych składników panowały w ciepłym i umiarkowanie wilgotnym roku 2011. Zastosowanie osłony z włókniny polipropylenowej w początkowym okresie wzrostu roślin nie powodowało zmian w składzie chemicznym owoców. Wpływ mulczu ze słomy na wartość odżywczą pomidora był zróżnicowany. Najwięcej suchej masy i kwasu askorbinowego zawierały pomidory uprawiane na mulczu ze słomy żytniej i gryczanej. Większą zawartość cukrów ogółem w owocach stwierdzono przy uprawie roślin na słomie kukurydzianej, a cukrów redukujących żytniej. Pomidory uprawiane na mulczu ze słomy gryczanej charakteryzowały się większą kwasowością miąższu.

Słowa kluczowe: sucha masa, kwas askorbinowy, cukry, kwasowość miąższu, włóknina polipropylenowa, słoma

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THE ROLE OF *Pseudomonas fluorescens* IN THE PROCESS OF *Rhizoctonia solani* GROWTH INHIBITION

UDZIAŁ *Pseudomonas fluorescens* W ZAHAMOWANIU WZROSTU *Rhizoctonia solani*

Abstract: The aim of conducted research was to determine influence of metabolites produced by *Pseudomonas fluorescens* on the growth of 4 phytopathogenic strains of *Rhizoctonia solani* marked R1, R2, R3 and R4 which infect sugar beetroot. The antagonistic properties were assessed with the culture-plate method on PDA medium for *P. fluorescens* cultured for 4, 6 8, 10 and 24 hours at 25 ^{o}C for 5 days. The fungistatic activity of *P. fluorescens* was determined against the growth rate index and the rate of mycelial growth inhibition. Obtained results prove, that *Rhizoctonia* spp. strains were both sensitive and resistant to metabolites produced by *P. fluorescens*. The highest inhibition of the linear growth of mycelium has been observed for *R. solani* R1 and R4 strains. In both cases the highest inhibition, reaching almost 60 %, has been recorded for the trials cultured for 4 hours and the lowest, amounting ca. 30 % after 24 hours of culturing. However, the other strains of *R. solani* marked R2 and R3 were resistant to the metabolites produced by *P. fluorescens* regardless of the length of culturing.

Keywords: antifungal activity, *Pseudomonas fluorescens*, *Rhizoctonia solani*, growth rate index

Introduction

Rhizoctonia solani Kühn is one of the most commonly recognised soil pathogens. It can persist in soil for a long time due to durable sclerotia and saprophytic manner of mycelial growth which infects host plants. *Rhizoctonia solani* is a destructive plant pathogen and can cause damage worldwide on more than 142 plant species, including many agricultural and horticultural crops. *Rhizoctonia solani* is an heterogeneous species composed of subspecific groups called anastomosis groups (AGs) defined on the basis of hyphal anastomosis reactions [1–2]. On sugar beet, *R. solani* is responsible for diverse pathologies. Isolates from AG-2 and AG-4 are known to be pathogenic on roots of adult plants and on seedlings, respectively. Additional anastomosis groups

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R. solani can be virulent on seedlings or have been isolated from soils or sugar beets in diseased fields [3–4]. In Poland, there have been the following anastomosis groups of *R. solani* Kühn recognized at sugar beetroot plantations: AG 5, AG 1 IB, AG 2-1, AG 2-2 IIIB, AG 4 HG II and AG 11, which infect mostly beetroot seedlings [5]. Economic losses were estimated to average 2 % in the United States; however, damage can vary greatly (0 to 50 %) from field to field depending on cropping history and environment [6-7]. There are limited possibilities to protect damaged plant roots with chemical substances, therefore the only way to reduce the danger of infecting the host plant by *R. solani* is an application of an appropriate agrar procedure, a selection of tolerant types of plants as well as searching for alternative methods of protection, such as biological control employing microorganisms [8]. Biological control method employ microorganisms being the natural antagonists of plants pathogens [9]. Such microbes are very often isolated from the same natural environment where they will be applied again.

It is worth considering bacteria residing in a rhizosphere of cultivated plants when searching for the appropriate bacteria strains. As shown in research papers, *P. fluorescens* may be useful as a potential antagonist towards phytopathogenic fungi. Certain members of the *P. fluorescens* have been shown to be potential agents for the biocontrol which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth promotion and reduce severity of many fungal diseases [10–12]. It is believed that biological activity of *P. fluorescens* results from their ability to produce biologically active compounds such as: hydrogen cyanide, salicylic acid, siderophores, lytic enzymes, proteases and secondary metabolites revealing strong antifungal properties – pyrrolnitrin, pyoluteorin, and 2,4-diacetylphloroglucinol [13–15]. Hass and Défago [12] reviewed the mechanisms by which *P. fluorescens* control pathogenic microorganisms in detail. During root colonization, these bacteria produce antifungal antibiotics, elicit induced systemic resistance in the host plant or interfere specifically with fungal pathogenicity factors. Before engaging in these activities, biocontrol bacteria go through several regulatory processes at the transcriptional and post-transcriptional levels. In this way, competitive exclusion of pathogens as the result of rapid colonization of the rhizosphere by *P. fluorescens* may also be an important factor in disease control.

In the research, the activity of soil strain *P. fluorescens* against four selected *R. solani* strains isolated from infected sweet beetroot roots has been examined.

Materials and methods

In the experiment, a fungistatic activity of *P. fluorescens* against 4 strains of *R. solani* marked R1, R2, R3 and R4 has been assessed. The strain *P. fluorescens* was isolated from soil and identified with the use of ID32GN tests (bioMérieux) and Bergey's Manual of Systematic Bacteriology [16]. The strains of tested fungi were isolated from the infested bulbs of sugar beetroot and diagnosed on the basis of their macro- and microscopic features.

The bacteria were cultured in the broth medium for 48 hours at 30 ^{o}C. Next, the broth was inoculated with the suspension of 10^6 cfu/ml density and incubated for the time period from 4 to 24 hours. After the incubation it was centrifuged at 10 000 rpm and obtained supernatant underwent further analysis.

Conducted tests employed a culture-plate method applied on PDA growth medium consisting of (g/dm^3): glucose 20.0, potato extract 4.0, agar 15.0. Tested growth media were inoculated with supernatants obtained after 4, 6, 8, 10 and 24 hours of culturing of *P. fluorescens* rods. Next, the media were inoculated with 10 mm discs overgrown with 7-days old mycelium of tested *R. solani* strains. The control trials contained only tested *R. solani* strains with no addition of supernatant. All plates were incubated at 25 ^{o}C for 5 days. The diameters on the plates were measured every day until the mycelium of *R. solani,* in the control trial, reached the edge of the plate. The experiment was conducted in six replicates, where one trial was represented by one culturing plate with the growth medium and the mycelial disc.

The influence of metabolites produced by *P. fluorescens* on the growth of *R. solani* strains was determined against the growth rate index, calculated according to the formula [17]:

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \frac{b_2}{d_2} + \ldots + \frac{b_x}{d_x}$$

where: T – the growth rate index,
A – is a mean value of diameter measurements [mm],
D – is the length of the experiment (number of days),
b_1, b_2, b_x – denote an increase in a diameter size since the last measurement,
d_1, d_2, d_x – are the number of days since the last measurement.

The fungistatic properties of the supernatant have been assessed on the basis of the linear growth inhibition of the fungus.

Statistical significance was determined using an analysis of variance (ANOVA) followed by Duncan's test. Values were considered significantly different at $p < 0.05$.

Results and discussion

The paper presents a pilot research which tests *P. fluorescens* strain against its ability to synthesize exocellular metabolites possessing fungistatic abilities in relation to phytopathogenic strains of *Rhizoctonia* spp. Conducted tests revealed the direct influence of metabolites produced by *P. fluorescens* on the growth rate of the fungi under study. It has been noted, that an addition of the supernatant collected at different development phases of bacteria prompted responses which differed for every tested strain. Obtained results are presented by the values of the growth rate index of the mycelium (including the mycelial growth in time) and the degree of the linear growth inhibition of fungi. On the basis of obtained results and statistical analysis, strains of *Rhizoctonia* spp. under study may be divided into two groups due to a lack of statistically significant differences between the strains in test trials. Strains *R. solani* R1 and R4 may be described as sensitive strains, whereas strains *R. solani* R2 and R3 as resistant (Table 1).

Table 1

Influence of *P. fluorescens* on the growth rate index of tested fungi

Culturing time [h]	*R. solani* R1		*R. solani* R2		*R. solani* R3		*R. solani* R4	
	control	trial	control	trial	control	trial	control	trial
4	99.53[a]	36.67[a]	94.10[a]	94.22[a]	95.35[a]	67.33[a]	99.28[a]	36.86[a]
6	99.28[a]	43.14[ab]	93.92[a]	94.47[a]	95.44[a]	95.20[b]	98.89[a]	45.97[b]
8	98.42[a]	50.92[b]	93.94[a]	95.60[b]	93.78[a]	94.24[b]	98.78[a]	45.58[b]
10	97.69[a]	49.25[ab]	93.41[a]	93.70[a]	93.85[a]	93.09[b]	98.19[a]	51.39[b]
24	99.97[a]	54.22[b]	95.42[a]	95.95[b]	98.17[a]	99.39[b]	100.64[a]	70.44[b]

Different letters indicate significant differences (ANOVA, $p < 0.05$, Duncan's test).

Presented paper shows that prolonging the culturing time and at the same time increasing the amount of metabolites do not affect the inhibition rate of the linear growth index. For the strains of *R. solani* R1 and R4 described as sensitive strains the reduction rate of the growth rate index decreases as the culturing time of bacteria is longer (Table 1). The measured values of the indexes for the control trials employing these strains ranged between 97.69 and 100.64, while for the proper trials they amounted from 36.86 to 70.44. The highest inhibition of the growth rate index of *R. solani* R1 was noted in case of 4 and 6-hour culturing of *P. fluorescens* and amounted 63.16 % and 56.55 % respectively. The highest reduction in the growth rate index of *R. solani* R4 was recorded after inoculation with the bacteria cultured for 4, 6 and 8 hours. The amounts obtained are: 62.87 %, 53.51 % and 53.85 % respectively. Prolonging the culturing time did not contribute to a higher reduction of the growth rate index. Therefore, for the both strains *R. solani* R1 and R4 the reduction of the growth rate index was the lowest in case of metabolites obtained after 24-hours of culturing of *P. fluorescens* (45.76 % and 30.01 % respectively) (Fig. 1). For the strains presented in

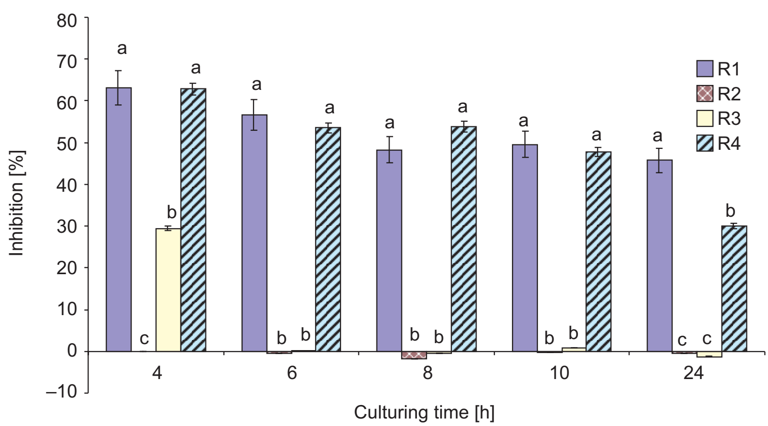

Fig. 1. Reduction of the growth rate index of tested fungi. Different letters indicate significant differences (ANOVA, $p < 0.05$, Duncan's test)

the paper and described as resistant, *ie R. solani* R2 and R3 (Table 1), the values of the growth rate indexes in the control trials ranged between 93.41 and 98.17, and for the proper trials they amounted from 67.33 to 99.39. Recorded values of the growth rate indexes were so high that in most trials their reduction was not noted. Only in case when the culturing medium was inoculated with the bacteria grown for 4 hours, the reduction of the mycelial growth index, lower than 30 %, was noted for *R. solani* R3 (Fig. 1).

The negative values of the reduction of the growth rate index noted during conducted research prove, that metabolites produced by *P. fluorescens* stimulate the mycelial growth of *R. solani* R2 and R3. It would be impossible to draw such conclusion by only analysing the linear growth inhibition of the mycelium (Fig. 2). The chart presents clearly, that introduction of the metabolites produced by *P. fluorescens* did not inhibit the mycelial growth of *R. solani* R2 at all, while the growth of strain R3 was inhibited in 25 % after introduction of bacteria cultured for 4 hours. Significantly higher values were noted for *R. solani* R1 and R4 strains. For the both strains, it has been observed that the longer the bacteria were cultured for, the lower the inhibition rate of the linear growth of mycelium was. The values of noted reduction amounted between 60 % when applying bacteria cultured for 4 hours to 42 % (for *R. solani* R1 train) and 27 % (for *R. solani* R4 strain) when introducing the bacteria cultured for 24 hours.

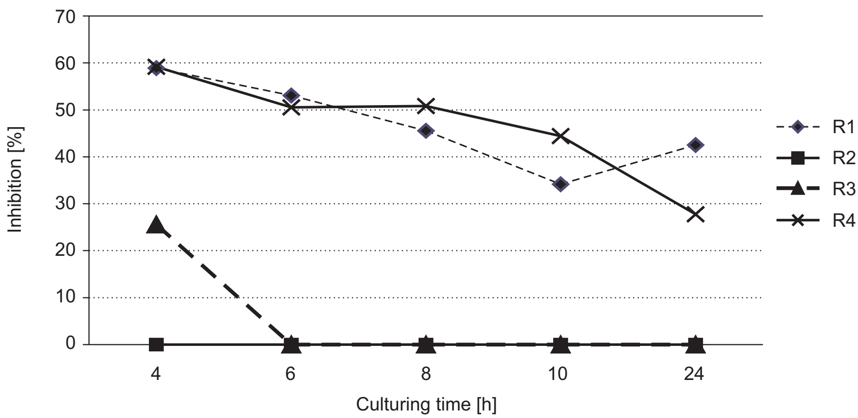

Fig. 2. Influence of *P. fluorescens* on the linear growth of *R. solani* strains

Conducted pilot research showed varied and selective activity of *P. fluorescens* strain against indicative fungi development and thus it is difficult to identify clearly its phytopathogenic activity against soil fungi. Also some differences in the growth rate inhibition of phytopathogenic fungi caused by strains of *P. fluorescens* have been obtained by Jankiewicz [15]. The value of inhibition rate for *P. fluorescens* F1 amounted between 30–80 % and for the strain *P. fluorescens* F2 between 40–90 %. Singh and Sinha [18] found that *P. fluorescens* of higher rate (8 g/dm^3) was highly effective in reducing disease severity (60.0 %) and incidence (35.6 %) and increasing grain yield (33.8 %) and 1000-grain weight (12.9 %). In this study, the lower rate of the bio-agents though effective against the disease but was found inferior as compared to higher rates. Grosch et al [19] analyzed two strains of *P. fluorescens*, B1 and B2, were evaluated in a growth chamber and in the field against *R. solani* in potato and in lettuce. The greatest disease suppression effect on potato was achieved by strain B1 (37 %), followed by B2 (33 %), whereas the marketable tuber yield increased up to 12 % (B1) and 6 % (B2). Whereas, research by Stachowiak and Dach [20] show that strains of *R. solani* are resistant to bacteria isolated from compost. It seems that inhibitory activity depends on both bacterial strain and indicative fungus. It may result from different sensitivity to antifungal substances produced by bacteria and concentration of such metabolites in the culturing medium. Selective activity of *P. fluorescens* and other bacteria against the development of phytopathogens has been described in many research papers [10, 21–28]. It has been proved that antifungal activity of *P. fluorescens* is closely related to antibiotics and lytic enzymes production. Hammer et al [14] proved, that *P. fluorescens* BL915 strain which produces pyrrolnitrin is an efficient micro-organism in the process of biological control of *R. solani* development, whereas Corbell and Loper [13] showed in their research that the growth inhibition of *R. solani* depends on pyrrolnitrin, pyoluteorin, 2,4-diacetylphloroglucinol and hydrogen cyanide. They employed *P. fluorescens* Pf-5 and proved that mutants of Pf-5 ApdA^{-} produced significantly lower amounts of the above substances and did not inhibit the growth of *R. solani.* Among fourteen strains of *P. fluorescens* tested by Nagarajkumar et al. [29], one marked PfMDU2 was most efficient in terms of mycelial growth inhibition of *R. solani.* At the same time, tested strain showed high activity of β-1,3-glucanase and production of salicylic acid, siderophore and hydrogen cyanide. The authors proved in their research that antifungal properties of *P. fluorescens* depend on the level of substances mentioned above. Vivekananthan et al. [30] proved, that the enhanced expression of defence-mediating lytic enzymes chitinase and β-1,3-glucanase of *P. fluorescens* FP7 may collectively contribute to suppress the anthracnose pathogen, leading to improved yield attributes. Nielson and Sörensen [31] demonstrated that isolates of *P. fluorescens* antagonistic to *R. solani* and *Pythium ultimum,* produced endochitinase and chitobiosidase. Detailed studies of three selected isolates showed that extracellular release of endochitinase activity also took place in stationary phase (corresponding to 25–50 h of incubation). These results differ from author's own results as the highest antifungal activity was noted after the application of supernatants obtained after 4 and 6 hours of culturing, which corresponds to the early logarithmic growth phase. It allows the authors to assume that since in own research there has been no inhibitory activity of *P. fluorescens* R2 and R3 strains recorded, then the strains are not active in terms of β-1,3-glucanase, salicylic acid, hydrogen cyanide and siderophore as well as do not produce pyrrolnitrin.

Conclusions

To sum up, it has been stated that *P. fluorescens* shows antagonistic activity to different strains of *R. solani.* Their potential in suppressing fungal growth should be exploited as a complement or an alternative to chemical control for sheath blight disease in plants. Significant differences in obtained results indicate that prior to field trials, extended laboratory tests including various strains of *P. fluorescens* are required. It should be noted that, there are some restrictions which may hinder a widespread application of this bacteria in the process of biological control in agriculture. The most important factor concerning their application is variable efficiency of *P. fluorescens* under field conditions, in which the inhibition of phytopathogens growth results from cooperation and complementarity of different mechanisms.

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UDZIAŁ *Pseudomonas fluorescens* W ZAHAMOWANIU WZROSTU *Rhizoctonia solani*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej
Uniwersytet Opolski

Abstrakt: Celem podjętych badań było określenie wpływu metabolitów *Pseudomonas fluorescens* na wzrost 4 fitopatogennych szczepów buraka cukrowego *Rhizoctonia solani* oznaczonych jako R1, R2, R3 oraz R4. Ocenę właściwości antagonistycznych metabolitów przeprowadzono metodą hodowlano-płytkową na podłożu PDA dla 4, 6, 8, 10 i 24 godzinnych hodowli *P. fluorescens*. Hodowle prowadzono w temperaturze 25 ^{o}C przez 4–7 dni. Na podstawie indeksu tempa wzrostu oraz stopnia zahamowania wzrostu grzybni określono aktywność fungistatyczną *P. fluorescens*. Wyniki doświadczenia wskazują, że wśród badanych szczepów *Rhizoctonia* spp. były zarówno szczepy wrażliwe, jak i oporne na działanie metabolitów *P. fluorescens*. Największą inhibicję rozrostu liniowego grzybni zaobserwowano dla szczepów *R. solani* R1 oraz R4. W obu przypadkach najwyższe, prawie 60 % zahamowania wzrostu grzybni uzyskano dla 4-godzinnej hodowli, a najniższe w granicach 30 % dla hodowli 24-godzinnej. Natomiast szczepy *R. solani* R2 i R3 były oporne na działanie metabolitów *P. fluorescens* niezależnie od wieku hodowli.

Słowa kluczowe: aktywność przeciwgrzybowa, *Pseudomonas fluorescens, Rhizoctonia solani,* indeks tempa wzrostu

Marcin NIEMIEC[1]

EFFICIENCY OF SLOW-ACTING FERTILIZER IN THE INTEGRATED CULTIVATION OF CHINESE CABBAGE

EFEKTYWNOŚĆ NAWOZU WOLNODZIAŁAJĄCEGO W INTEGROWANEJ UPRAWIE KAPUSTY PEKIŃSKIEJ

Abstract: The study aimed to assess the suitability of slow-acting fertilizer in cultivation of Chinese cabbage in the integrated production system. The assumed objective was realized on the basis of a strict field experiment set up on the soil with granulometric composition of medium loam. The test plant was Chinese cabbage (*Brassica rapa* L.), 'Parkin F1' cv. The experiment was set up on 17.08.2011 and the plants were harvested on 08.10.2011. The experimental factor was diversified fertilization. The cultivation and protection of the plants were conducted on the basis of methodology of Chinese cabbage integrated production approved by the Main Inspector for Plant Protection and Seed Science. Traditional fertilizers (triple superphosphate, potassium salt and ammonium nitrate) and NPKCaMg (18-05-10-4-2) multi-component fertilizer with nutrient slow-release were applied. The experiment comprised 8 fertilization variants and the control treatment. Doses of phosphorus and potassium, meeting the plants requirements at assumed yield amount, were applied in all fertilization variants. Nitrogen fertilization on subsequent objects differed both with the form and quantity of applied element. On the basis of the results obtained in conducted experiments, the indices showing nitrogen fertilization efficiency were computed: agronomic efficiency, partial factor productivity, physiological efficiency, nitrogen recovery efficiency and removal efficiency.

Fertilization significantly modified the quantity of obtained yield. On the control, without mineral fertilization, the crop yield was 44.22 $Mg \cdot ha^{-1}$. The largest yield, 120.7 $Mg \cdot ha^{-1}$, was obtained on the object with an admixture of 400 kg of slow-acting fertilizer, 89 kg of triple superphosphate and 177 kg of potassium salt. The best optimal values of fertilization efficiency were obtained in the objects where 400 and 500 $kg \cdot ha^{-1}$ of slow-acting fertilizer and full doses of phosphorus and potassium were applied. Computed indices of fertilization efficiency indicate that optimization of fertilization using slow-acting fertilizer, particularly in conditions of intensive cultivation, may improve fertilization efficiency several-fold in comparison with integrated production methods using traditional fertilizers. Results of conducted experiments show that optimization of fertilization in conditions of intensive production may enhance fertilizer nitrogen uptake by 50 % at maintained high crop yields, which greatly improves economic effectiveness of production and reduces the amount of biogens dispersed in the environment.

Keywords: Chinese cabbage, integrated production, slow-acting fertilizer, fertilization efficiency

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Introduction

Integrated production (IP) is the food quality system which bases on sustainable use of the environmental resources, energy and means of production to generate good quality yields at maintained profitability of production. The idea of integrated production emphasizes the environmental and health aspects in agricultural production. The environmental benefits, which may be reached through implementation of integrated production are associated with reducing the amount of xenobiotics supplied to the environment through a rational plant protection based in the first place on agrotechnical and biological methods. According to IP assumptions, the use of pesticides should be preceded by a thorough monitoring of the cultivated plants but also attempts should be made to use in the first place agrotechnical and biological methods for plant protection [1]. In the situation when the full knowledge in the field of pest biology and conditions of pathogen expansion in specified plantations using biological methods is applied, the results comparable with the systems using traditional plant protection may be obtained [1, 2]. Both the quantity of pesticides used and the date of their application are important, as well as the selection of an appropriate assortment of plant protection means. Optimization of plant protection may result in a diminishing of eco-toxicological effect of agriculture on the environment and consumers, as pointed to by many researchers dealing with this problem [4–6]. However, implementing plant protection in compliance with IP principles is problematic due to insufficient knowledge of producers about biological and environmental aspects of agronomic production and a lack of universally used technologies of production [7]. A necessity to employ a highly qualified staff responsible for plant protection, high labour outlays and greater risk of plantation destruction by agrophages, discourage producers from introducing integrated production [8]. Another problem preventing an efficient realization of integrated production is the system of sustainable nutrient management. Fertilization plays an important role in crop production because it shapes the quantity and quality of yield, affects physical, biological and physicochemical soil properties, influences quality of surface and groundwater and the air quality. From the producer's point of view, fertilization and plant protection are crucial factors in price formation. Too high or too low doses of components but also wrong technologies of fertilization (techniques and dates of fertilizer application) negatively affect the environment, the quantity and quality of yield [9]. Increase in the efficiency of plant irrigation is also an important aspect of integrated plant production [10]. Application of irrigation, despite greater energy and environmental outlays connected with the exploitation of water resources, often produces positive results through improving fertilization efficiency and use of soil productive potential. Improvement of the efficiency of fertilization and irrigation has been currently one of the priorities of agricultural sciences [11]. In the extensive agriculture, owing to simple techniques, crop yielding may be increased even by several dozen percent at low outlays of labour and means of production [12]. Improving fertilization efficiency in modern agriculture is much more difficult due to efficient methods of production already in use. However, improvement of fertilizer components utilization by several percent is profitable in the global scale [13, 14], therefore contemporary agriculture must be equipped with the technologies making use of the recent scientific achievements [15]. Implementing integrated production, despite the benefits resulting from the idea behind it, is risky at the present stage of knowledge and experiments because of a danger of destroying crops by pests or diseases or a hazard of plant malnourishment, mainly with nitrogen. These hazards result from inadequate knowledge and mistakes, which may be made during production process. Limited use of pesticides requires their more precise application, which is connected with a necessity of choosing the optimal date of their application and implementing phytosanitary crop rotations, which are usually troublesome from the producer's point of view. Effective application of pesticides may be associated with a necessary ongoing counselling in this respect, which raises the costs of production. The only effective way of implementing the principles of integrated agriculture is convincing farmers about a possible improvement of production owing to the application of methods in compliance with the principles of sustainable agriculture [16]. Implementing quality systems in a primary production is connected with necessary costs which must be borne by the society, therefore the strategic objective should be convincing consumers about the necessity of bearing costs of environment-friendly technologies implementation. Carlsson et al [17] point to the non-economic aspects of rationalization of agronomic production.

For many producers moral and social aspects are important. Farmers implementing integrated production systems achieve a higher social status on local markets and win greater confidence of consumers. Educational activities undertaken by non-governmental organization caused that integrated production is the food quality system winning increasingly greater confidence among consumers, whereas IP quality mark is more desired. In compliance with The Directive of the European Parliament and the Council of 21 October, 2009 no. 2009/128/WE [1], farmers are obliged to implement plant protection according to the principles of integrated production from 1.01.2014.

Agricultural production is to various degree affected by the soil properties, water availability in individual periods of vegetation and the weather conditions [18, 19]. A proper approach to agricultural production should be based on making decision about fertilization, plant protection or agrotechnology basing on the results of plantation monitoring. Formulating and unification of the principles of integrated production is difficult because of changeability of soil conditions depending on the region of production and climatic conditions in respective years. Because the efficiency of widely understood agrotechnology to a great extent depends on the temperature and water availability, cultivation technologies should be developed based on a thorough analysis of the atmospheric and soil conditions, which on the same time do not require a costly equipment or specialist knowledge.

The aim of presented investigations was determining the usability of a slow-acting fertilizer for the optimisation of Chinese cabbage production efficiency under conditions of integrated production. Efficiency of nitrogen fertilization was assessed on the basis of the following indices of fertilization efficiency: agronomic efficiency, physiological efficiency, partial factor productivity, efficiency of nitrogen recovery and efficiency of nitrogen removal [20]. The indices showing fertilization efficiency provide plenty of information about the environmental and productive aspects of nitrogen fertilization.

Material and methods

The field experiment was set up using randomised block method, on the soil with granulometric composition of medium loam; its properties were shown in Table 1.

Table 1

Selected properties of soil used for the experiments [$mg \cdot kg^{-1}$]

pH in H_2O	pH in KCl	N_{tot}	C_{org}	N_{min}	P	K	Mg	Ca
		[$g \cdot kg^{-1}$]		[$mg \cdot kg^{-1}$]				
7.01	6.65	3.14	46.5	366	147.8	459.5	199.4	1350

The test plant was Chinese cabbage (*Brassica rapa* L.), 'Parkin F1' cv. The forecrop for the Chinese cabbage was early potato. Organic fertilization with 35 Mg FYM $\cdot$ ha^{-1} was applied under the forecrop in autumn 2010. A full dose of mineral fertilization was applied under potatoes. The experiment was set up on 17.08.2011. The experimental factor was diversified fertilization. The experiment comprised the control and 8 fertilizer treatments in four replications, according to the design presented in Table 2.

Table 2

Experiment design

Object number	Fertilizer quantity				Component dose		
	Slow acting fertilizer NPKCaMg (18-05-10-4-2)	Ammonium nitrate	Triple superphosphate	Potassium salt	N	P_2O_5	K_2O
	[$kg \cdot ha^{-1}$]				[$kg \cdot ha^{-1}$]		
Control	0	—	—	—	—	—	—
1	200	—	108	213	36	60	150
2	400	—	89	177	72	60	150
3	500	—	76	158	90	60	150
4	600	—	65	140	108	60	150
5	800	—	43	103	144	60	150
6	—	150	130	250	50	60	150
7	—	225	130	250	100	60	150
8	—	300	130	250	150	60	150

A slow-acting multi component fertilizer NPKCaMg (18-05-10-4-2), ammonium nitrate, triple superphosphate and 60 % potassium salt were used for the experiment. The slow-acting fertilizer was applied in points under each plant during planting of seedlings prepared in cell VEFL trays. Phosphorus and potassium fertilizers were wholly used pre-sowing, whereas ammonium nitrate was applied at two terms: 60 % of nitrogen dose before and 40 % after the plants planting. The date of top dressing was selected on the basis of observations of meteorological conditions and plant condition monitoring. The plants were cultivated at the row spacing of 50 × 28 cm. Chinese cabbage was harvested on 08.10.2011.

Plants were cultivated and protected in compliance with the Methodology of integrated production of Chinese cabbage by art. 5, item 2 of the Act on plant protection of 18 December 2003, uniform text [21] approved by the Main Inspector for Plant Health and Seeds [22].

Production and technical documentation was kept for each fertilization variant, in compliance with the regulations of the appropriate legislation [23]. The plants were watered to the optimum moisture content in order to eliminate the effect of water stress on the experiment result.

Prior to the experiment outset, analyses of physiochemical and chemical properties were conducted for the soil on which the experiment was located. Soil pH, granulometric composition, organic matter content, mineral nitrogen and Kjeldahl's nitrogen content, available forms of P, K, Mg, Ca and Na contents and microelement content were assessed in the soil.

Results and discussion

In the integrated cultivation of brassica vegetables all doses of organic and mineral fertilizers are determined on the basis of fertilizer needs resulting from the expected crop yield, the kind of soil, its abundance in nutrients and position in a crop rotation. Particular attention is paid to the use of organic fertilizers as the basic source of soil humus and nutrients for plants. At a low level of organic fertilization there may be problems with a necessary application of larger doses of mineral fertilizers, which poses a hazard of a occasionally higher supply of components for plants and higher soil salinization. It results in a greater amount of fertilizer components dispersed in the environment and their utilization by plants to a lower degree. The optimum content of nutrients for Chinese cabbage is 160–200 mg of mineral N, 90–105 mgP, 240–270 mgK and 80–100 mgMg per 1 kg of soil. Chinese cabbage requirements, *ie* the amount of components taken up with 10 Mg yield are: 20 kg nitrogen (N), 10 kg phosphorus (P_2O_5), 35 kg potassium (K_2O) and 3 kg magnesium (MgO) [22]. Plant nutrient requirements were computed for the assumed yield of 100 Mg $\cdot$ ha^{-1}, as: 160 kgN, 36 kgP, 270 kgK and 21 kgMg $\cdot$ ha^{-1}. Fertilizer needs were calculated on the basis of the content of available element forms in soil and nutrient needs of plants, with regards to the history of the field, following the methodology of Chinese cabbage integrated production system [22]. The fertilizer needs were determined on the level of 100 kgN, 60 kgP_2O_5 and 150 kgK_2O $\cdot$ ha^{-1}. While designing the fertilization system a negative balance of the main fertilizer components was assumed due to a high content of their available forms in soil (Table 1). The fertilizers used for the experiment meet the standards stated in the Directive of the European Parliament and the Council of 24 November 1997 [24].

The doses of phosphorus and potassium applied in all variants of the conducted experiment corresponded to the fertilizer needs calculated according to the methodology of integrated production, while individual objects differed with the form of applied fertilization. In case of nitrogen fertilization the variable factor was the form and dose of this element.

The yield of Chinese cabbage aboveground parts obtained on the control was on the level of 44.22 Mg $\cdot$ ha^{-1} (Fig. 1).

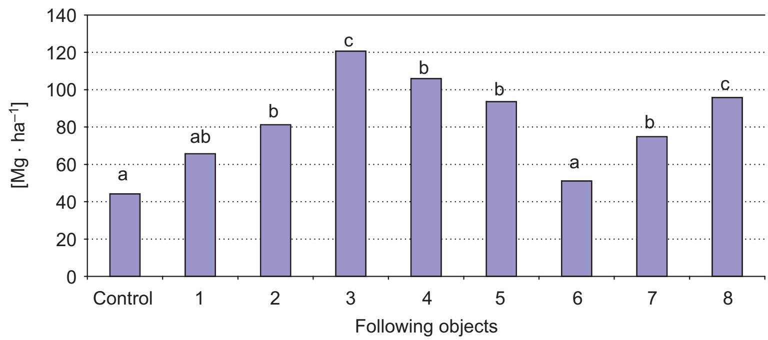

Fig. 1. The yield of Chinese cabbage aboveground parts in the successive objects of experiment

Fertilization with 200 kg of slow-acting fertilizer per 1 hectare and application of a full dose of phosphorus and potassium led to an increase in yield by over 20 Mg $\cdot$ ha^{-1}. The biggest yield of 120.7 Mg $\cdot$ ha^{-1} was obtained in the 3rd fertilization variant in which 500 kg of slow-acting fertilizer was applied with a complementary phosphorus and potassium treatment. Further increasing the amount of slow-acting fertilizer (objects 4 and 5) caused a decline in the crop yield. The effect of decline in yields might have been connected with worsening of growth conditions caused by a large quantity of fertilizer applied immediately under the root. It results in increased salinization of the soil solution in the root zone, which always affects negatively plant growth [25]. Nitrogen fertilization with ammonium nitrate (objects 6–8) influenced the crop yield of Chinese cabbage to a lesser extent, however statistically significant differences were observed between the objects fertilized with growing nitrogen doses applied as ammonium nitrate.

The way of fertilizer application is crucially important for achieving the assumed productive objectives [12]. The use of nitrogen in contemporary farming systems is insufficient and methods of fertilizer efficiency improvement should be implemented, both through the use of more specialist fertilizers and fertilizing technologies [26, 27]. Efficiency of nitrogen recovery in the individual fertilization variants ranged from 13.29 to 130 % (Fig. 2).

In the variant with three first levels of fertilization the highest efficiency of nitrogen recovery was observed. The reason was high soil abundance in available nitrogen

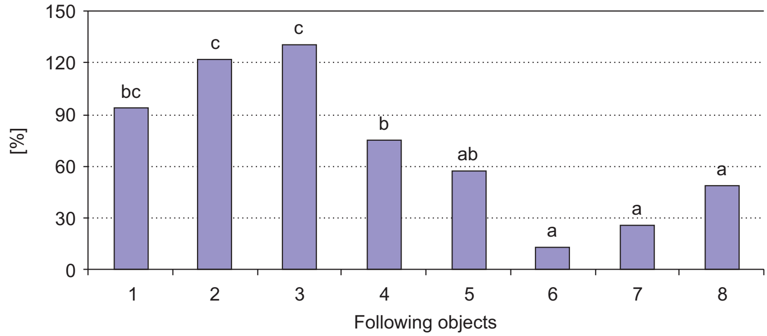

Fig. 2. The values of recovery efficiency in the successive variants of fertilization

compounds. The highest value of this parameter was noted in the 3rd experimental variant in which the slow-acting fertilizer was applied in the amount of 400 kg · ha^{-1} and supplementary fertilization with phosphorus and potassium. Treatment with ammonium nitrate did not have any marked effect on the efficiency of nitrogen recovery. In ammonium nitrate treatments (objects 6–8) value of this parameter was the lowest, ranging from 13.29 to 49.26 %.

Recovery efficiency is an index of the uptake of nitrogen supplied to the soil ecosystem with fertilizers. Generally, the higher value of this index, the more efficient fertilization. While interpreting the value of recovery coefficient one should consider also the crop yield. At very low fertilization level, value of nitrogen recovery coefficient may be high, even above 100 %, yet the obtained yield will be small, not corresponding with productive potential of the site. In such case production per area unit will not be effective. Under regular conditions this parameter value ranges from 30 to 50 %. At low level of fertilization and high nitrogen contents in soil it may be from 50 to 80 % [20]. Cassman et al [28] report that the efficiency of nitrogen removal on farms producing wheat, rice and corn in Asia and the US ranged from 18–49 %, depending on farming system. The authors point to a significant effect of the source of nitrogen applied in mineral fertilizers. The soil on which the experiment was set up revealed a high content of mineral nitrogen, therefore high value of recovery coefficient is advantageous from the environmental point of view.

Partial factor productivity determines the yield obtained following the application of 1 kg of nitrogen as fertilizer. In Author's own investigations the factor expressed in the yield of fresh mass ranged from 650 to 1825 kg · kg^{-1} of applied N (Fig. 3). Partial factor productivity in conversion to dry weight of yield was from 22.91 to 66.63 kg · kg^{-1} N.

The highest value of this parameter (80.83 kg · kg^{-1} N) was registered in the object which received the smallest dose of slow-acting fertilizer, 200 kg (object 1). Dua et al [29] reported the value of this parameter for potato production in India in 1968–2000 ranging from 111 to 428 kg · kg^{-1} N, however at the beginning of the investigations period it was about 30 % lower in comparison with the results from the 90-ties of the

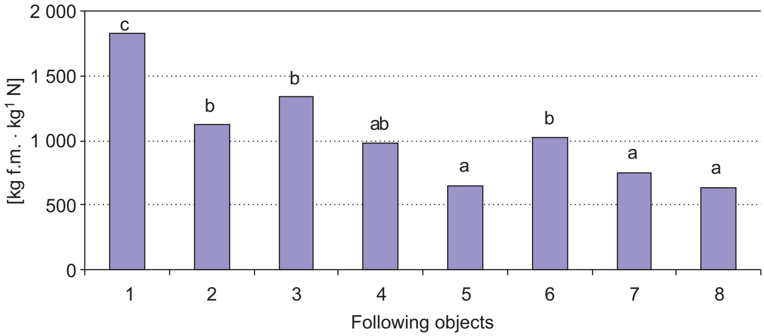

Fig. 3. The values of partial factor productivity in the successive variants of fertilization

20th century. The same authors point to a negative correlation between the value of partial factor productivity and fertilization amount.

Cassman et al [28] indicate a significant relationship between the quantity of applied fertilizer and the value of partial factor productivity. In farming systems using small doses of fertilizers, value of this coefficient is generally high, which does not evidence a high efficiency. On the basis of the analysis of 2000 farms the authors state that in 1965–2000 partial factor productivity calculated for corn in the US increased by about 15 %, but at the same time, average yield of this crop per hectare increased by over 100 %. Assessment of agronomic efficiency based on the analysis of partial factor productivity should be always connected with the obtained yield. Partial factor productivity is the most important index, because it considers the efficiency of nutrient utilization, which translates into profitability of production [30]. The most frequent values of this parameter in traditional farming systems in conversion to dry mass range from 40 to 80 kg $\cdot$ kg^{-1} N [20]. Values exceeding 60 kg d.m. $\cdot$ kg^{-1} N are noted in well managed systems, at low nitrogen concentrations in soil.

Agronomic efficiency is an index describing increase in the crop yield after the application of 1 kg of nitrogen fertilizer. Its value best shows the efficiency of farming systems. With development of agriculture, the value of this parameter increases [31]. The value of agronomic efficiency for cereal production in developing countries, in conversion to yield dry mass, ranges from 10 to 30 kg $\cdot$ kg^{-1} of fertilizer [32]. In the Author's own investigations, value of agronomic efficiency in the individual variants of the experiment, expressed in Chinese cabbage dry weight, ranged from 6.16 to 35.92 kg $\cdot$ kg^{-1} of fertilizer (Fig. 4). Significantly lower value of this parameter was registered in the object receiving traditional fertilization in the amount of 50 kg N as ammonium nitrate and full doses of phosphorus and potassium (object 6).

The highest value of this parameter was obtained in variant 3, where 500 kg $\cdot$ ha^{-1} of slow-acting fertilizer was applied. In general, in the objects receiving this fertilizer, except its highest dose, significantly higher values of agronomic efficiency were noted in comparison with traditional treatment with ammonium nitrate. Point application of slow-acting fertilizer caused a considerable increase in the yield of experimental crops. When corresponding doses were applied as ammonium nitrate, much lower yields were

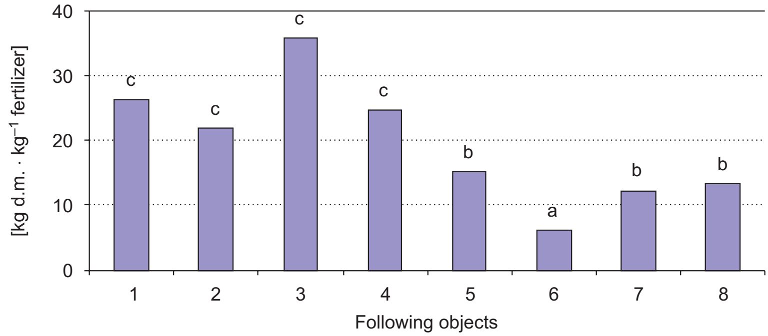

Fig. 4. The values of agronomic efficiency in the successive variants of fertilization

produced. These results indicate a better efficiency of fertilization using the slow-acting fertilizer under conditions of crop cultivation in the soils with very high nutrient content.

A fuller view of fertilization efficiency will be available after several years of fertilization using this type of fertilizers. Applied point fertilization caused a better utilization of nitrogen from the soil resources, which is an important factor improving economic effectiveness of production and reducing a negative environmental impact of production [33]. Helander and Delin [34] state that the content of nitrogen available forms in soil after plant harvesting should not exceed 30 kg $\cdot$ ha^{-1}. If available nitrogen concentration in soil after crop harvesting is below 30 kg $\cdot$ ha^{-1}, there is hardly any hazard of this element leaching into the soil profile or to the surface waters. Small amount of nitrogen in soil after crop harvesting evidences a proper use of this element applied in fertilizers.

Physiological efficiency shows the increase in agronomic production per 1 kg of nutrient absorbed by plants in result of applied fertilization. Its value in the highest degree depends on plant genotype and environmental conditions, but also on the strategy of fertilization management. Therefore, it is usually used for an assessment of farming systems efficiency [28]. Its low values indicate occurrence of a stressor for plants (nutrient deficit, drought, thermal stress, occurrence of a disease or pests). Physiological efficiency in the subsequent experimental variants ranged from 16.43 to 36.79 kg d.m. $\cdot$ kg^{-1} of nitrogen taken up by plants from the applied fertilizers (Fig. 5).

Significantly the highest value of physiological efficiency was obtained in the 7th variant of the experiment in which traditional fertilization was applied with a medium dose of nitrogen as ammonium nitrate. A decrease in this coefficient value was observed after the application of 50 kgN as ammonium nitrate. The environmental conditions were the same in all experimental variants, therefore the registered differences result from different fertilization in the respective objects. The lowest physiological efficiency was observed in the object where 400 kg of slow-acting fertilizer was applied together with the full dose of phosphorus and potassium. The most usual values of this parameter are 40–60 kg d.m. $\cdot$ kg^{-1} of applied N. In well managed

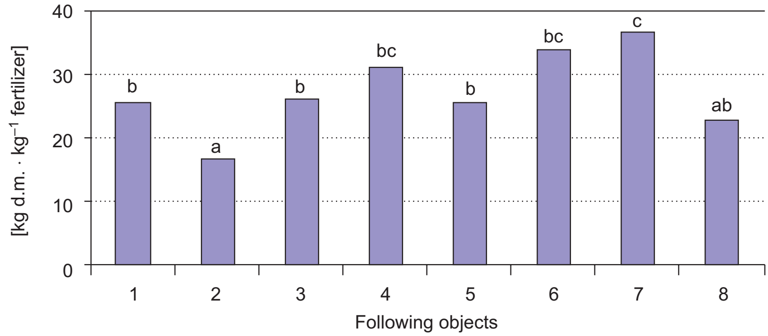

Fig. 5. The values of physiological efficiency in the successive variants of fertilization

systems under good conditions of plant growth and development, value of this parameter exceeds 50 kg d.m. · kg^{-1} N [ISA 2007].

Removal efficiency is an important indicator determining a potential environmental impact of agriculture. It informs which part of the component applied in fertilization is removed from the ecosystem with yield. At removal coefficient equaling 1, the quantity of nitrogen absorbed with yield is equal to its amount applied in mineral fertilizers. It is particularly important in case of nitrogen fertilization because it is the element poorly bound to soil sorption complex, particularly after the transformation into nitrate form. Mineral nitrogen which was not incorporated into the biomass during vegetation season is to a great extent dispersed in the environment, which negatively affects all of its elements but does not constitute this element reserve for the subsequent year. A very high nitrogen removal efficiency is encountered in the systems showing this element deficit. Low values of this index are characteristic for the cultivation in which plant growth limiting factors appeared, such as nutrient deficiency, drought, thermal stress, diseases or pests. The most frequently noted values of this parameter fluctuate from 0.3 to 0.9, whereas the values from 0.55 to 0.65 kg · kg^{-1} are regarded as optimal [20]. In the presented experiment the values of removal efficiency ranged narrowly between 0.93 and 2.77 kg · kg^{-1} (Fig. 6).

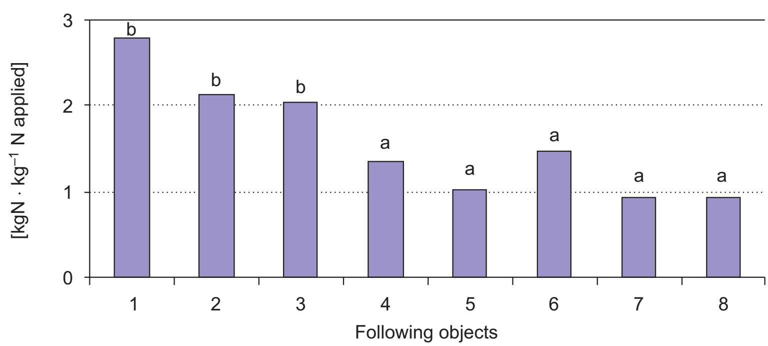

Fig. 6. The values of removal efficiency in the successive variants of fertilization [kg · kg^{-1} N]

The highest value of removal efficiency was registered in object 1, where 200 kg · ha^{-1} of the slow-acting fertilizer was applied. In objects 2 and 3 the values of this parameter were similar. A significantly lower nitrogen removal efficiency was noted in the other fertilizer variants. Values of nitrogen removal efficiency registered in all objects are very high and evidence a negative nitrogen balance. However, on the basis of the plant organoleptic and chemical analysis as well as their crop yield, no deficiency of this element was found. Very high nitrogen content in soil on which the experiment was conducted, was the cause of reduced nitrogen fertilization. It led to this element utilization by plants from the soil reserves and depletion of its reserves in soil and dispersion in the environment.

All quality systems in the primary agronomic production are implemented in the first place in vegetable and fruit growing because of a considerable impact of these branches of production on the environment, as well as bigger than in case of agronomic plants, hazard of obtaining product with elevated content of xenobiotics [35]. Fruit and vegetables are destined for immediate consumption to a greater extent than the other plants, therefore implementing quality systems in their production processes has a marketing importance. Rationalization in agriculture requires numerous experiments and observations to find solutions and developing a technology, which will make possible realization of integrated production.

Conclusions

1. The effect of applied fertilization on the obtained crop yield of Chinese cabbage was observed under conditions of the conducted experiment.

2. The most advantageous agronomic efficiency and nitrogen recovery efficiency were obtained in the combination of 500 kg · ha^{-1} of slow-acting fertilizer with supplementary traditional PK fertilizers. Generally higher values of these indices, in comparison with fertilization using exclusively traditional fertilizers, were observed after the application of a slow-acting fertilizer and traditional PK ones.

3. The highest nitrogen physiological efficiency was noted when traditional treatment with ammonium nitrate (100 kgN · ha^{-1}) was applied.

4. Application of fertilization technology based on the proportion of a slow-acting multi component fertilizer may improve efficiency of agronomic production.

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EFEKTYWNOŚĆ NAWOZU WOLNODZIAŁAJĄCEGO W INTEGROWANEJ UPRAWIE KAPUSTY PEKIŃSKIEJ

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Abstrakt: Celem pracy było określenie przydatności nawozu wolnodziałającego wykorzystywanego w uprawie kapusty pekińskiej w systemie integrowanej produkcji. Realizację założonego celu osiągnięto w oparciu o wyniki ścisłego doświadczenia polowego, założonego na glebie o składzie granulometrycznym gliny średniej. Rośliną testową była kapusta pekińska (*Brassica rapa* L.) odmiany 'Parkin F1'. Doświadczenie założono 17.08.2011 r. Rośliny zebrano 08.10.2011 r. Czynnikiem doświadczenia było zróżnicowane nawożenie. Uprawę oraz ochronę roślin prowadzono w oparciu o metodykę integrowanej produkcji kapusty pekińskiej zatwierdzonej przez Głównego Inspektora Ochrony Roślin i Nasiennictwa. W doświadczeniu zastosowano nawozy konwencjonalne (superfosfat potrójny, sól potasowa oraz saletra amonowa) oraz nawóz wieloskładnikowy NPKCaMg (18-05-10-4-2) o spowolnionym uwalnianiu składników pokarmowych. Doświadczenie obejmowało 8 wariantów nawożenia i obiekt kontrolny. We wszystkich wariantach nawożenia zastosowano dawki fosforu i potasu odpowiadające zapotrzebowaniu roślin przy założonej wielkości plonów. Nawożenie azotem w kolejnych obiektach różniło się zarówno formą, jak i ilością zastosowanego składnika. Na podstawie wyników przeprowadzonych doświadczeń obliczono wskaźniki obrazujące efektywność nawożenia azotem: efektywność agronomiczną, współczynnik produktywności, efektywność fizjologiczną, efektywność odzysku oraz efektywność usunięcia azotu.

Nawożenie w istotny sposób modyfikowało wielkość uzyskanego plonu. Plon roślin w obiekcie kontrolnym, bez nawożenia mineralnego, wynosił 44,22 Mg · ha^{-1}. Największy plon, wynoszący 120,7 Mg · ha^{-1} uzyskano w obiekcie z dodatkiem 400 kg nawozu wolnodziałającego, 89 kg superfosfatu potrójnego oraz 177 kg soli potasowej. Najbardziej optymalne wartości wskaźników efektywności nawożenia uzyskano w obiektach, w których zastosowano nawóz wolnodziałający w ilości 400 i 500 kg · ha^{-1} oraz pełne dawki fosforu i potasu. Obliczone wskaźniki efektywności nawożenia wskazują, że optymalizacja nawożenia z udziałem nawozu wolnodziałającego, szczególnie w warunkach intensywnej uprawy, może kilkakrotnie poprawić efektywność nawożenia w stosunku do integrowanych metod produkcji z wykorzystaniem nawozów konwencjonalnych. Wyniki przeprowadzonych badań wskazują, że optymalizacja nawożenia w warunkach intensywnej produkcji może zwiększyć pobieranie azotu zastosowanego w nawozach mineralnych o 50 % przy utrzymaniu wysokich plonów roślin, co znacznie poprawia efektywność ekonomiczną produkcji i zmniejsza ilość biogenów rozpraszanych w środowisku.

Słowa kluczowe: kapusta pekińska, integrowana produkcja, nawóz wolnodziałający, efektywność nawożenia

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CONTENT OF ZINC IN PLANTS DEPENDING ON ITS FRACTIONAL COMPOSITION IN SOIL AND PROPERTIES OF SOIL

ZAWARTOŚĆ CYNKU W ROŚLINACH W ZALEŻNOŚCI OD JEGO SKŁADU FRAKCYJNEGO W GLEBIE ORAZ WŁAŚCIWOŚCI GLEBY

Abstract: Metals in soils occur in different forms, which are more or less mobile, which means bioavailable. The aim of this study was to assess the content of zinc in cultivated plants, depending on soil properties but also its content in soil and fractions in typical and unpolluted region, which is the Podlasie Province. The research material consisted of samples taken from arable soil in 81 points of Podlasie Province. Basic physicochemical properties and content of soil fraction < 0.02 mm in samples were determined. Pseudo-total content of zinc and its fractional composition in soil as well as zinc content in plants was evaluated. Correlation coefficients between the content of zinc in plants and physicochemical properties of soil were calculated.

It was found that examined soil contained natural amount of zinc, characteristic for uncontaminated soils. The most of zinc was associated with the organic matter fraction and the least with residual fraction. Among the analysed plants from light soils, the most of zinc was found in rye. Plants from medium-heavy soils contained similar amount of zinc, with the exception of maize. Correlation coefficients do not confirm the significant relationship between soil properties and zinc in plants. Significant relationship was stated between bioaccumulation factors and the content of zinc in plants.

Keywords: zinc in plant, soil, BCR method, zinc fraction

Introduction

Zinc – apart from boron, manganese, molybdenum and copper, belongs to the most important micronutrients for plants, but together with its increasing content, it belongs

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to dangerous elements for living organisms. It is one of the most mobile metals in soil [1, 2]. It is known that in soils it occurs in a variety of forms that are more or less mobile, and in the same way bioavailable. Zinc mobility is mainly determined by the physicochemical properties of soils, pH, organic matter content and granulometric composition [3]. The intensity of agrotechnical management is not without significance. Zinc uptake by plants is proportional to the content of available forms in soil [4, 5].

The aim of this study was to assess the content of zinc in plants, depending on soil properties but also its content and fractional composition in soils of unpolluted region, which is Podlasie Province.

Materials and methods

The research material consisted of samples taken from arable soil in 81 points of Podlasie Province. One point was selected in the majority of its districts. Each point was located on mineral soil used as arable land, without external source of contamination, like roads or industrial plants. The samples were collected from arable layer (0–30 cm) after plant harvest. Maize was cultivated in 14 points, cereals in 57 points and grass in 10 ones. The following basic physicochemical properties in soil samples were determined: granulometric composition by particle analyser, content of organic carbon by Tiurin's method and pH potentiometrically in 1 mol $\cdot$ dm^{-3} KCl suspension. Based on the content of soil fraction < 0.02 mm, soils were divided into two groups: very light and light and medium-heavy soils. The pseudo-total content of zinc was determined after mineralization in *aqua regia*, by means of FAAS technique using Varian AA-100 apparatus. Zinc fractions were evaluated using the BCR method. Extraction included four stages: acid soluble and exchangeable fraction (fraction I), reducible fraction, bound to Fe/Mn oxides (fraction II), oxidizable fraction, bound to organic matter (fraction III) and residual fraction (fraction IV). The scheme of extraction was described in detail in the article, which is in press [6].

From the points where soil samples were collected to the analysis, the following plants were also taken: cereal grains, maize for silage and grass on field crops. The content of zinc in plants was determined, after previous digestion in microwave oven, by means of FAAS technique using Varian AA-100 apparatus. Bioaccumulation factors were calculated as the ratio of zinc content in plants to the pseudo-total zinc content in soils. Correlation coefficients between zinc content in plants and physicochemical properties of soil, zinc fractions and bioaccumulation factors were determined.

Results and discussion

The granulometric composition of soil from both groups was slightly differentiated. The content of soil fraction < 0.02 mm in very light and light soil ranged from 4.0 to 17.0 %, 12.0 % on average, and in medium-heavy soil – 22.0 % on average, with the range of 21.0–28.0 % (Table 1).

Table 1

Physicochemical properties of soils

Soil	Value	Content of soil fraction < 0.02 mm [%]	pH	Organic carbon [$g \cdot kg^{-1}$]	Pseudo-total content of Zn [$mg \cdot kg^{-1}$]
Very light and light n = 35	range	4.0–17.0	4.0–7.6	6.0–31.0	13.5–52.5
	$\bar{x}$	12.0		16.0	32.4
Medium-heavy n = 46	range	21.0–28.0	4.1–7.8	7.0–42.0	14.5–58.8
	$\bar{x}$	22.0		24.0	35.1

The pH of the first group of studied soils was very differentiated and varied from 4.0 to 7.6. Among 35 soil samples, twelve were very acid (pH < 4.5), eight were acid (pH 4.6–5.5), nine were slightly acid (pH 5.6–6.5) and the rest 6 soil samples were neutral (pH 6.6–7.2). Medium-heavy soils were characterized by higher pH values. Only three samples were counted to very acid soils, eleven samples were acid and the rest were light acid and neutral. Investigated soils were typical for Podlasie Province, which typical soils are light and acid ones.

The content of organic carbon in both groups of soil was differentiated. In very light and light soils ranged from 6.0 to 31.0 $g \cdot kg^{-1}$ (16.0 $g \cdot kg^{-1}$ on average). In medium-heavy soils, there was more carbon – 24.0 $g \cdot kg^{-1}$ on average, with the range of 7.0–42.0 $g \cdot kg^{-1}$.

The pseudo-total content of zinc in very light and light soils ranged from 13.5 to 52.5 $mg \cdot kg^{-1}$ (32.4 $mg \cdot kg^{-1}$ on avarage). In the second group of soils (medium--heavy) the average pseudo-total content of zinc was slightly higher than in very light and light group and ranged from 14.5 to 58.8 $mg \cdot kg^{-1}$. This diversity indicates the correlation of pseudo-total zinc and granulometric composition of soil. The pseudo-total content of zinc in studied soils corresponds to the natural content in soils of Poland [8]. It is in terms of the results given by Skorbilowicz et al [9]. He claims that total zinc in acid soils of Podlasie Province was in the range from 13.2 to 54.8 $mg \cdot kg^{-1}$. The content of zinc in analysed soils in individual fractions was differentiated (Fig. 1). In a group of very light and light soils, the content of fraction I (exchangeable) ranged from 5.0 to 19.1 $mg \cdot kg^{-1}$ (10.2 $mg \cdot kg^{-1}$ on average), which makes 32 % of total content. This fraction contains the most available part of zinc, which consists of zinc in soil solution and the exchangeable one but also bound with carbonates. In medium-heavy soils, the content of this fraction was slightly less than in very light and light soils. While more zinc in medium-heavy soils was bound with iron and manganese oxides (fraction II), the organic matter (fraction III) and residual fraction (fraction IV). Average content in fraction II was 13.1 $mg \cdot kg^{-1}$ (from 2.4 to 26.9 $mg \cdot kg^{-1}$), and in fraction III – 14.9 $mg \cdot kg^{-1}$ on avarage. The least amount of zinc was in residual fraction and the most in fraction of organic matter.

Kalembasa and Pakula [10] determined the content of zinc in grey-brown podzolic soils of south Podlasie Province with the use of Tessier's method and obtained different

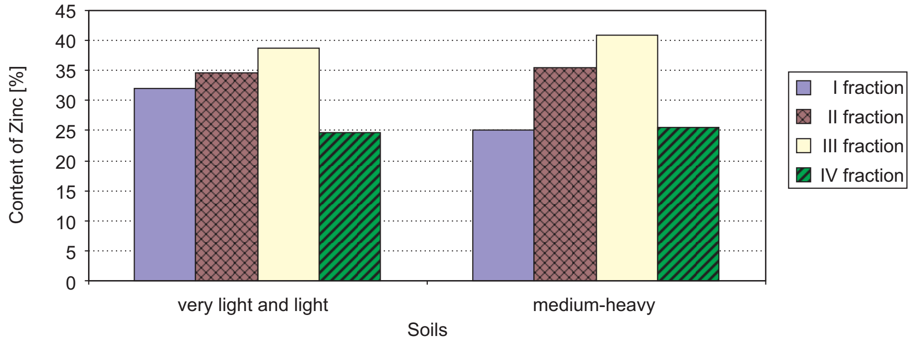

Fig. 1. Zinc percentage in pseudo-total content, in very light, light and medium-heavy soils

results in relation to those shown in Authors' own studies. In the humus layer, the most of zinc was in the residual fraction, and less in the fraction bonded with the organic matter and exchangeable fraction.

The content of zinc in cultivated plants reached the range from 11.0 to 40.2 mg · kg^{-1} d.m. depending on the species and the content of soil fraction < 0.02 mm (Table 2). The average content of this microelement was similar in the analysed species grown on very light and light soils. In cultivated plants from medium-heavy soils, content of zinc was less (a few mg on average) than in plants from very light soils. Among cereal plants, the most zinc contained rye grain cultivated on very light and light soils, and other cereals had similar content. Similar contents of this element among the crops grown on medium-heavy soils had a mixture of cereals, triticale and oat. The least Zn amount contained wheat, which appeared only on medium-heavy soils.

Table 2

Content of zinc in plants

Soils	Value	Maize for silage	Grasses	Mixture of corns	Triticale	Oat	Rye	BF*
		[mg · kg^{-1} d.m.]						
Very light and light n = 35	range	19.6–36.2	21.3–36.7	22.9–35.2	15.7–36.5	19.0–34.5	23.9–36.7	0.4–2.1
	$\bar{x}$	27.7	25.6	26.5	27.5	27.3	29.8	2.9
	n	7	5	7	5	6	5	
							Wheat	
Medium-heavy n = 46	range	11.0–30.1	16.1–24.5	21.2–33.9	19.6–40.2	22.7–29.9	17.4–27.8	0.3–2.1
	$\bar{x}$	19.6	23.5	25.0	25.7	25.5	23.2	0.7
	n	7	8	15	7	4	5	

* BF – Bioaccumulation factor.

The content of zinc in plants is determined by many factors and according to Terelak and Lipinski [11], there are mainly pH of soils, clay content and organic matter. The difference of zinc content between plants from light soils and those from medium-heavy soils is caused by the physicochemical properties of two groups of soils. It is confirmed by the contents of this element in maize. Zinc content in maize from very light and light soils was higher than in the one from the medium-heavy soil, and this is mainly caused by the reaction of these soils (there were more acid soils). These results are consistent with observations of Kaniuczak et al [12]. The authors have proved that liming causes the reducing of zinc content in potato tubers and forage of sunflower.

The average zinc content in analysed cereals was located in the lower level of content for cereals grown in Poland, which in wheat ranged from 24.2 to 47.0 mg · kg^{-1} d.m., according to Terelak and Lipinski [11]. Similar content in cereals in other countries was determined by Cubadda et al [13] as well as by Vijayan et al [14], while Wlasniewski [15] provides a wider range of Zn content in wheat, from 23.8 to 72.7 mg · kg^{-1} d.m. According to many authors [4, 5, 11, 15] the content of zinc in plants depends on this element content in soil, and especially on the content of its available forms.

Correlation coefficients between zinc content in plants and content in fractions, in both groups of soils, were low and not significant (Table 3).

Table 3

Correlation coefficients

Variable	Zn in plant [mg · kg^{-1} d.m.]	
	Very light and light soils	Medium-heavy soils
Pseudo-total Zn [mg · kg^{-1}]	0.06	–0.08
Fraction I [%]	–0.21	0.03
Fraction II [%]	–0.30	–0.11
Fraction III [%]	0.04	0.03
Fraction IV [%]	0.27	–0.01
Content of soil fraction < 0.02 mm [%]	0.29	0.02
Bioaccumulation factor	0.50*	0.71*
pH	0.26	–0.07
Organic carbon [g · kg^{-1}]	–0.27	–0.09

* – significant for $p \leq 0.05$.

Jeske and Gworek [16] discussing the method of bioavailability determination of heavy metals in soils, claim that the results of the research does not indicate the existence of strong relationship between the occurrence of bioavailability of metals and their contents in plants. The authors argue that the correlations are not observed very commonly or are not significant, which is proved by the obtained results. Correlation coefficients do not confirm the significant relationship between soil properties such as pH, content of soil fraction < 0.02 mm and the content of organic carbon and zinc in plants. Significant relationship between the value of bioaccumulation factor and zinc content in plants was stated (Table 3, Fig. 2).

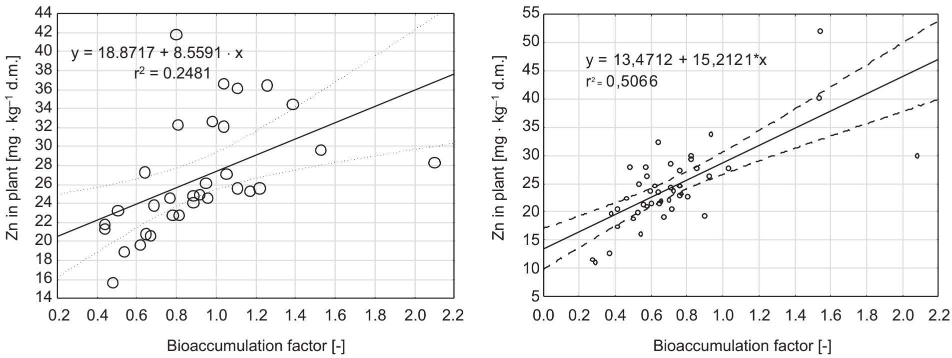

Fig. 2. Relationship between the content of Zn in plants and the bioaccumulation factor (a – very light and light soils, b – medium-heavy soils)

Conclusions

1. Analysed soils contained natural amount of zinc, which is characteristic for uncontaminated soils.
2. The highest content of zinc was stated in fraction bound with organic matter, and the lowest in residual fraction in both groups of soils.
3. Among analysed plants from light soils, the highest content of zinc was observed in rye. Plants from medium-heavy soils contained similar amount of zinc, except of maize.
4. Significant relationship between zinc content in plants, soil properties and zinc fractions was not stated. Significant correlation was observed only in the case of bioaccumulation factors.

Acknowledgements

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ZAWARTOŚĆ CYNKU W ROŚLINACH W ZALEŻNOŚCI OD JEGO SKŁADU FRAKCYJNEGO W GLEBIE ORAZ WŁAŚCIWOŚCI GLEBY

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Abstrakt: Metale występują w glebie w różnych formach, które są bardziej lub mniej rozpuszczalne, a tym samym w różnym stopniu biodostępne. Celem pracy było określenie zawartości cynku w roślinach uprawnych w województwie podlaskim, w zależności od właściwości gleby oraz jego zawartości w glebie i w poszczególnych frakcjach. Materiał badawczy stanowiły próbki gleb i roślin uprawnych pobrane w 81 punktach woj. podlaskiego. W próbkach oznaczono podstawowe właściwości fizykochemiczne gleby, określono kategorię agronomiczną gleb i podzielono je na dwie grupy: gleby bardzo lekkie i lekkie oraz gleby średnie. Oznaczono zawartość cynku w roślinach oraz zbliżoną do ogólnej jego zawartość w glebie i skład frakcyjny. Obliczono współczynniki korelacji między zawartością cynku w roślinach a właściwościami fizykochemicznymi gleb i frakcjami cynku w glebie oraz jego współczynnikami bioakumulacji.

Stwierdzono, że badane gleby zawierały naturalne ilości cynku, charakterystyczne dla gleb niezanieczyszczonych. Zawartość cynku w poszczególnych frakcjach układała się w następujący szereg: frakcja związana z substancją organiczną > frakcja związana z tlenkami i wodorotlenkami > frakcja wymienialna > frakcja rezydualna. Wśród analizowanych roślin uprawianych na glebach lekkich najwięcej cynku zawierało żyto. Rośliny uprawiane na glebach średnich, z wyjątkiem kukurydzy, zawierały zbliżone ilości cynku. Nie stwierdzono istotnych zależności pomiędzy zawartością cynku w roślinach a właściwościami gleb. Istotne zależności wystąpiły tylko w przypadku współczynnika bioakumulacji.

Słowa kluczowe: cynk w roślinie, gleba, metoda BCR, frakcje cynku

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EVALUATION OF SUSCEPTIBILITY OF PHYTOPATHOGENIC *Fusarium culmorum* STRAIN ON SELECTED ESSENTIAL OILS

OCENA WRAŻLIWOŚCI FITOPATOGENNEGO SZCZEPU *Fusarium culmorum* NA WYBRANE OLEJKI ETERYCZNE

Abstract: The prevalence in environment, high pathogenicity, toxigenicity and acquisition of resistance to used fungicides by *Fusarium culmorum* leads to a search for alternative methods to combat them. The solution to this problem may be essential oils. The aim of the study was to evaluate the sensitivity of the phytopathogenic *Fusarium culmorum* 187 (Mart.) Sacc. strain on selected essential oils such as thyme, lemongrass, Litsea cubeba, cajeput, tea tree, verbena and grapefruit in comparison to Funaben T seed preparation. The research material was a species of *Fusarium culmorum* 187 (Mart.) Sacc., isolated from infected cereals. The tested essential oils such as thyme, lemongrass, Litsea cubeba, cajeput, tea tree, verbena and grapefruit oils were introduced into the substrate at concentrations of 0.025; 0.05; 0.125; 0.25; 0.5; 1.00, 2.0 %, while Funaben T at concentrations of 0.125; 0.25; 0.5 %. The control was the development of the fungus in a medium free from these substances. Biotic activity of essential oils in reducing the linear growth of *F. culmorum* 187 (Mart.) Sacc. was assessed using the method of poisoned substrates. The activity of oils in reducing the linear growth of mycelium was evaluated by measuring the growth of fungal colonies (growth rate index) while the fungistatic activity was evaluated on the basis of the percentage growth inhibition of fungal colony calculated according to Abbott's formula. The susceptibility of *Fusarium culmorum* 187 strain was differentiated and depended on the type and concentration of tested oils. Thyme oil at all concentrations tested (0.025–2.0 %) completely inhibited the growth of mycelia while other oils caused a total inhibition of growth at concentrations of 0.5–2.0 %. The effect of essential oils was similar to that of Funaben T preparation.

Keywords: *Fusarium culmorum,* thyme oil, lemongrass oil, Litsea cubeba oil, cajeput oil, tea tree oil, verbena oil, grapefruit oil, antifungal activity

Introduction

One of the most widespread perpetrators of economically important diseases of crops are polyphagous fungi *Fusarium*. Fusariosis of cereals diseases caused by these fungi

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are a threat in different regions of the world. *Fusarium* pathogens systemically affect plants at all stages of their development. In wheat they cause such diseases as pre- and post-emergence blight, ear blight and head blight [1, 2]. They can lead to a loss of yields in the range of 7–70 % depending on the development phase of grains [3] and reduction in their quality, especially by mycotoxin contamination [4–8]. One of the most frequently occuring perpetrators of cereals diseases in all climate zones is a species of *Fusarium culmorum* (W.G. Smith) Sacc. [9, 10]. Chemical fungicides are often used to combat the perpetrators of economically important diseases. However, in recent years a growing interest in finding new formulations containing in their composition active substances of natural origin is noticeable [11–13]. An alternative to chemical formulations may be essential oils extracted from plants which have a number of valuable biological properties including antibacterial, antifungal and insecticidal activities [14]. These mixtures of different compounds, mainly monoterpenes, monoterpenoids, sesquiterpenes and fragrances (esters, ketones, phenols, alcohols, aldehydes, ethers, hydrocarbons, coumarin and organic acids) effectively restrict or inhibit the growth of phytopathogenic fungi of *Fusarium* type. Since the biocidal effect depends on chemical composition and concentration of essential oils and the susceptibility of phytopathogenic fungi strains, constant studies towards the use of essential oils as effective biofungicides are conducted [8, 14–17].

The purpose of this study was to evaluate the susceptibility of the phytopathogenic strain of *Fusarium culmorum* 187 (Mart.) Sacc. on selected essential oils of different chemical composition compared to the seed treatment Funaben T.

Materials and methods

The research material was the strain of *Fusarium culmorum* 187 (Mart.) Sacc. isolated from infected cereals on PDA medium. The strain was identified on the basis of morphological characteristics [19].

Essential oils such as: thyme, lemongrass, Litsea, cajeput, verbena, tea tree and grapefruit oil widely available in the course of trade were tested. These oils were inserted into a substrate in the following concentrations: 0.025; 0.05; 0.125; 0.25; 0.5; 1.00; 2.00 %. However, Funaben T (20 % carbendazim + 45 % thiocarbamate) was used in the following concentrations: 0.125; 0.25; 0.5 %. The control was carried out through the growth of tested strain in the PDA medium (without oils). The GC/MS analysis of tested oils using HP 6890 gas chromatograph incorporated with HP 5973 mass spectrometer Hewlett-Packard were conducted [20].

The biotic activity of oils in reducing the linear growth of the fungus *F. culmorum* 187 (Mart.) Sacc. was assessed by using the method of poisoned substrates [21]:

– Cultures were grown in PDA medium for 14 days at 25 ^{o}C.

– Inoculum. The spore suspension of *F. culmorum* 187 in 0,01 % sterile Tween 80 were obtained from 14 days old culture. The hemocytometer Thoma was used to obtain a spore suspension of $1 \cdot 10^6$ CFU $\cdot$ cm^3. Petri dishes (9 cm diameter) containing 20 cm^3 PDA medium were inoculating this spore suspension and stored at 25 ^{o}C for 14 days. Inoculum – rings having a diameter of 10 mm overgrown by mycelium.

– Absolute control was the culture of the fungus on PDA medium without oils, and relative control with a preparation Funaben T.

– On the basis of measurements of fungal colony a linear growth of mycelium index (T) was and fungistatic activity of essential oils and preparation was calculated.

The growth rates index of *Fusarium culmorum* 187 strain was calculated using the following formula:

$$T = \frac{A}{D} + \frac{b1}{d1} + \ldots + \frac{bx}{dx} \tag{1}$$

where: T – index of linear growth;
A – average measurement value of diameter colonies [mm];
D – duration of the experiment;
$b1...bx$ – increase in colonies diameter [mm];
$d1...dx$ – numer of days since last measurement.

Fungistatic activity of tested oils was assessed basing it on the percentage of fungus colonies growth inhibition and calculated using Abbott's formula:

$$I = \frac{C - M}{C} \cdot 100 \tag{2}$$

where: I – fungus linear growth inhibition index [%];
C – fungus colony diameter in the control combination [mm];
M – fungus colony diameter on a control plate with a given oil in combination containing a tested substance concentration in the agar [mm].

Results and discussion

The inhibition of fungal strains growth is related to volatile compounds contained in essential oils [22] and the sensitivity of microorganisms.

The degree of growth limitation of *F. culmorum* 187 (Mart.) Sacc. strain was differentiated; it depended on the kind and concentration of tested oils. The biological activity and effectiveness of oils are determined by qualitative and quantitative composition of active substance contained in the raw material [23] and the technology of their production. Bialon et al [20] claimed on the basis GC/MS analysis that citrus oils produced by various manufacturers differed in chemical composition and fungicidal activity.

The main component of lemon essential oils was limonene. However, fungicidal activity depended on the content of oxygen monoterpenes derivatives. Strong antifungal properties show only the oils that contain more than 10 % oxygen monoterpenes derivatives in their composition [20, 24, 25].

Oils containing cis- and trans-citral isomers, geraniol verbenol and γ-terpinene, and other valuable components such as carvone, 1-terpinen-4-ol or γ-terpinene – are known for their fungicidal properties [24–27].

To assess the fungicidal activity of essential oils the knowledge of their chemical composition is not without significance (Table 1). The main components of studied oils were the following:

– citral – more than 60 % of it was contained in lemongrass and Litsea-cubeba),
– citral, α-terpineol i eucalyptol – verbena oil,
– thymol and p-Cymene, limonene, eucalyptol – thyme oil,
– limonene – grapefruit oil,
– 1-terpinen-4-ol – of tea tree,
– cineol – cajeput oil.

Table 1

Percentage yield of oils used in the tests

Tested oils	The main active ingredients of essential oils [%]	
Grapefruit	Limonene (35.38)	1-terpinen-4-ol Gardenol, cis-Geraniol beta-Citral (neral) α-terpineol
Cajeput	Cineol (55.0)	α-terpineol, terpinyl acetate, pinene
Thyme	Thymol (45.75) p-Cymene, limonene, eucalyptol (15.15)	Terpineol α-terpinen bergamol
Verbena	Citral (36.0) α-terpineol (18.26) eucalyptol (13.46)	linalol, borneol
Litsea-cubeba	Citral (61.72) Limonene (20.54)	borneol
Lemongrass	Citral (68.95)	linalol, borneol
Tea tree	1-terpinen-4-ol (26.0) α-terpinen (11.6)	terpineol, eucalyptol

Funaben T preparation (carbendazim + thiocarbamate used in conventional agriculture) completely inhibited the development of *Fusarium culmorum* 187 strain regardless of the dose used. In practice barley grains treated with Funaben T product were not colonized by pathogenic *Fusarium culmorum* [12].

All tested oils when compared to a relative control decreased the growth of *Fusarium culmorum* 187 mycelium at concentrations of 0.05 to 2.0 %. Complete inhibition of mycelium growth in all the concentrations (0.025–2.0 %) showed thyme oil (Fig. 1) containing 45.75 % of thymol (Tables 1, 2).

Strong fungicidal properties of the oil were confirmed by Zambonelli *et al* (1996; 2004) [28, 29]. In turn, slightly higher concentrations (from 0.05 to 2.0 %) the growth of mycelium was completely inhibited by lemongrass oils (Fig. 2) and Litsea cubeba (Fig. 3), which contained a high concentration of citral in their comoposition – 68.95 %

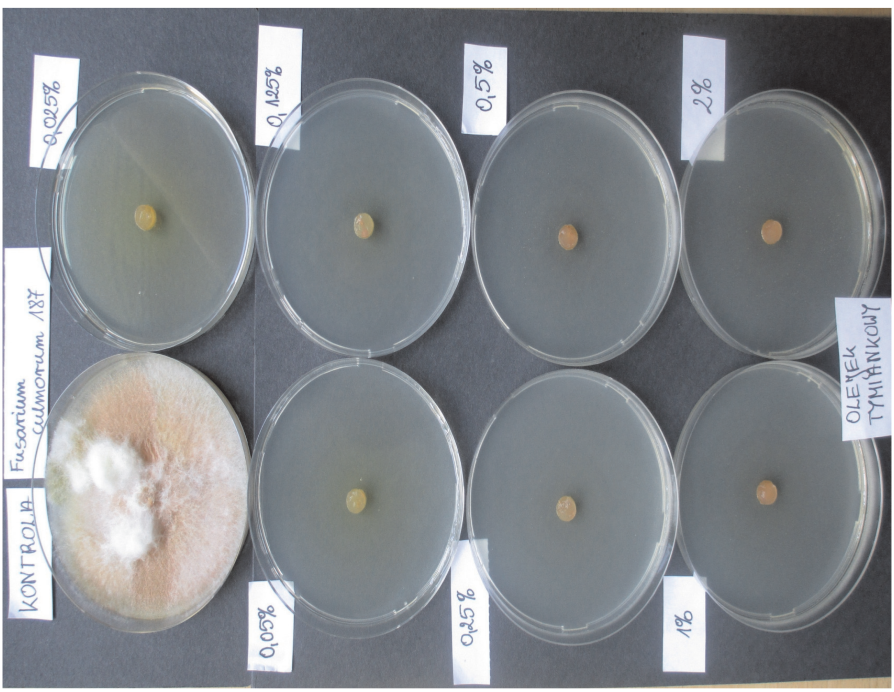

Fig. 1. Thyme oil in the following concentrations 0.025–2.0 %

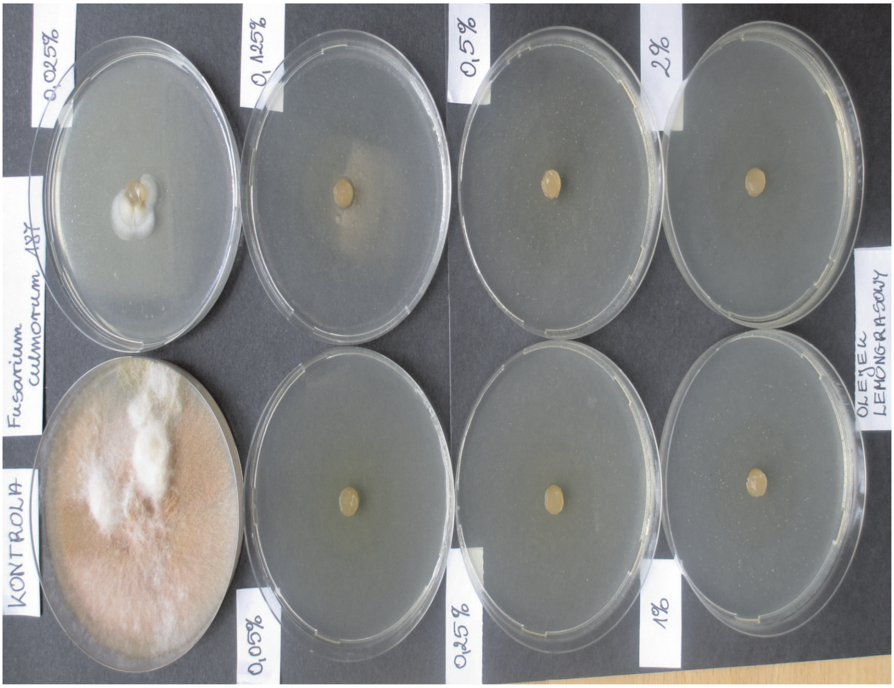

Fig. 2. Lemongrass oil in the following concentrations 0.025–2.0 %

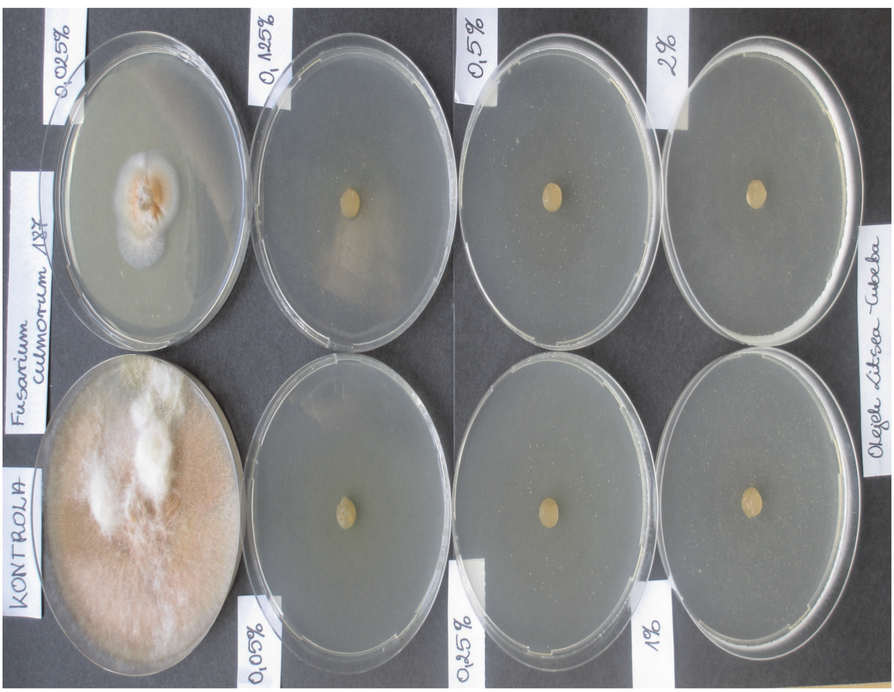

Fig. 3. Litsea cubeba oil in the following concentrations 0.025–2.0 %

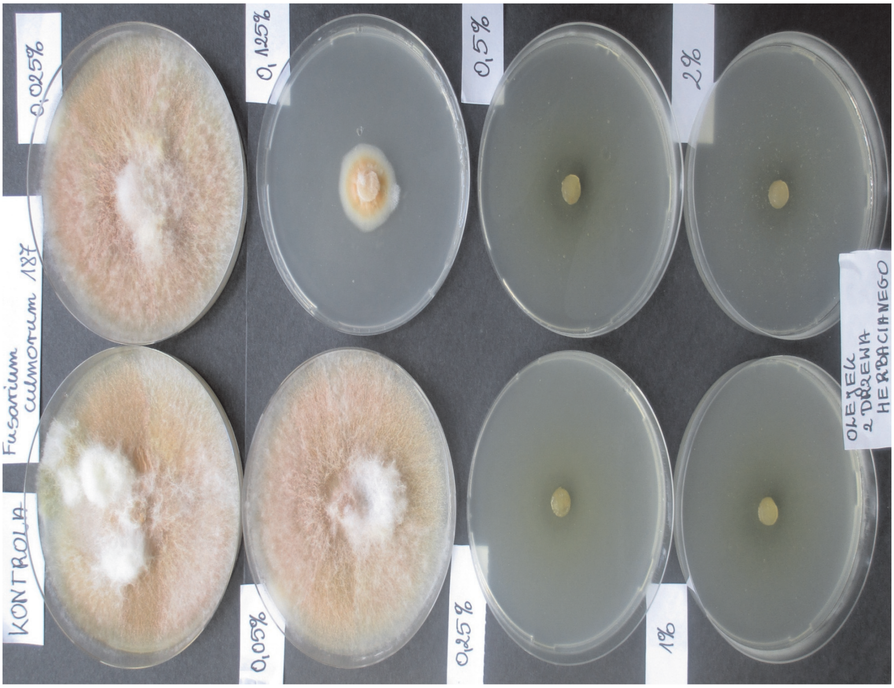

Fig. 4. Tea tree oil in the following concentrations 0.025–2.0 %

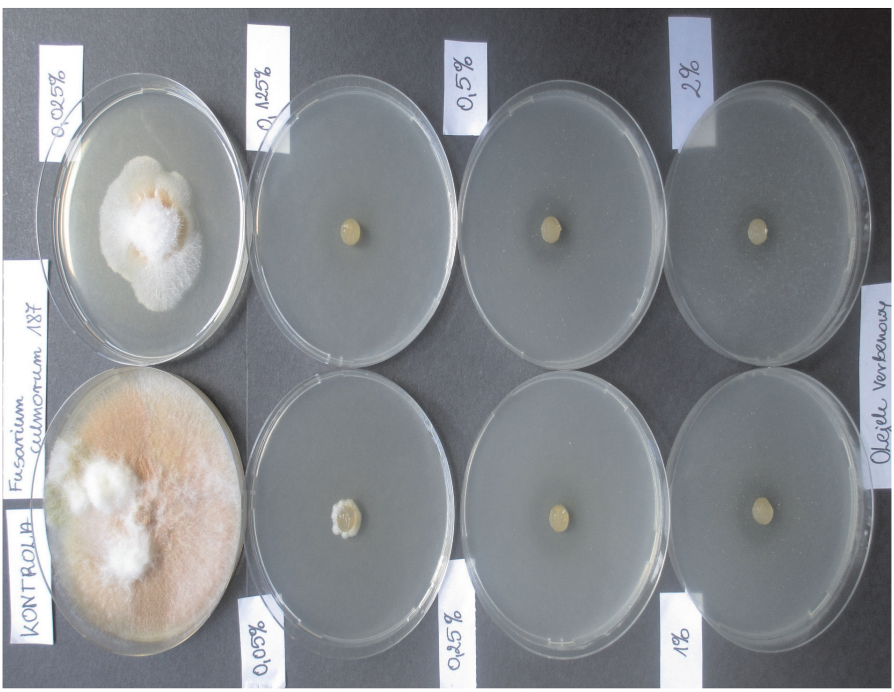

Fig. 5. Verbena oil in the following concentrations 0.025–2.0 %

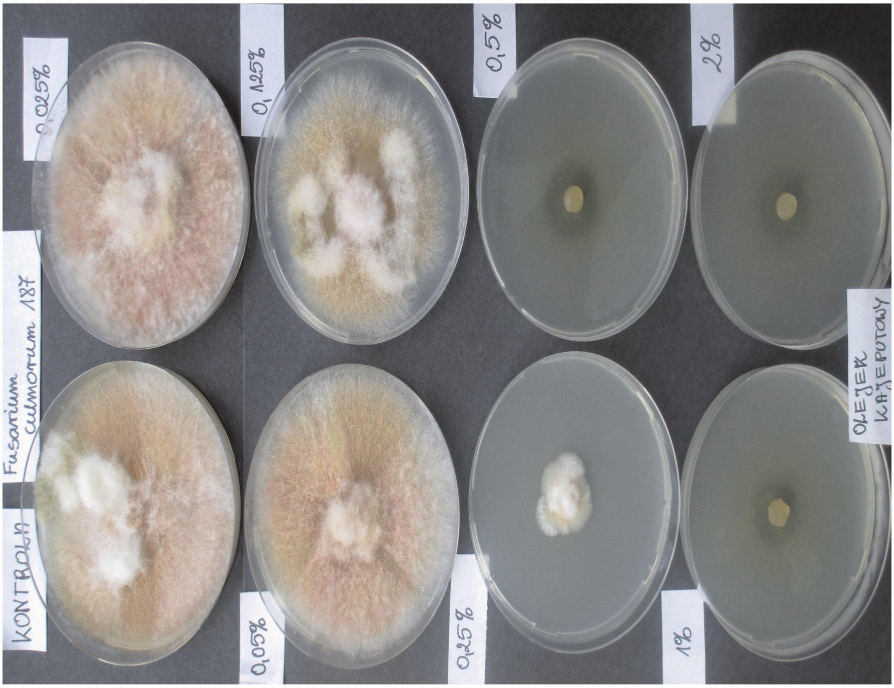

Fig. 6. Cajeput oil in the following concentrations 0.025–2.0 %

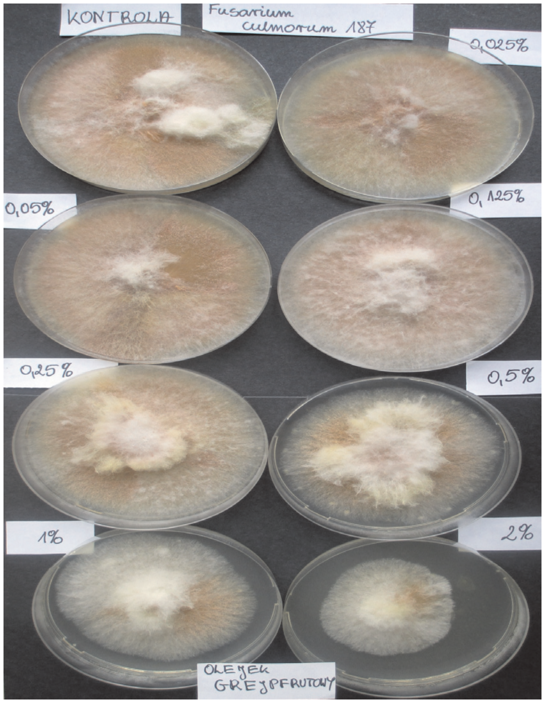

Fig. 7. Grapefruit oil in the following concentrations 0.025–2.0 %

and 61.72 %, respectively (Tables 1, 2). The sensitivity of fungi of the genus *Fusarium* on the active substance contained in lemongrass oil and Litsea cubebe oil were confirmed in research [30, 31].

Table 2

Index of *Fusarium culmorum* 187 growth (T)

Oils concentration [%]	Index of Fusarium culmorum 187 growth (T)							
	Oils							
	Funaben T	Thyme	Litsea-cubeba	Lemon-grass	Verbena	Cajeput	Tea tree	Grapefruit
Control	69.23	69.23	69.23	69.23	69.23	69.23	69.23	69.23
0.025	—	0.00	15.77	10.31	23.02	69.92	91.92	67.15
0.05	—	0.00	0.00	0.00	6.97	67.52	68.02	68.38
0.125	0.00	0.00	0.00	0.00	0.00	62.25	21.27	67.5
0.25	0.00	0.00	0.00	0.00	0.00	19.08	0.00	50.71
0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	48.42
1.0	—	0.00	0.00	0.00	0.00	0.00	0.00	45.46
2.0	—	0.00	0.00	0.00	0.00	0.00	0.00	33.23

Tea tree oil whose main active compounds were 1-terpinen-4-ol and α-terpinen showed much weaker effect in limiting the growth of *F. culmorum* 187 strain (Tables 1, 2). It completely inhibited the growth of mycelium at a concentration of only 0.25 % (Fig. 4), 10 times higher than Litsea cubeba and lemongrass oils. Terzi et al [32] analyzed the impact of individual components of tea tree oil, among others on *F. culmorum* and demonstrated that 1-terpinen-4-ol is mainly responsible for antifungal effect which confirms our research.

The same effect as Funaben T preparation a total inhibition of mycelium growth was caused by oils: thyme (regardless of concentration), lemongrass (Fig. 2) and Litsea cubeba (Fig. 3) (from 0.05 %) verbena (Fig. 5) (from 0.125 %) and cajeput (from 0.5 %) (Fig. 6). Grapefruit oil showed the weakest effect (Fig. 7).

The highest fungicidal activity in relation to *F. culmorum* 187 was shown by thyme oil (Fig. 1) regardless of concentration and Litsea cubeba (Fig. 3) oil 78 to 100 %, and lemongrass (Fig. 2) (55–100 %). The minimum fungicidal activity against a tested *Fusarium culmorum* 187 strain showed grapefruit oil (Fig. 7), which used in a concentration of 2 % limited the increase of *F. culmorum* 187 at a level of 38 % (Fig. 8).

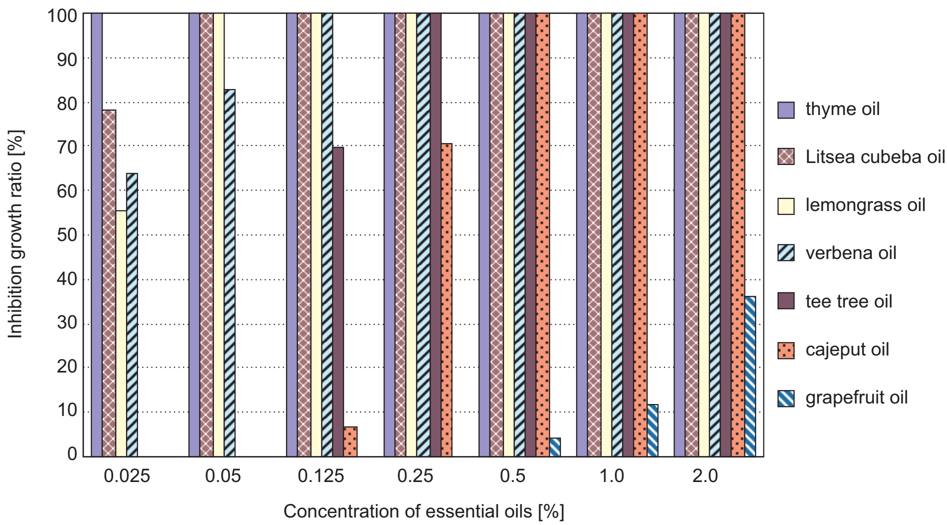

Fig. 8. Inhibition growth ratio in [%] of *Fusarium culmorum* 187 strain

Conclusions

The sensitivity of *Fusarium culmorum* 187 strain was varied; dependent on the type and concentration of tested oils.

1. The tested strain showed the highest sensitivity to thymol and citral-principal active ingredient oils, such as thyme, lemongrass and Litsea-cubeba.

2. *Fusarium culmorum* 187 strain demonstrated the lowest sensitivity towards grapefruit oil whose main component was limonene.

3. Oils: thyme, lemongrass, Litsea cubeba and verbena at concentrations (0.125–0.5 %) were acting similar to Funaben T preparation completely inhibiting the development of mycelium.

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OCENA WRAŻLIWOŚCI FITOPATOGENNEGO SZCZEPU *Fusarium culmorum* NA WYBRANE OLEJKI ETERYCZNE

Samodzielna Katedra Biotechnologii i Biologii Molekularnej
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Abstract: Rozpowszechnienie w środowisku, wysoka patogenność i toksynotwórczość oraz nabywanie oporności na stosowane fungicydy przez grzyby *Fusarium culmorum* prowadzi do poszukiwania alternatywnych metod ich zwalczania. Rozwiązanie problemu mogą stanowić olejki eteryczne. Celem pracy była ocena wrażliwości fitopatogennego szczepu *Fusarium culmorum* 187 na wybrane olejki eteryczne, takie jak: tymiankowy, z trawy cytrynowej, z Litsea cubeba, kajeputowy, z drzewa herbacianego, werbenowy i grejpfrutowy w porównaniu do zaprawy nasiennej Funaben T.

Materiał badawczy stanowił gatunek grzyba *Fusarium culmorum* 187 (Mart) Sacc., wyizolowany z porażonych zbóż. Testowane olejki eteryczne, takie jak: tymiankowy z trawy cytrynowej, z Litsea cubeba, kajeputowy, z drzewa herbacianego, werbenowy i grejpfrutowy, wprowadzano do podłoża w stężeniach: 0.025; 0.05; 0.125; 0.25; 0.5; 1.00; 2.0 %. Natomiast Funaben T w stężeniach; 0.125; 0.25; 0.5. Kontrolę stanowił rozwój grzyba w podłożu wolnym od tych substancji. Aktywność biotyczną olejków w ograniczaniu wzrostu liniowego grzyba *F. culmorum* 187 Mart.(Sacc.) oceniano metodą zatrutych podłoży. Aktywność olejków w ograniczaniu wzrostu liniowego grzybni oceniano na podstawie pomiarów przyrostu kolonii grzybów (indeks tempa wzrostu), a fungistatyczną – na podstawie procentu zahamowania wzrostu kolonii grzyba obliczonego zgodnie ze wzorem Abbotta.

Wrażliwość szczepu *Fusarium culmorum* 187 była zróżnicowana i zależała od rodzaju i zastosowanego stężenia testowanych olejków. Olejek tymiankowy we wszystkich badanych stężeniach (0.025–2.0 %) całkowicie hamował rozwój grzybni, a pozostałe olejki całkowite zahamowanie wzrostu wywoływały w stężeniach 0.5–2.0 %. Efekt działania olejków eterycznych był zbliżony do działania zaprawy Funaben T.

Słowa kluczowe: *Fusarium culmorum,* olejek tymiankowy, olejek lemongrasowy, olejek Litsea cubeba, olejek kajeputowy, olejek z drzewa herbacianego, olejek verbenowy, olejek grejpfrutowy, antyfungistatyczna aktywność

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STATE OF SCOTS PINE NEEDLES' EPICUTICULAR WAXES AND CONTENT OF MICROELEMENTS IN BIOINDICATION

STAN WOSKÓW EPIKUTYKULARNYCH IGIEŁ SOSNY ZWYCZAJNEJ ORAZ ZAWARTOŚĆ MIKROELEMENTÓW W BIOINDYKACJI

Abstract: Studies on the chemical composition and variability of morphological structure of Scots pine (*Pinus sylvestris* L.) allow to assess the health state of trees growing in conditions of exposure to alkaline dust emission. The aim of the studies was the morphological analysis of the epicuticular waxes and the content of microelements in the needles of Scots pine growing in conditions of alkaline pressure, compared to the needles from emission-free areas. The studies were conducted on two-year-old needles collected in the vicinity of cement mills in Sitkowka and Ozarow, located in the Swietokrzyskie Province. The chemical composition analysis of needles indicated higher contents of Cu, Pb and Sr as well as lower content of Mn in the needles from alkalised areas. They were also characterised by higher contents of pH_{KCl} than in the needles from emission-free areas. The images of needles, taken under a Scanning Electron Microscope (SEM), showed definite signs of epicuticular wax erosion in comparison to the needles collected from the control area. According to Turunen's classification, third and fourth degrees of epicuticular wax coverage atrophy were found in two-year-old needles. The analysis of the chemical composition of the needles and the morphology of their surface structure indicated a negative impact of cement dust pollution on Scots pine's assimilation apparatus.

Keywords: alkaline pressure, pine needles, microelements, epicuticular wax structure

Introduction

Scots pine is a species that is highly valued and widely used in bioindicative studies [1, 2]. Its main characteristic as a good bioindicator is, apart from common occurrence,

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a low tolerance to toxic factors [3]. The studies of the variability of anatomical structure of needles and their chemical composition allow to precisely assess the health state of tress growing in conditions of various anthropogenic pressures [4–10]. What is more, the bioindication studies on Scots pine ensure the comparability of results, what distinguishes them from subjective scientific methods including, among others, defoliation [1, 11].

The role of plant epicuticular waxes is not yet known exactly, but a wealth of chemical composition indicates that they have a variety of ecological functions [12, 13]. Chemically, epicuticular waxes are strongly hydrophobic polymers being important mechanical and physiological barriers between the plant and the environment. Epicuticular waxes of coniferous trees are considered to be a good biomarker of air pollution, indirectly indicating the health state of a particular tree [4, 8, 9, 13, 14]. Exposing needles to direct contact with pollution reduces general resistance of trees, affects photosynthesis, respiration and transpiration, as well as causes premature defoliation [15–19]. Describing many changes in structure and chemical composition of epicuticular waxes due to anthropogenic and natural factors is possible thanks to using a Scanning Electron Microscope (SEM). SEM allows to identify damage in epicuticular waxes at an early stage, even before the occurrence of visible symptoms. The most commonly reported changes in the structure of epicuticular waxes include: their underdeveloped crystal structure, coalescence of wax particles, wax erosion, transformation of crystal structured waxes into an amorphous form, as well as changes in chemical composition [8, 9, 13, 20].

The aim of the studies was the morphological analysis of the epicuticular waxes and the content of selected microelements in the needles of Scots pine growing in conditions of constant alkaline pressure, compared to the needles of Scots pine collected from the areas free of emissions.

Object and study methods

The studies were conducted in the years 2008 and 2012 in the forest areas situated in the vicinity of cement mills in Sitkowka and Ozarow, the communes located administratively in the Swietokrzyskie Province (Poland). All study sites were localised at a similar distance of about 0.5 km from the dust emitter. The study sites were in the coniferous forests from the *Dicrano-Pinion* community (degenerative forms) in the fresh coniferous forest habitat with a pine forest stand at the age between 50 and 70 years. For comparative studies, there was selected a site with the *Leucobryo-Pinetum* community located in Wymyslow within the boundaries of the Cisowsko-Orlowinski Landscape Park (Swietokrzyskie Province) which is beyond the reach of industrial emissions.

The study material consisted of two-year-old pine needles collected from each surface of eight randomly selected trees. The samples were dried in a desiccator at 40 ^{o}C for 24 hours, and then crushed in a Fritsch planetary mill and incinerated in an electric oven at 450 ^{o}C. After digesting the samples with HCl-HNO_3, the total contents of selected elements were determined by using ICP-AES method. The state of needles' surface structure was studied as well. The central parts of five randomly selected needles were sputtered with gold and photographed under a scanning electron microscope JSM-540 with magnification of 2000, 1000 and 500 times [21]. The study results were compared to Turumen's scale of epicuticular wax erosion, which involves six classes:

– 0 (underdeveloped wax structure),
– I (100 % of stomatal wax cover),
– II (71–100 % of stomatal wax cover),
– III (31–70 % of stomatal wax cover),
– IV (0–30 % stomatal wax cover),
– I (0 % of stomatal wax cover).

Results and discussion

The average contents of studied microelements in the needles collected in the study areas indicated considerable variation depending on their locations (Table 1). The pine needles from the alkalised areas were characterised by definitely higher pH_{KCl} values (neutral and slightly acidic pH) in comparison with the needles from the control site (acidic pH). The pH values for the material from the areas surrounding cement mills were also higher than those reported for the areas free of emissions [22, 23]. This indicates a change in the chemical character of the tree stand growing in the areas influenced by the cement mills.

Table 1

Content of microelements and pH_{KCl} values in two-year-old needles of *Pinus sylvestris* (n = 24)

Area		Parameter						
		pH_{KCl}	Ba	Cu	Mn	Pb	Sr	Zn
			[mg/kg d.m.]					
Sitkowka	average		6.3	8.8	71.5	29.5	18.3	61.5
	range	6.1–6.4	6.0–7.1	7.2–10.1	55.0–92.5	20.1–42.5	15.2–22.3	59.5–65.7
	coefficient of variation CV [%]		15.2	41.0	39.0	19.5	11.7	22.1
Ozarow	average		3.5	8.0	125.8	17.8	8.8	46.3
	range	5.5–5.9	3.2–5.9	7.3–8.5	120.5–151.0	8.0–23.5	4.2–8.3	40.6–48.1
	coefficient of variation CV [%]		17.0	35.2	29.5	17.2	20.0	16.5
Control area	average		10.0	5.8	890.8	8.0	3.5	63.1
	range	3.9–4.1	9.0–12.5	4.3–7.0	850.5–990.5	5.1–9.5	3.0–4.6	52.1–65.5
	coefficient of variation CV [%]		12.2	18.0	8.2	17.1	20.2	7.6

The material from the alkalised areas was characterised by higher values of Cu, Pb and Sr as well as lower levels of Mn, Ba and Zn.

The content of Cu was at a similar level in the needles from the areas with confirmed alkaline immission, while the greater dispersion of values was observed for the areas located in Sitkowka. Significantly lower contents of this element were noted for the control area, where its average content was lower by 31 % than that in the needles from the alkalised areas. Copper is a microelement that is essential for proper plant functioning; however, its excess leads to chlorosis. Moreover, if this element is contained in dusts, it has the ability to deposit itself on the surface of leaf blades by binding to the cuticle, and therefore it negatively affects its protective function [24]. The content of heavy metals in pine needles were also being determined by Pomierny [25] who conducted bioindicative studies in the vicinity of Katowice Steelworks. She [25] found that the content of Cu was in the range between 5.4 and 6.6 mg/kg. Nevertheless, these values were lower than those concerning the needles from the alkalised areas in the Swietokrzyskie Province.

The content of most heavy metals in bioindicator's needles is significantly correlated with the concentration of heavy metals in the air [26–29]. That is why, the high content of lead in the needles from the alkalised areas, that was on average three times greater than in the needles from the control area, indicated considerable soil and air pollution by this element [1, 30]. The content of Pb at the level of 43 mg/kg, reported for the material from the vicinity of cement mill in Sitkowka, causes wilting of plants [24]. Hence, the content of this element in the needles collected from the areas with confirmed alkaline immission seems to be the most alarming.

The average content of zinc in aerial parts of plants from the emission-free areas was included in the range between 10 and 70 mg/kg d.m. [24]. Zn, just like Cu, is an essential element in the functioning of organisms and plays an important role in the biosynthesis of enzymes, auxin, and a part of proteins. Zinc deficiency has a destructive impact on a tree stand [1], but its excess (above 100 mg/kg) causes chlorosis [24]. Scots pine is classified as a good Zn accumulator [31]. The needles collected from the control area were characterised by the content of Zn at the level between 52.1 and 65.5, with the average content at 63.1 mg/kg. Similar content of Zn in pine needles (62.5 mg/kg) was observed by Parzych who conducted studies in the area of the Slowinski National Park [32]. The lower content of Zn, even by 26.6 %, was reported for the alkalised areas. Similar value distribution of this element was indicated by Bajorek-Zydron's studies, in which the content of Zn in the needles collected from coal dumps of the mine in Belchatow was at the level of 69,0 mg/kg, but beyond the reach of pollution, the obtained values were relatively lower, *ie* 61.4 mg/kg d.m. and 41.4 mg/kg d.m. [33].

The average content of Mn for the controlled area was at the level of 890.8 mg/kg. Ceburnis and Steines [34], who conducted biomonitoring studies in Lithuania, indicated similar values of this element in pine needles from emission-free areas. The needles collected in the vicinity of cement mills in Sitkowka and Ozarow were characterised by much lower content of Mn, which was included in the range between 55.0 and 151.0 mg/kg. Similar values for Mn in two-year-old needles, exposed to influence of pollution, were noted in Pomierny's measurements [25] in which the needles contained from 73.5 to 132.0 mg/kg of this element. The content of microelements in pine needles was also studied by Miglaszewski, whose studies were conducted in the area of the

Swietokrzyski National Park [35]. According to these results, the content of Mn in two-year-old needles was at the level of 968 mg/kg, and this value was similar to that obtained in the control area located in Wymyslow. The values of other analysed elements, *ie* Pb 3 mg/kg, Cu 4 mg/kg, Ba 4 mg/kg, Sr 5 mg/kg, and Zn 58 mg/kg were at the levels comparable to those observed by Miglaszewski in the control area.

The contents of other analysed elements, *ie* Sr and Ba, were also characterised by considerable variability. However, the content of Ba was lower for the alkalised areas, while the content of Sr was characterised by completely opposite distribution trend. Strontium belongs to the group of elements whose contents in the air are reflected in their contents in plant assimilation apparatuses [34]. Low content of this element in the needles collected from the control area, which was observed at the level between 3.0 and 4.6 mg/kg, coincided with literature data [24]. In the case of Ba, alkaline immission reduces the assimilation of this element by pine needles. The values obtained for the control area, just like for Sr, coincided with literature data [24, 35].

Among the areas remaining under alkaline pressure, the area located in Sitkowka was characterised by higher contents of analysed microelements in the needles (except for Mn). This may be caused by greater pollution emissions from the cement mill than the close proximity to the City of Kielce. High contents of heavy metals in pine needles reduce their durability, which was observed by Lamppu i Hutterm [36]. Mandre et al [37, 38] proved that the needles collected from highly polluted areas are from 26 % to 50 % shorter than the needles collected from control areas.

The high variation of chemical composition of the needles was evidenced by calculated coefficients of variation (CV). The parameter was included in the range of CV = 11.7–41.0 % for the alkalised areas. In the case of the control area, the parameter was relatively lower and was included in the range of CV = 7.6–20.2 %. The statistical analysis showed that the value of pH_{KCl} is significantly correlated by $p \le 0.05$ with the content of Cu ($r = 0.673$), Sr ($r = 0.793$), and Pb ($r = 0.602$). Significant correlations were also found for the following pairs: Sr – Cu ($r = 0.703$) and Zn – Ba ($r = 0.696$).There were no strong and very strong statistical relationships for other analysed heavy metals. Also, Cord and Cord [39], who conducted bioindicative studies, demonstrated significant correlations among selected heavy metals in the areas remaining under alkaline pressure.

It was found, through observing the state of needles' epicuticular waxes, that cement dusts strongly affect their structures, causing coalescence of well-developed wax particles until complete disappearance of structural forms. The wax layer became poorer and gradually eroded. In the case of coniferous trees, the process of stomata filling with a network of wax fibres, under natural conditions, begins in the second year of a needle's life, and then it undergoes gradual erosion that lasts from 4 to 6 years [4]. The SEM images, which were taken under 500 and 1000 times (Fig. 1–4), and present the needles collected from the alkalised areas in comparison to the needles from the control areas, indicated definite erosion of crystal epicuticular waxes. Observation of needles' wax structure degradation within the study sites located in Sitkowka and Ozarow allows to assess the degree of surface erosion. The needles' structure, changed in such a way, may be classified into third and fourth degrees of erosion by Turunen [5],

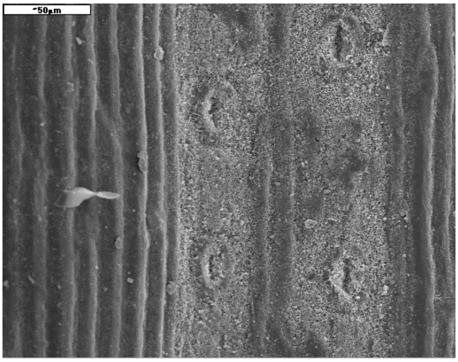

Fig. 1. Natural microstructure of the pine needle's epidermis surface; crystal wax, stomata, control area (SEM image, magnification of 500 times)

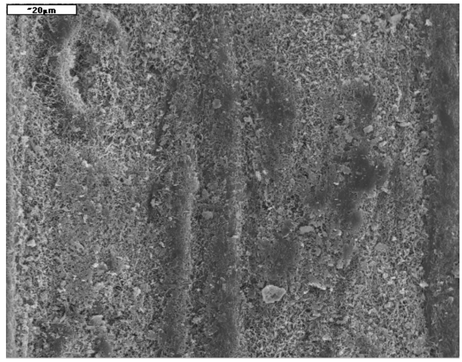

Fig. 2. Natural microstructure of the pine needle's epidermis surface; crystal wax, stomata, control area (SEM image, magnification of 1000 times)

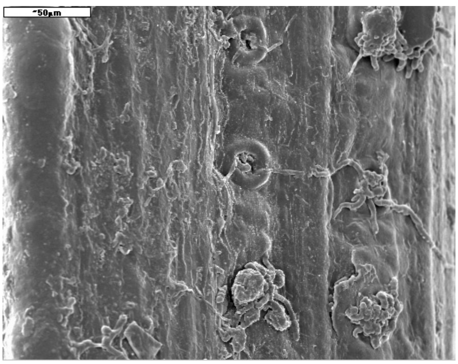

Fig. 3. Changed microstructure of the pine needle's epidermis surface; crystal wax, stomata, study site in Ozarów (SEM image, magnification of 500 times)

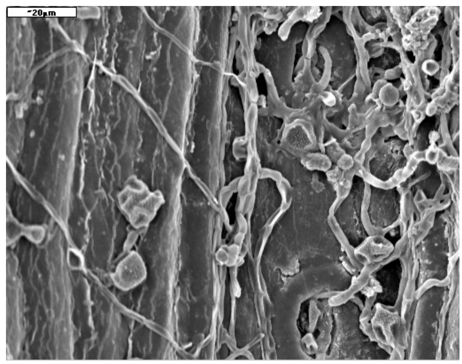

Fig. 4. Changed microstructure of the pine needle's epidermis surface; crystal wax, stomata, study site in Ozarów (SEM image, magnification of 1000 times)

which suggests almost complete decay of wax structures in interstomatal space. The wax layer was discontinued, strongly eroded, locally collapsed, and originally crystal wax became amorphic. Similar observations were the subjects of Staszewski, Bacic et al and Swiercz's studies [9, 17, 40, 42]. Equally interesting were also studies on the epicuticular wax structure conducted by Burkhardt [43] who noticed an amorphous form of epicuticular wax occurring after treatment of its intact form with saline solution. According to the author, the process of wax degradation may be the result and – at the same time – the indicator of a high concentration of aerosols in the air.

Deposition of dust aerosols weakens the health condition of needles, whereas the scale of damage depends on the size of emission and time of exposure [44]. Cement dusts contain particles with a diameter from 20 to 30 µm [40], hence, there is additional risk of bunging the stomata which have a diameter between 8 and 10 µm. Degradation of needles' wax surface structure affects their average longevity as well. It was observed that Scots pine growing in the alkalised areas sheds elder needles earlier. According to Mäkelä and Huttunena's studies [15], this is a necessity that allows trees to maintain their water balance as elder needles are characterised by higher cuticular than stomatal transpiration. What is more, due to defoliation, the crowns of some pine trees growing within the study sites in the vicinity of cement mills were markedly thinned. The type of thinning the tree crowns may be classified as arch-shaped or bottom-up [45]. Observations made under SEM confirmed that the needles of Scots pine collected from the control area in Wymyslow have a well-preserved network of wax structures (Fig. 1 and 2). Their crowns were dense and had a complete set of needles.

Conclusions

Cement dusts have a strong impact on the chemical state of Scots pine's assimilation apparatuses and on their general state. It was claimed that there are differences in accumulation of elements between the needles collected from the alkalised areas and those from the control areas. Significantly higher contents were noted for Cu, Sr and Pb, whereas the content of Mn was considerably lower. The analysis of the needles' morphological surface properties and various degrees of epicuticular wax preservation, made under SEM, may be useful for indicating degrees of assimilation apparatus deformation through deposition of dust pollutions causing wax layer erosion. It was determined, on the basis of needles' surface morphology observations, that according to Turunen's classification [1992] there are third and fourth degrees of epicuticular wax coverage atrophy. Needles, which lack of natural protection, are easily attacked by filamentous fungi, and severe degradation of wax surface structure involves more frequent defoliation of elder needles.

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STAN WOSKÓW EPIKUTYKULARNYCH IGIEŁ SOSNY ZWYCZAJNEJ ORAZ ZAWARTOŚĆ MIKROELEMENTÓW W BIOINDYKACJI

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Abstrakt: Badania składu chemicznego oraz zmienności struktury morfologicznej igieł sosny zwyczajnej (*Pinus sylestris* L.) pozwalają ocenić stan zdrowotny drzew wzrastających w warunkach narażenia na emisję pyłów alkalicznych. Celem badań była analiza morfologiczna wosków epikutykularnych oraz zawartości mikroelementów w igłach sosny zwyczajnej wzrastającej w warunkach presji alkalicznej, w porównaniu do igieł z terenów wolnych od emisji. Badania prowadzono na igłach dwuletnich, pobranych z okolic cementowni w Sitkówce oraz Ożarowie w województwie świętokrzyskim. Analiza składu chemicznego igieł wykazała wyższą zawartość Cu, Pb i Sr oraz niższą zawartość Mn w igłach z powierzchni alkalizowanych. Cechowały je także wyższe wartości pH_{KCl} niż w igłach z powierzchni wolnych od zanieczyszczenia. Zdjęcia igieł, wykonane pod SEM (elektronowym mikroskopem skaningowym), wskazały na wyraźną erozję wosków epikutykularnych w porównaniu do igieł pobranych z powierzchni kontrolnej. Według klasyfikacji Turunena stwierdzono III i IV stopień zaniku pokrycia wosków epikutykularnych na igłach 2-letnich. Analiza składu chemicznego igieł oraz morfologii ich powierzchni, wskazują na niekorzystny wpływ zanieczyszczenia pyłami cementowniczymi na aparat asymilacyjny sosny.

Słowa kluczowe: presja alkaliczna, igły sosny, mikroelementy, struktura wosków epikutykularnych

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NEURAL NETWORK MODEL PREDICTION OF CHROMIUM SEPARATION IN POLYELECTROLYTE-ENHANCED ULTRAFILTRATION

MODELOWANIE EFEKTYWNOŚCI SEPARACJI CHROMU W ULTRAFILTRACJI WSPOMAGANEJ POLIELEKTROLITAMI

Abstract: Effectiveness of polyelectrolyte-enhanced ultrafiltration in chromium recovery from its aqueous solutions was tested experimentally. Two chromium species, Cr(III) and Cr(VI) ions, were the subject of ultrafiltration processes enhanced with two water-soluble, ion-exchanging polyelectrolytes. These were: poly(sodium 4-styrenesulfonate) – PSSS (for Cr(III) ions recovery) and poly(diallyldimethylammonium chloride) – PDDAC (for Cr(VI) ions recovery). Experimental ultrafiltration tests with two different membranes and model solutions of appropriate chromium ions (5 and 50 mg · dm^{-3}), at different pH and with various polyelectrolyte doses, provided numerical data for the artificial neural networks training procedure. Numerical neural network models made prediction of chromium retention coefficient (*R*) under different process conditions (pH, polymer dose, concentration of selected Cr form) possible. Strongly nonlinear dependences of retention coefficient (*R*) on pH and polymer : metal concentration ratio for both chromium species, represented by experimental data, were identified and modeled by neural networks correctly. Good compatibility between experimental data and neural network predictions was observed.

Keywords: polyelectrolyte-enhanced ultrafiltration (PEUF); Cr(III); Cr(VI); poly(sodium 4-styrenesulfonate); poly(diallyldimethylammonium chloride)

Introduction

Over the last years, the polymer-enhanced ultrafiltration (PEUF), used to separate heavy metal ions from their water solutions, has been gaining in popularity. The process is based on binding the metal ions by water-soluble chelating polymers or polyelectrolytes containing ion-exchange groups, followed by their retention on an ultra-

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filtration membrane. Normally, the ultrafiltration separation of metals is enhanced by macromolecules containing amino (chitosan, polyethyleneimine), carboxyl (poly(acrylic acid), its salts and copolymers) or hydroxyl (poly(vinyl alcohol)) groups [1–3]. Polymer-enhanced ultrafiltration has been successfully applied to the separation of chromium from aqueous solutions, both Cr(III) and Cr(VI) [4–9]. Promising effects corresponded to binding Cr(III) with poly(acrylic acid) or its copolymer with maleic acid [5, 6], chitosan, pectin, polyethyleneimine and ethoxylated polyethyleneimine [4, 6, 7]. Polyethyleneimine appeared also to be an effective agent in enhancing the ultrafiltration separation of Cr(VI), due to the anion-exchange nature of the primary, secondary and tertiary amino groups present in its structure [5, 6]. Similar effect was achieved in ultrafiltration of Cr(VI) with addition of modified starch having cationic functional groups, capable of reacting with contaminants of anionic nature [8]. Based on literature reports and own research [5], the authors suggested ultrafiltration removal of Cr(III) and Cr(VI) ions enhanced with polyelectrolytes which contain functional groups of strong ion-exchange properties. Poly(sodium 4-styrenesulfonate) – PSSS, a water--soluble polymer which contains sulfonic groups capable of binding metal cations (Cr(III)) and poly(diallyldimethylammonium chloride) – PDDAC, whose quaternary ammonium groups exhibit anion-exchange properties (Cr(VI)) were selected for the process [9].

The work demonstrates possibility of application of artificial neural networks trained on experimental data sets for prediction of the effectiveness of Cr(III) and Cr(VI) separation (expressed by chromium retention coefficient, *R*) in diversified process conditions (environment's pH, polymer : metal concentration ratio, initial Cr concentration and its chemical form).

Materials and methods

Model solutions of Cr(III) and Cr(VI) ions, of concentrations 5 and 50 mg · dm^{-3}, were prepared using analytical grade chromium nitrate $Cr(NO_3)_3 \cdot 9H_2O$ and potassium dichromate $K_2Cr_2O_7$ (POCh S.A., Gliwice, Poland).

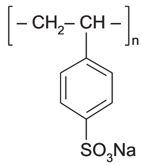
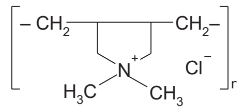
Water soluble polyelectrolytes: poly(sodium 4-styrenesulfonate) – PSSS (M_w 70000, 30 % solution) and – PDDAC (M_w 100000–200000, 20 % solution), Sigma-Aldrich, were used as Cr(III) and Cr(VI) binding agents. In order to remove small molecules, polyelectrolytes were preliminarily diafiltrated using UF membrane.

The set of polymer–chromium solutions of different molar concentration ratios (mol of mer unit per mol of metal) were prepared by mixing the appropriate doses of suitable polyelectrolyte with the proper chromium species. Process environment's pH adjustments to the required level, within the 1–10 range, were done with the use of 1 mol · dm^{-3} NaOH and HNO_3 solutions (POCh S.A.), respectively. More detailed characteristic of the model solutions used is presented in Table 1.

Prepared model solutions were a subject of ultrafiltration tests in AMICON 8400 membrane cell, equipped with magnetic stirrer. Two ultrafiltration membranes of different transport and separation characteristics were used in the PEUF process. A polysulfone membrane HZ20 (cut-off 50 kDa) and a modified polyacrylonitrile membrane

Table 1

Characteristic of the model solutions used in ultrafiltration tests

Chromium ion type	Cr(III)		Cr(VI)	
Polyelectrolyte	 PSSS poly(sodium 4--styrenesulfonate)		 PDDAC poly(diallyldimethylammonium chloride)	
pH	1; 2; 4; 6; 8; 10		1; 2; 4; 6; 8; 10	
Chromium concentration	$5\ mg \cdot dm^{-3}$	$50\ mg \cdot dm^{-3}$	$5\ mg \cdot dm^{-3}$	$50\ mg \cdot dm^{-3}$
Polymer : metal molar concentration ratio, *Pol.:Met.*	2.5; 5; 7.5; 10	5; 7.5; 10; 12.5	0.5; 1; 2.5; 5; 7.5; 10	1; 2.5; 5; 7.5; 10; 12.5

MX50 (cut-off 100 kDa) were provided by GE Osmonics. Water permeability coefficients, determined experimentally, were $2.1 \cdot 10^{-10}$ and $6.4 \cdot 10^{-10}\ m^3 \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$, respectively. Transmembrane pressure of 1 bar was applied in the ultrafiltration tests. Effectiveness of polymer enhanced ultrafiltration was evaluated by measurement of chromium concentrations in the permeate and in the feed (Atomic Absorption Spectrometer SpectrAA 880, Varian) followed by calculation of chromium retention coefficient (R) according to the formula: $R = 1 - C_P/C_F$, where: C_P, C_F – chromium concentrations in the permeate and in the feed, respectively. Detection limit for chromium ions analysis was $0.005\ mg \cdot dm^{-3}$.

Calculations

Raw experimental data matrixes collected, matching chromium separation efficiencies (R) with various combinations of Cr initial concentrations, *Pol.:Met*. molar ratios and ultrafiltration process environment's pH provided basis for the artificial neural networks creation, followed by their training, validating and testing procedures. Computations were done with the use of *STATISTICA Neural Networks software*. Various network types were preliminary tested, including radial basis function (RBF) networks, general regression neural networks (GRNN), multilayer perceptrons (MLP) and linear ones. Each net structure was trained with appropriate algorithms (pseudo-inversion, sub-sample, conjugate gradient and backpropagation error), both independently or in various sequences. Four optimal net structures were identified using appropriate statistical indicators.

For the network modeling ultrafiltration process on HZ20 membrane with PSSS polymer in a form of $R = f(C_{met}, Pol.:Met., pH)$ it was MLP type network of 3-9-8-1 structure (three inputs representing: C_{met}, *Pol.:Met.*, *pH*, one output neuron (R), with two hidden neuron layers with 9 neurons in the first and 8 in the second one), trained 100 iterations with backpropagation error algorithm (BP) followed by additional training covering 59 cycles with the conjugate gradient (CG) algorithm (resulting mean deviation MD = –0.0085, root mean square deviation RMSD = 0.0552). In case of artificial neural network modeling ultrafiltration behavior of HZ20 – PDDAC – Cr(VI) system, the best model structure proved to be a multilayer perceptron of 3-3-1 structure (3 inputs, one hidden layer with 3 neurons and one output neuron), trained with 100 backpropagation error iterations followed by 61 conjugate gradient cycles (resulting MD = 0.0074, RMSD = 0.0654).

In case of network modeling ultrafiltration behaviour of MX50 – PSSS – Cr(III) system, the optimal net configuration proved to be 3-6-1 configuration, trained with 100 iterations of backpropagation error algorithm with adjustment of the net's weight matrix using additional training with 20 iterations of conjugate gradient algorithm (MD = = 0.0273, RMSD = 0.1028). On the other hand it was identified that the MX50 – PDDAC – Cr(VI) system performance in various process conditions was the best described using multilayer perceptron of 3-5-4-1 topology trained with 100 initial iterations of backpropagation error algorithm supplemented with 63 cycles of conjugate gradient training (MD = –0.0029, RMSD = 0.0553).

These four optimal neural network configurations were then used for numerical simulation of the polyelectrolyte enhanced ultrafiltration process, especially for the identification of complex influence of various combinations of PEUF parameters on chromium forms retention effectiveness, *R*.

Results and discussion

Fig. 1 presents neural network simulation results for the two membranes used (MX50, HZ20) with respect to Cr(III) ultrafiltration enhanced by PSSS. Fig. 2 depicts the same simulation schedule for the Cr(VI) – PDDAC system.

The $R = f(Pol.:Met., pH)_{\mathrm{Cmet.}}$ response surfaces, based on neural network predictions for selected matrixes of assumed PEUF process conditions, demonstrate essential, strongly nonlinear influence of both parameters under study (pH, polymer dose), as well as concentration, chemical form of chromium in its initial solution and the added polyelectrolyte type on the ultrafiltration process effectiveness. Observed steep increase in $R = f(\mathrm{pH})_{\mathrm{Pol:Met,Cmet}}$ dependency values with the increase of the solution alkalinity, compatible with the trends within the experimental data, is demonstrated clearly, especially within the pH 1–4 (Cr(III)) and 1–6 (Cr(VI)) ranges at small polyelectrolyte doses.

Similar $R = f(Pol.:Met.)_{\mathrm{pH,Cmet}}$ function dependency can be observed, especially for all Cr(III) solutions and for higher Cr(VI) concentrations. As a result, systematic increase in *R* is observed, corresponding to the increase in polyelectrolyte dose, up till attaining the maximal, stable metal retention effect, which for Cr(VI) solutions of concentration 50 mg · dm^{-3} corresponds to *Pol.:Met.* > 5 and pH value > 4, whereas in case of Cr(III) solutions it is attained at various *Pol.:Met.* values, depending in

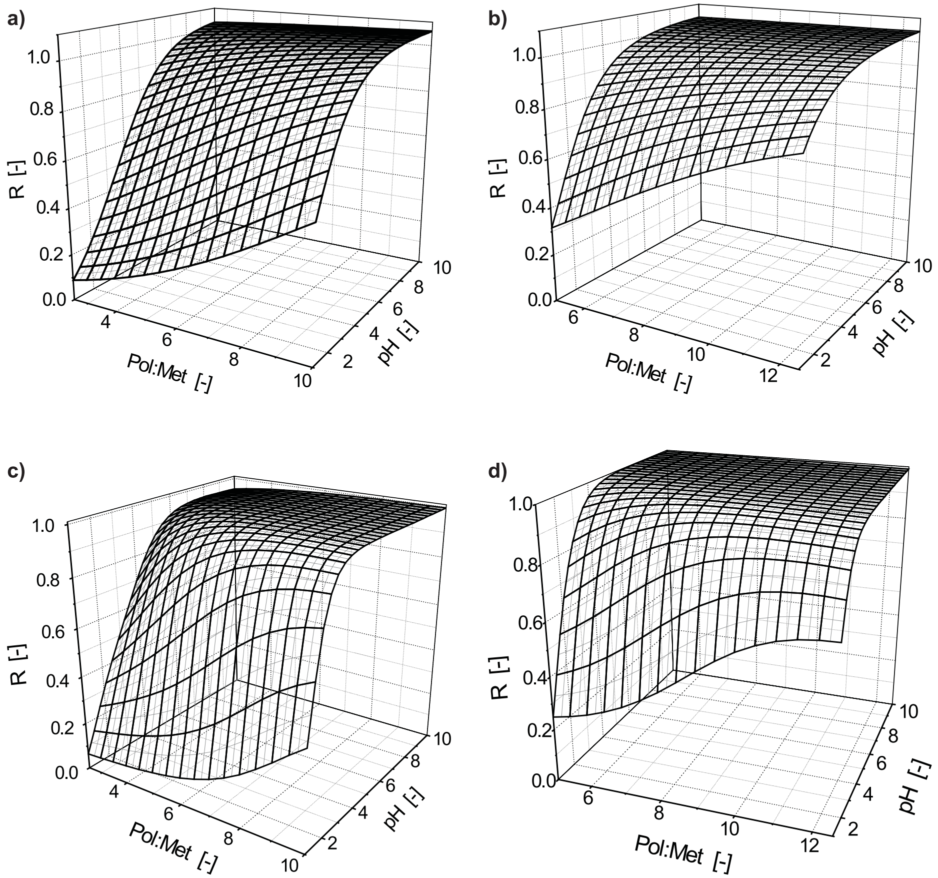


Fig. 1. Neural network model predictions – dependence of Cr(III) retention coefficient (*R*) on polymer: metal concentration ratio (*Pol.:Met.*) and process environment's pH. Membrane MX50, Cr(III) concentrations: 5 mg · dm^{-3} (a) and 50 mg · dm^{-3} (b); membrane HZ20, Cr(III) concentrations: 5 mg · dm^{-3} (c) and 50 mg · dm^{-3} (d); polyelectrolyte – PSSS

a complex way also on pH value, concentration of metal ions in initial solution and the membrane type used.

In case of Cr(VI) solution of concentration 50 mg · dm^{-3}, the $R = f(\mathrm{pH})_{\mathrm{Pol:Met}}$ dependency for *Pol.:Met.* < 5 reaches clear maximum, corresponding to pH approx. 5–6 range. In a more alkaline environment some slight decrease in *R* value is observed, particularly visible for MX50 membrane (Fig. 2b). For *Pol.:Met.* > 6 this extreme becomes more and more diffuse and after reaching the maximum only small decline in *R* is reported (Fig. 2a) – more stronger effect is visible in Fig. 2b. Comparing both neural network response surfaces, attributed to two concentrations (5 and 50 mg · dm^{-3}) and corresponding to some selected chromium form, in solutions of higher Cr concentrations within the low pH values (*ca.* 1–2) – for a constant *Pol.:Met.* ratio – one can notice also higher values of metal retention coefficient, *R*. On the other hand it

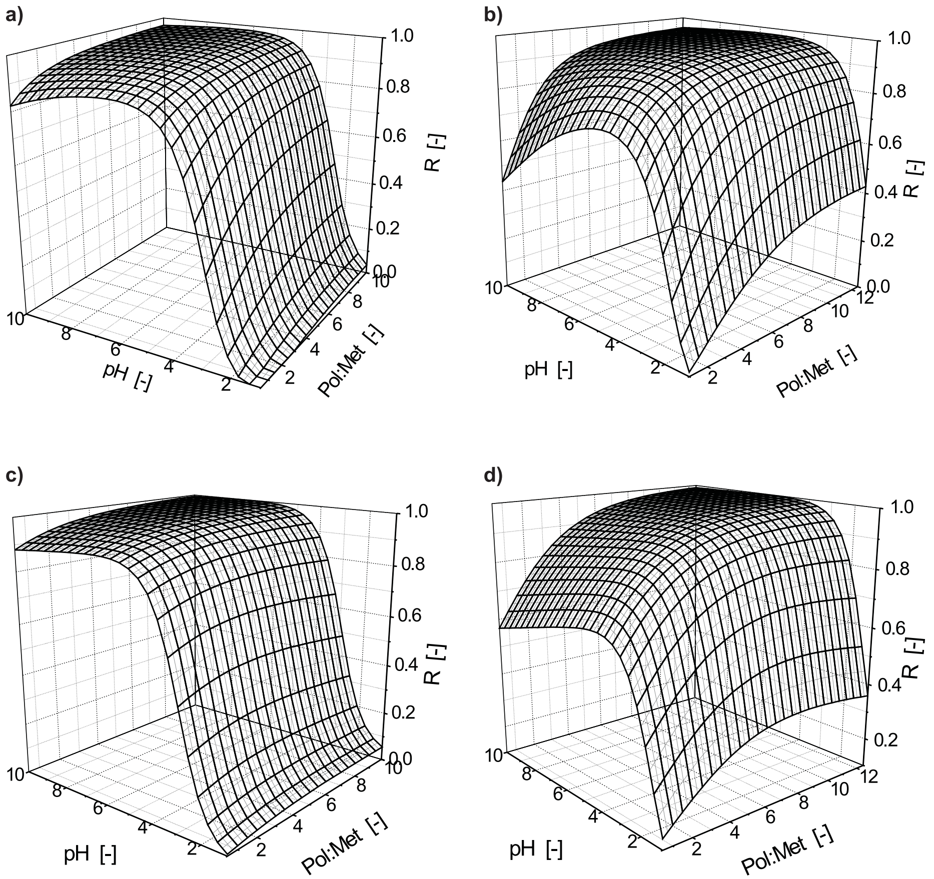


Fig. 2. Neural network model predictions – dependence of Cr(VI) retention coefficient (*R*) on polymer : metal concentration ratio (*Pol.:Met.*) and the process environment's pH. Membrane MX50, Cr(VI) concentrations: 5 mg · dm^{-3} (a) and 50 mg · dm^{-3} (b); membrane HZ20, Cr(VI) concentrations: 5 mg · dm^{-3} (c) and 50 mg · dm^{-3} (d); polyelectrolyte – PDDAC

should be noted, that Cr(III) solutions of concentration 50 mg · dm^{-3} required application of larger polyelectrolyte doses than Cr(III) solutions of concentration 5 mg · dm^{-3}, resulting from formation of precipitates, observed within the range of *Pol.:Met.* < 2.5 and < 5, for 5 and 50 mgCr(III) · dm^{-3}, respectively.

In general, spatial courses of the object's response surfaces (neural networks simulation results), attributed to MX50 and HZ20 membranes, were considered similar within all pH and *Pol.:Met*. ranges tested experimentally, for both chemical chromium forms and both metal concentrations (compare Fig. 1a–d and 2a–d). Insignificant differences between the model-predicted *R* values for MX50 and HZ20 membrane were observed in solutions of higher Cr(III) and Cr(VI) concentrations, within the lowest pH and *Pol.:Met*. ratio values tested, demonstrated thus a little better efficiency of chromium separation on HZ20 membrane.

Fig. 3 demonstrates comparison of retention coefficients *R* predicted by artificial neural network model for the Cr(III)-PSSS and Cr(VI)-PDDAC systems, respectively, and the corresponding original experimental values (model target values).

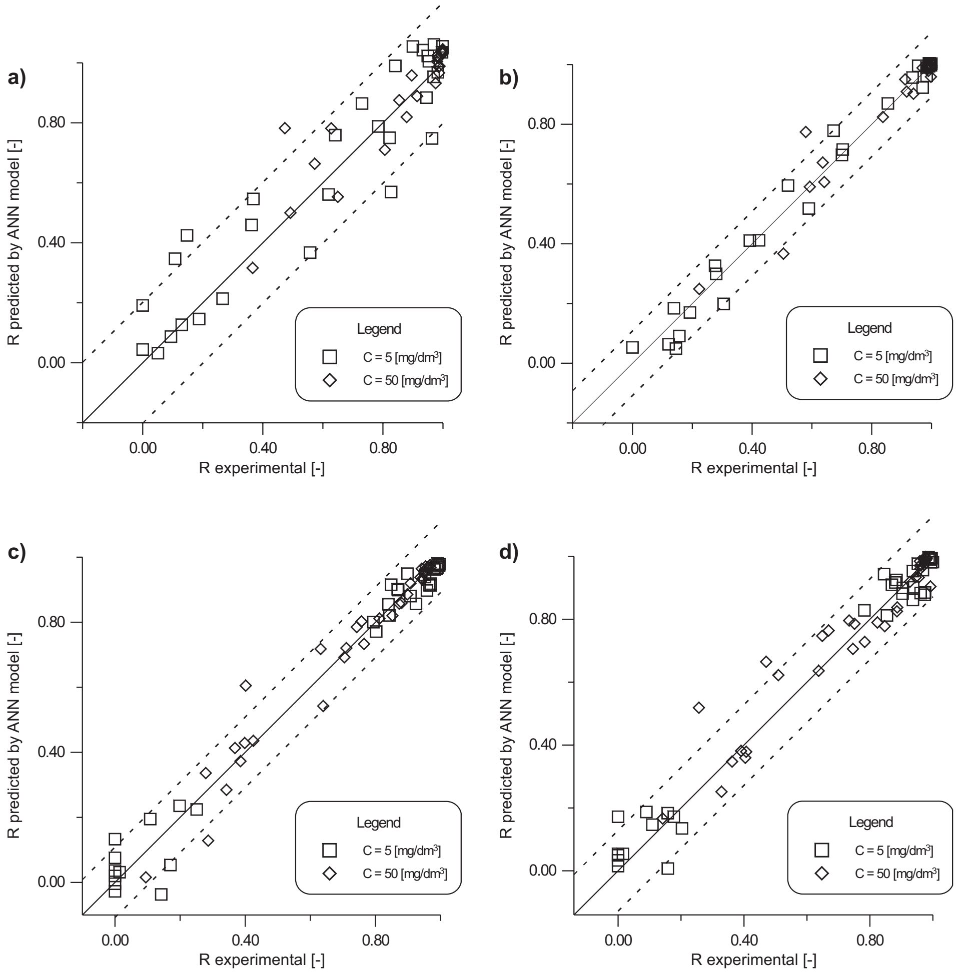


Fig. 3. Retention coefficients *R* – neural network model predictions vs. experimental data for the examined systems: (a) Cr(III)/PSSS, membrane MX 50 – confidence limits ± 0.2015; (b) Cr(III)/PSSS, membrane HZ 20 – confidence limits ± 0.1082; (c) Cr(VI)//PDDAC, membrane MX 50 – confidence limits ± 0.1084; (d) Cr(VI)//PDDAC, membrane HZ 20 – confidence limits ± 0.1282

From Fig. 3 it can be concluded, that artificial neural network models provide relatively good approximation of the real ultrafiltration systems behaviour, additionally confirmed by MD and RMSD values. The observed differences between experimental and predicted *R* values can be attributed to the unavoidable experimental procedure/analytical apparatus errors resulting in scattering of the laboratory output values. However, it should be noted that main, strongly nonlinear trends within the experimental data, with some experimentally proved extremes, were identified by ANN models properly.

Conclusions

Significant influence of polymer dose and environment's pH on Cr(III) and Cr(VI) separation efficiency in the ultrafiltration process enhanced by polyelectrolytes of strong ion-exchange properties was demonstrated.

Numerical $R = f(Pol.:Met., pH)_{\mathrm{Cmet}}$ function response surfaces, elaborated on the basis of neural network models simulation results, identified clearly (for both chromium forms and their concentrations used) the existence of some pH – *Pol.:Met.* regions, within which metal retention processes can run with very high efficiency, $R \approx 1$ (corresponding to plateau ranges noticeable in Fig. 1 and 2).

In the case of both membrane types used, within the Cr(III) and Cr(VI) concentrations tested, artificial neural network model provided good compatibility between the predicted values of chromium retention coefficient (*R*) and the corresponding experimental values for the defined PEUF process conditions.

Numerical artificial neural network models presented can be regarded as a helpful tool in design works, especially concerning optimization of metals removal in polyelectrolyte enhanced ultrafiltration processes in various environment protection applications. It should be, however, noted that the elaborated neural models can be effectively used for modeling only the systems represented by experimental data used for the neural nets training, validating and testing.

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MODELOWANIE EFEKTYWNOŚCI SEPARACJI CHROMU W ULTRAFILTRACJI WSPOMAGANEJ POLIELEKTROLITAMI

Politechnika Śląska, Gliwice

Abstrakt: Przedstawiono możliwości prognozowania efektywności separacji chromu z roztworów wodnych w procesie ultrafiltracji wspomaganej działaniem polielektrolitu w oparciu o sztuczną sieć neuronową. Badaniom poddano układy zawierające dwie różne formy chromu – Cr(III) i Cr(VI). Do wspomagania ultrafiltracyjnej separacji metalu wykorzystano dwa rozpuszczalne w wodzie polimery o właściwościach jonowymiennych – poli(4-styrenosulfonian sodu), PSSS (separacja Cr(III)) oraz poli(chlorek diallilodimetyloamoniowy), PDDAC (separacja Cr(VI)). Wyniki testów ultrafiltracji przeprowadzonej dla wodnych roztworów modelowych obu form chromu o stężeniach 5 i 50 mg · dm^{-3}, przy różnych wartościach pH środowiska oraz zróżnicowanych dawkach właściwych polielektrolitów, stanowiły podstawę uczenia i testowania struktur obliczeniowych sztucznych sieci neuronowych, umożliwiających predykcję współczynnika retencji (*R*) danej formy chromu dla różnych warunków procesowych (pH, dawka wybranego polimeru, stężenie odpowiedniego jonu Cr). Na podstawie wyników doświadczalnych oraz opracowanych sieciowych modeli numerycznych zidentyfikowano istotny, mocno nieliniowy wpływ pH oraz stosunku stężeń polimer : metal na wartości współczynników retencji (*R*) obu form chromu. Uzyskano dobrą zgodność danych eksperymentalnych z wartościami wyznaczonymi przy pomocy sztucznej sieci neuronowej.

Słowa kluczowe: ultrafiltracja wspomagana polielektrolitem (PEUF); Cr(III); Cr(VI); poli(4-styrenosulfonian sodu), poli(chlorek diallilodimetyloamoniowy)

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Elementary steps in heterogeneous catalysis: the basis for environmental chemistry

will be delivered by the Nobel Prize Winner in Chemistry 2007 Professor Dr. Gerhard ERTL

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