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INFLUENCE OF FOLIAR NITROGEN AND MAGNESIUM FERTILIZATION ON CONCENTRATION OF ASH MICRONUTRIENTS IN POTATO TUBERS

WPŁYW NAWOŻENIA DOLISTNEGO AZOTEM I MAGNEZEM NA ZAWARTOŚĆ MAKROSKŁADNIKÓW POPIELNYCH W BULWACH ZIEMNIAKA

Abstract: The paper contains a discussion of the results of an experiment concerning the effect of foliar nitrogen and magnesium fertilization on the concentration of ash macronutrients in edible potato tubers of a medium-early cultivar called Zebra. The trials were based on a three-year, two-factorial field experiment, carried out in 2005–2007 at the Research Station in Tomaszkowo, owned by the University of Warmia and Mazury in Olsztyn. The applied fertilization consisted of 80 kgN, 35 kgP and 100 kgK · ha^{-1}. The first experimental factor comprised foliar nitrogen fertilization in the range of doses 8–40 kgN · ha^{-1} accompanied by simultaneously diminished doses of soil nitrogen fertilization. The second factor included three series: without magnesium, with magnesium introduced to soil in a rate of 24 kg · ha^{-1} and with magnesium sprayed over potato leaves in a rate of 12 kgMg · ha^{-1}. Tuber samples were analyzed for the concentrations of phosphorus, potassium, magnesium and sodium. The content of these macronutrients tended to decrease under the influence of the increasing nitrogen fertilization, with the exception of phosphorus, whose concentration rose in the series unfertilized with magnesium under the effect of 8 and 16 kgN, and the concentration of sodium, which continued to increase in the Mg fertilized series up to the rate of 24 kg of N applied as a foliar fertilizer. The mean Ca : P = 0.28, Ca : Mg = 0.39 and K : Ca = 11.9 ratios suggest very poor calcium supply of the potato cultivar. In contrast, very broad ratios between K : (Ca + Mg) = 3.32 and K : Mg = 4.60 prove that the concentrations of potassium and magnesium were relatively high. The foliar application of nitrogen, tested in this experiment, had a significant effect on the ratios between ash elements in tubers. It has been demonstrated that as the top-dressing rate of nitrogen increased, the Ca : P and Ca : Mg ratios narrowed while the ratios of K : (Ca + Mg) and K : Ca were broader. The applied fertilization had no effect on the K : Mg ratio.

Keyword: macronutrients, mineral fertilizers, magnesium, nitrogen, *Solanum tuberosum*, potato

Chemical composition of tubers is the major determinant of potato quality and value as food or raw produce for processing. The chemical composition is a cultivar-specific

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trait which can be modified by the climate, soil and agronomic practice. Among the cultivation treatments, fertilization has the strongest influence on the quality of potato tubers [1–4]. Owing to rational fertilization of potato, including foliar nutrition, quick supply of deficient nutrients is possible, either when their uptake from soil by potato roots is impaired or when their soil concentrations are low. The main advantage of foliar fertilization of plants is the rapid effect that the applied nutrients have on fertilized crops. Another positive aspect is that they are highly effectively used up by plants [1, 3, 5–9]. Moreover, foliar fertilization means that less nitrogen and magnesium fertilizers can be used, which is good for the environment and the economics of potato cultivation. The up-to-date research on foliar nutrition of potato has focused mainly on investigating the effect of such fertilization on potato yield. Less attention has been paid to the quality of tubers.

The purpose of the present study has been to clarify the influence of foliar nitrogen fertilization as well as foliar and soil magnesium fertilization on the concentrations and ratios between ash macronutrients (phosphorus, potassium, calcium, magnesium and sodium) in potato tubers.

Material and methods

The results originate from a three-year field experiment, set up at the Experimental Station in Tomaszkowo near Olsztyn (53°42′35″ N, 20°26′01″ E) in 2005. The experiment was established on proper brown soil developed from weak loamy sand class IVb in the Polish soil valuation system, classified as good rye complex. According to the FAO/WRB (*World Reference Base for Soil Resources*) [10], this soil belonged to Cambisols – Brown Soils. The effect of foliar nitrogen fertilization combined with foliar and soil magnesium fertilization on concentrations of chlorophyll in leaves of a medium-early potato cultivar Zebra (Plant Breeding Station in Szyldak, Ltd.) was examined. The study involved a two-factorial experiment in random blocks with four replications, including different nitrogen and magnesium fertilization variants, either applied to soil or sprayed over leaves. The experiment consisted of three series: in the first one, nitrogen fertilization alone was applied in a rate of 80 kgN · ha^{-1}, with a gradually increasing share of foliar nutrition (0, 8, 16, 24, 32, 40) kgN · ha^{-1} at the expense of soil fertilization, which equalled (80, 72, 64, 56, 48, 40) kgN · ha^{-1}; the other two series included additional magnesium fertilization. In the second series, magnesium was introduced to soil in a rate of 24 kgMg · ha^{-1} and in the third one, it was sprayed over leaves in an amount of 12 kgMg · ha^{-1}. Phosphorus and potassium fertilization rates were constant in all the treatments and equalled 35 kgP and 100 kgK · ha^{-1}. The phosphorus fertilizer, granular triple superphosphate 20 % P ($Ca(H_2PO_4)_2$), and the potassium one, potassium salt 50 % K (KCl), were applied in a single dose to soil before planting potatoes. Nitrogen was used as urea 46 % N ($CO(NH_2)_2$), and magnesium in the form of magnesium sulphate ($MgSO_4 \cdot 7H_2O$). Whole amounts of the fertilizers introduced to soil were applied before planting potatoes, and the ones used for foliar fertilization were sprayed in five doses during the plants' vegetative season. The first spraying treatment was performed after the rows of potato plants became compact

and the first flower buds formed. The subsequent treatments were carried out at 7-day intervals. The working concentration of the solutions of fertilizers applied to leaves was 6.9 % of urea and 10.0 % of magnesium sulphate. The rows were spaced at 62.5 cm and the distance between planted potatoes in a row was 40 cm. Thus, the calculated plant density was 40 thousand plants per ha^{-1}. The area of plots for harvest was 12.96 m^2.

After harvest, averaged samples of tubers were collected from each plot for chemical analyses. The samples were washed, dried at 65°C, grounded and mineralized. Samples weighing 1 g each were wet digested according to the EPA Method 3052 (*Microwave Assisted Acid Digestion of Siliceous and Originally Based Matrices*) [11], using microwave heating with a suitable microwave system (MARS-5, CEM Corporation). The K, Ca, Mg, and Na concentrations were determined by *flame atomic absorption spectroscopy* (FLAAS) (VARIAN model SpectrAA – FS240, Varian Inc. Australia). The vanadium molybdate colorimetric method was used to determine the phosphorus content P [12]. Absorbance was measured at the wavelength $\lambda = 470$ nm in a 1 cm path length quartz cuvette using flow spectrophotometer type Specol 220 (Carl Zeiss Jena). Ratios between the nutrients contained in tubers were expressed as equivalent (K, Na, Ca and Mg) and as molar ratios (Ca, P). The equivalent ratios were calculated from the content of the nutrients expressed in $g \cdot kg^{-1}$ d.m. and their gram equivalent weight(+) (K = 39.098, Na = 22.989, Ca = 20.039 and Mg = 12.153 $g \cdot val(+)^{-1}$). Due to the varying valence of phosphorus in chemical compounds, the ratios Ca : P were expressed as molar ones. This is how the Ca : P ratio is presented in available research papers [13, 14]. This ratio was calculated from the concentrations of Ca and P in tubers expressed in $g \cdot kg^{-1}$ d.m. and their molar mass (Ca = 40.078, P = 30.972 $g \cdot mol^{-1}$).

The results were processed statistically with ANOVA at the level of significance of $\alpha = 0.05$, using a Statistica v. 9.0 software package [15]. The correlation between the analyzed factors was established using a simple linear correlation model, with the Microsoft Excel programme [16].

Results and discussion

The foliar nitrogen fertilization in the series without magnesium generally contributed to a decrease in the levels of the analyzed ash nutrients. There were two exceptions, however, such as phosphorus and sodium, which slightly fluctuated under the influence of the above fertilization. The concentration of phosphorus was raised by 5 % at the most under the effect of the foliar application of 16 $kgN \cdot ha^{-1}$ compared with its level in tubers which had only received soil nitrogen fertilization (Fig. 1). The concentration of sodium increased nearly linearly under the applied foliar nitrogen fertilization (Fig. 2).

The applied magnesium fertilization did not demonstrate any significant effect on the content of phosphorus, although it produced such impact on the concentrations of sodium. In the treatments with a soil magnesium fertilization dose of 24 $kg \cdot ha^{-1}$, the concentration of sodium in potato tubers was significantly positively correlated with the rate of the applied foliar dose of nitrogen ($r = 0.83^*$). With respect to the series with foliar magnesium fertilization, a reverse correlation was determined ($r = -0.90^*$). In the light of the available literature, it can be concluded that the results of the present trials

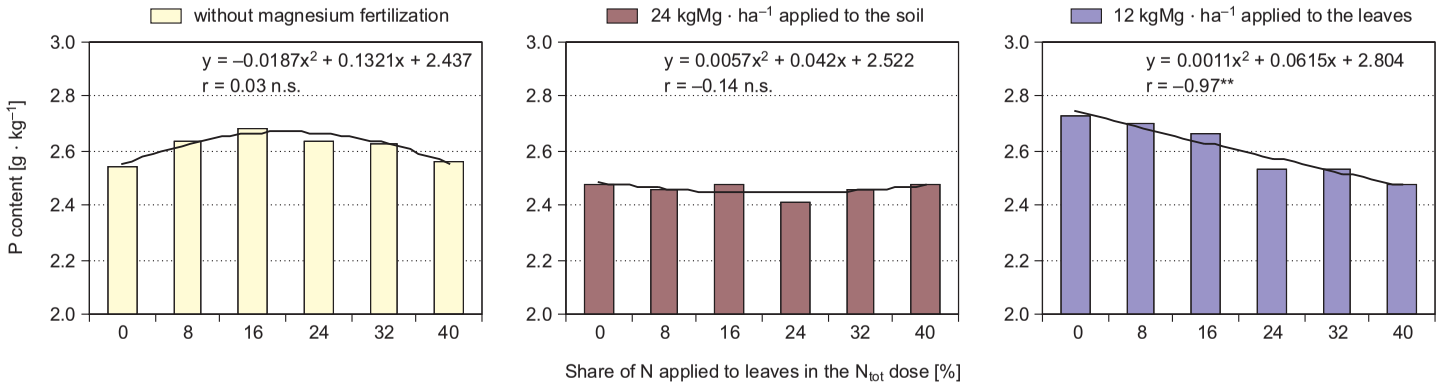

Fig. 1. Effect of foliar nitrogen and two magnesium fertilization technologies on the phosphorus (P) content in potato tubers
* – correlation coefficient significant at $\alpha = 0.05$, ** – correlation coefficient significant at $\alpha = 0.01$

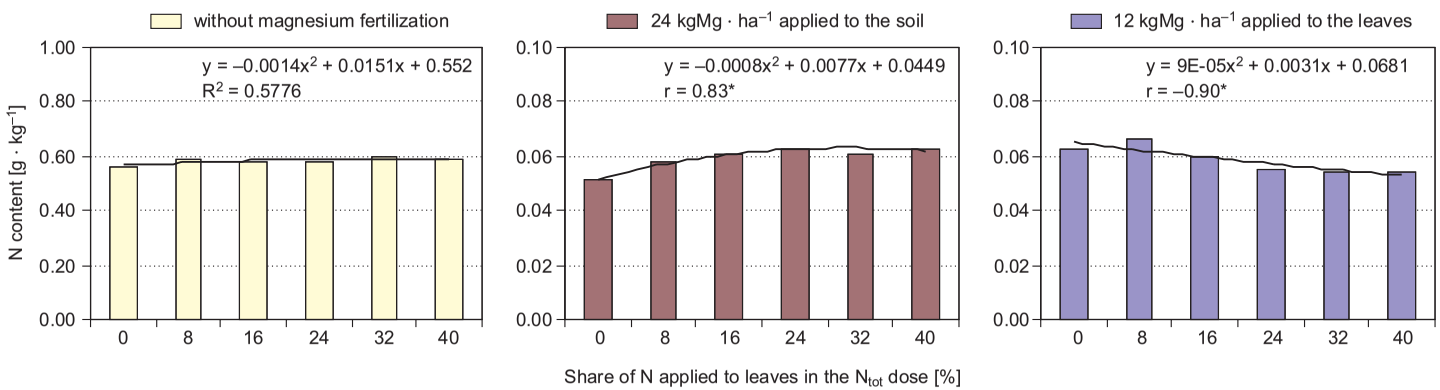

Fig. 2. Effect of foliar nitrogen and two magnesium fertilization technologies on the sodium (Na) content in potato tubers
* – correlation coefficient significant at $\alpha = 0.05$, ** – correlation coefficient significant at $\alpha = 0.01$

are partly congruous with the reports which state that nitrogen fertilization has no influence on the content of phosphorus in potato tubers [17–20], although there are also such data which suggest that under the influence of rising nitrogen fertilization the accumulation of this element in potato tubers is enhanced [5, 21, 22].

The concentrations of the other ash nutrients, *ie* potassium, calcium and magnesium, under the influence of increasing foliar nitrogen fertilization tended to decrease, both in the series without magnesium nutrition and in the ones where magnesium was applied to soil or to leaves (Figs. 3, 4 and 5). At the same time, significant negative correlation coefficients were found between the volume of the foliar nitrogen dose and the concentrations of K, Ca and Mg, which suggest that the applied nitrogen had strongly diminished the content of these macronutrients in potato tubers. These coefficients ranged from r = –0.83* to r = –0.99**.

A highly significant negative correlation (r = –0.92** – r = –0.99**) between the rate of nitrogen sprayed over leaves and the content of in tubers suggests that there is a strong relationship between the rate of the applied nitrogen potassium fertilization and the effect consisting in depressed accumulation of potassium in potato tubers. Numerous studies indicate that nitrogen fertilization causes a decrease in the content of K in tubers [20, 24], or else has no effect on potassium levels [23]. However, reverse relationships were demonstrated in some previous studies [19], in which the accumulation of potassium was stimulated by foliar and soil nitrogen fertilization. In this experiment, the concentration of potassium in tubers was not affected by magnesium fertilization, which coincides with the data reported in the literature [19, 25]. Nevertheless, there are also reports suggesting a large decrease in the concentration of potassium in plants under the effect of magnesium fertilization [26].

The concentration of calcium in the analyzed potato tubers tended to decline under the influence of foliar nitrogen fertilization. No such effect had been found in some earlier investigations [19, 20]. Many researchers claim that magnesium fertilization has had some effect on the increased accumulation of calcium in potato [19, 26, 31]. In the present experiment, no effect of magnesium on the accumulation of calcium in potato tubers has been evidenced.

In respect to the concentration of magnesium, no significant decrease in its content in potato tubers was noted under the effect of foliar nitrogen fertilization. Many researchers claim that the concentration of magnesium does not change significantly under the influence of nitrogen fertilization [23]. The concentration of magnesium in plants tends to increase in response to nitrogen nutrition [27, 28]. What is worrying is that in both our earlier studies [19] and in the present trials, magnesium fertilization in any of the tested technologies did not lead to an increase in the concentration of this nutrient in tubers. Potatoes are one of the staple foodstuffs in an average diet in Poland and therefore are an important source of minerals, including magnesium. Magnesium is the second to potassium intercellular cation which occurs in the human organism. It has been experimentally demonstrated that Poles, compared with citizens of Western Europe, consume too little magnesium, and its deficit may lead to such disorders as hypercalcaemia, tetany, paraesthesia, tremor of the limbs, hypomagnesaemia, etc. [29, 30].

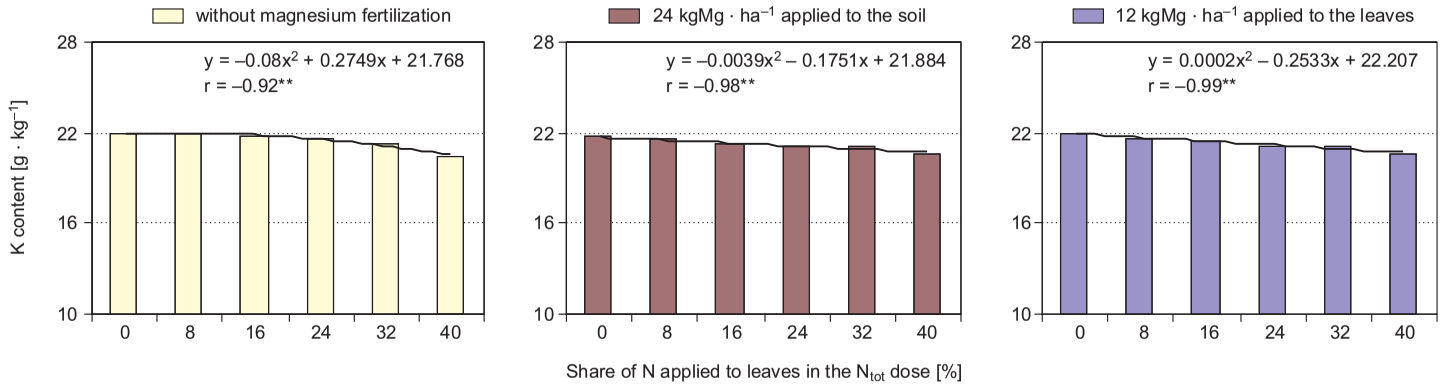

Fig. 3. Effect of foliar nitrogen and two magnesium fertilization technologies on the potassium (K) content in potato tubers
* – correlation coefficient significant at $\alpha = 0.05$, ** – correlation coefficient significant at $\alpha = 0.01$

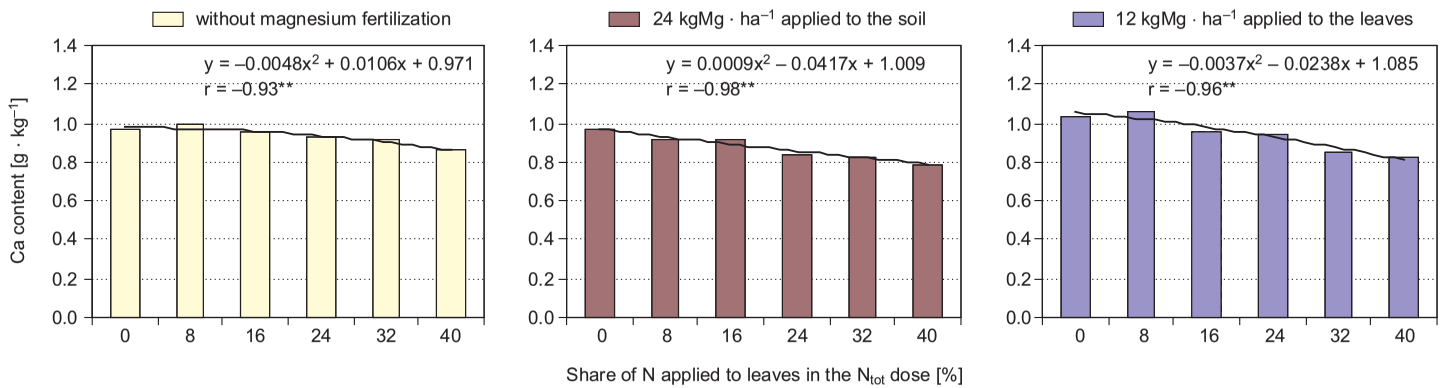

Fig. 4. Effect of foliar nitrogen and two magnesium fertilization technologies on the calcium (Ca) content in potato tubers
* – correlation coefficient significant at $\alpha = 0.05$, ** – correlation coefficient significant at $\alpha = 0.01$

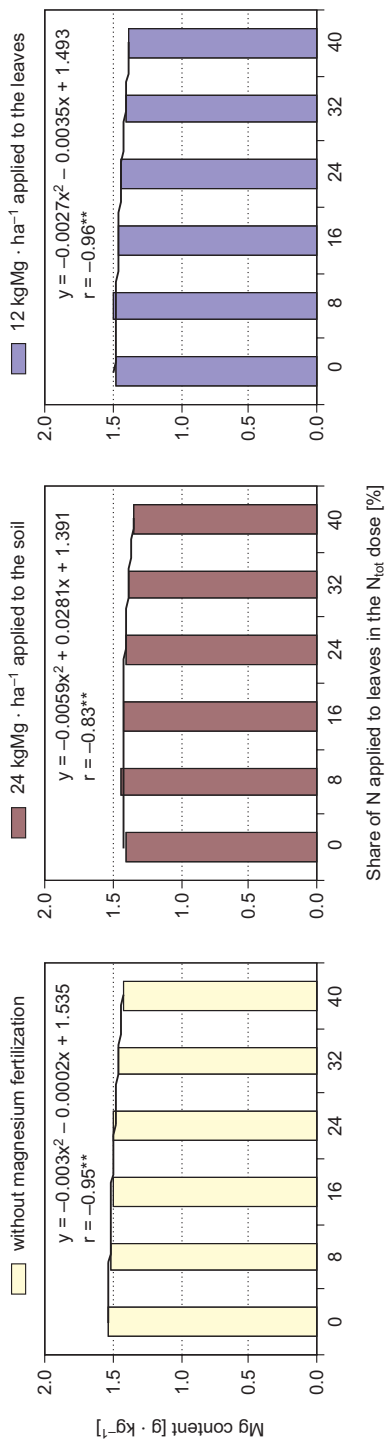

Fig. 5. Effect of foliar nitrogen and two magnesium fertilization technologies on the magnesium (Mg) content in potato tubers
* – correlation coefficient significant at $\alpha = 0.05$, ** – correlation coefficient significant at $\alpha = 0.01$

In general, the decrease in the content of most ash macronutrients in potato tubers, as demonstrated by the present study, was negatively correlated with the rate of nitrogen sprayed over leaves. This effect is attributable to the accumulation of potassium by potato plants, as during this process some kind of 'dilution' of minerals occurred as a result of the growing mass of tubers, in which starch and water accumulated. This dilution effect was particularly evident in the treatments receiving a higher rate of foliar nitrogen fertilizer, which led to a decrease in the dry matter of tubers and the ash nutrients it contained.

Beside the general content of macronutrients in tubers, another important determinant of the potato tuber quality is the mutual ratios between these macronutrients, which characterize proportions of particular components in plant products [13]. Ionic ratios in plants are typically highly correlated with soil abundance in nutrients, and the synergistic or antagonists responses between ions in soil solution have a direct influence on the product such as the chemical composition of plants. In plant production, analysis of plant chemical composition may reveal which elements require special attention when making fertilization plans. The ionic ratios in plants cited in many papers [32–34] are mainly the ones found in fodder crops. The authors who are most often cited in the above articles are Korzeniowski [35], Underwood [36], Czuba and Mazur [13] and Falkowski et al [14]. The optimum values of such ratios, as suggested by the above authors, are K : (Ca + Mg) = (1.6–2.2) : 1, K : Mg = 6 : 1, K : Ca = 2 : 1, K : Na = (5–10) : 1, Ca : Mg = (2–3) : 1, K : Ca = 2 : 1 and a molar ratio of Ca : P = 2 : 1.

The optimum value of the Ca : P ratio determined by Underwood [36] is within the range of (1–7). As this proportion in the bone system is 2 : 2, many researchers quote the latter as an optimum value [14]. In the present study, the average value of the Ca : P molar ratio was very narrow, and on average equalled 0.28:1 (Table 1). Such a narrow Ca:P ratio suggests that the analyzed potato tubers had a very poor calcium supply.

This poor calcium supply can be confirmed by a very broad K : Ca ratio, which in fodder crops should equal 2 : 1 [13], whereas in the tubers analyzed in our study the average value of this ratio fell within the range of (11.6–12.4) : 1. The applied nitrogen fertilization contributed to a slight narrowing of the Ca : P ratio. For nutrition, the ratio between divalent cations Ca and Mg is very important. In the analyzed tubers, the value of this ratio was within (0.38–0.40) : 1, which is also indicative of a very poor calcium supply. The applied magnesium nutrition, whether to soil or to leaves, has not been demonstrated to improve this ratio. Potassium plays a very important role in potato fertilization. It is responsible for carbohydrate metabolism in plants. This element improves potato yields, simultaneously raising the content of starch in tubers. However, it is an element which plants take up luxuriously, which may lead to some unbalance between the content of the other elements, especially magnesium. When comparing the optimum values of the K : (Ca + Mg) ratio to the values obtained after potato harvest, very high accumulation of potassium was found in each fertilization series at the expense of divalent cations. The value of the K : (Ca + Mg) ratio ranged on average between (3.41–3.25) : 1, which was double the optimum value, *ie* (1.6–2.2) : 1 [13, 14]. The increasing foliar nitrogen fertilization contributed to the further broadening of this ratio. The computed K : Mg ratios suggest that magnesium was another element present

Table 1

Effect of foliar nitrogen fertilization and two magnesium fertilization technologies on the ionic ratios in potato tubers

Objects			Molar ratio	mval(+) ratios			
NPK fertilization to the soil	foliar N fertilization	Mg fertilization	Ca : P	Ca : Mg	K : (Ca+Mg)	K : Mg	K : Ca
1. N_{80} P_{35} K_{100}	—	series without Mg fertilization	0.30	0.38	3.21	4.44	11.6
2. N_{72} P_{35} K_{100}	N_8		0.29	0.40	3.24	4.53	11.4
3. N_{64} P_{35} K_{100}	N_{16}		0.27	0.38	3.26	4.52	11.8
4. N_{56} P_{35} K_{100}	N_{24}		0.27	0.38	3.25	4.47	11.9
5. N_{48} P_{35} K_{100}	N_{32}		0.27	0.38	3.29	4.53	12.0
6. N_{40} P_{35} K_{100}	N_{40}		0.26	0.37	3.28	4.48	12.2
Mean:			0.28	0.38	3.25	4.49	11.8
Correlation coefficient between: share of N applied to leaves and ionic ratio			–0.97**	–0.74	0.89*	0.25	0.90*
7. N_{80} P_{35} K_{100}	—	24 kgMg · ha^{-1} applied before planting to the soil	0.30	0.42	3.38	4.79	11.5
8. N_{72} P_{35} K_{100}	N_8		0.29	0.39	3.37	4.68	12.0
9. N_{64} P_{35} K_{100}	N_{16}		0.28	0.39	3.35	4.65	12.0
10. N_{56} P_{35} K_{100}	N_{24}		0.27	0.36	3.42	4.65	12.9
11. N_{48} P_{35} K_{100}	N_{32}		0.26	0.36	3.47	4.74	13.0
12. N_{40} P_{35} K_{100}	N_{40}		0.25	0.35	3.51	4.75	13.4
Mean:			0.28	0.38	3.41	4.71	12.4
Correlation coefficient between: share of N applied to leaves and ionic ratio			–0.99**	–0.94**	0.88*	–0.02	0.97**

Table 1 contd.

Objects			Molar ratio	mval(+) ratios			
NPK fertilization to the soil	foliar N fertilization	Mg fertilization	Ca : P	Ca : Mg	K : (Ca+Mg)	K : Mg	K : Ca
13. N_{80} P_{35} K_{100}	—	12 kgMg · ha^{-1} applied as foliar spraying	0.29	0.43	3.24	4.62	10.8
14. N_{72} P_{35} K_{100}	N_8		0.30	0.43	3.15	4.51	10.5
15. N_{64} P_{35} K_{100}	N_{16}		0.28	0.40	3.29	4.61	11.5
16. N_{56} P_{35} K_{100}	N_{24}		0.29	0.40	3.27	4.56	11.5
17. N_{48} P_{35} K_{100}	N_{32}		0.26	0.37	3.41	4.67	12.7
18. N_{40} P_{35} K_{100}	N_{40}		0.26	0.36	3.42	4.65	12.9
Mean:			0.28	0.40	3.30	4.60	11.6
Correlation coefficient between: share of N applied to leaves and ionic ratio			–0.89*	–0.96**	0.86*	0.55	0.93**

* – correlation coefficient significant at $\alpha = 0.05$, ** – correlation coefficient significant at $\alpha = 0.01$.

in tubers in relatively high quantities. The K : Mg ratio was on average (4.49–4.71) : 1, whereas the optimum value has been cited to equal 6 : 1 [13]. By analyzing the relationships between the increasing foliar nitrogen fertilization and the calculated ratios, it was concluded that the Ca : P and Ca : Mg ratios were negatively correlated with the top-dressing rate of N. In turn, positive correlations between the N rate applied as top-dressing fertilizer were determined for the K : (Ca + Mg) and K : Ca ratios. At the same time, it was demonstrated that nitrogen fertilization had no effect on the K : Mg ratio.

Conclusions

1. In general, foliar nitrogen fertilization had a negative effect on the concentrations of phosphorus, potassium, calcium and magnesium in potato tubers. The concentration of sodium in tubers was an exception in that it rose nearly linearly ($r = 0.83^*$) from 0.051 gN · kg to 0.063 gN · kg of tubers^{-1} in the series with soil magnesium fertilization under the effect of foliar application of nitrogen.

2. Magnesium fertilization did not have any significant effect on the concentration of phosphorus, potassium, calcium or magnesium in the tubers of the tested potato cultivar.

3. The calculated ratios of Ca : P, Ca : Mg and K : Ca suggest that the grown potato cultivar was very poorly supplied with calcium; in contrast, the values of K : (Ca + Mg) and K : Mg indicate a relatively high concentration of potassium and magnesium.

4. A negative correlation has been determined between the top-dressing N fertilization and the values of the Ca : P and Ca : Mg ratios and a positive one between the N rate and the values of K : (Ca + Mg) and K : Ca ratios.

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WPŁYW NAWOŻENIA DOLISTNEGO AZOTEM I MAGNEZEM NA ZAWARTOŚĆ MAKROSKŁADNIKÓW POPIELNYCH W BULWACH ZIEMNIAKA

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Abstrakt: W pracy przedstawiono wyniki badań dotyczące oddziaływania dolistnego nawożenia azotem i magnezem na zawartość makroskładników popielnych w bulwach ziemniaka jadalnego średnio wczesnej odmiany Zebra. Za podstawę badań przyjęto 3-letnie, II-czynnikowe doświadczenie polowe, które realizowano w latach 2005–2007 na polu Ośrodka Dydaktyczno-Doświadczalnego w Tomaszowie, należącego do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Zastosowane nawożenie wynosiło 80 kgN, 35 kgP · ha^{-1} i 100 kgK · ha^{-1}. Czynnik I doświadczenia obejmował nawożenie dolistne azotem w zakresie (8; 40) kgN · ha^{-1} zmniejszając jednocześnie doglebową dawkę azotu. Czynnik drugi uwzględniał trzy serie: bez magnezu, z magnezem stosowanym doglebowo 24 kgMg · ha^{-1} oraz z magnezem stosowanym dolistnie w dawce 12 kgMg · ha^{-1}. Próby bulw analizowano na zawartość fosforu, potasu, wapnia, magnezu i sodu. Zawartość makroskładników w bulwach pod wpływem wzrastającego dolistnego nawożenia azotem generalnie ulegała obniżeniu. Wyjątek stanowiła zawartość fosforu, która wzrosła w serii nienawożonej magnezem pod wpływem 8 i 16 kgN oraz zawartość sodu, która w serii nawożonej Mg rosła do dawki 24 kgN stosowanego dolistnie. Średnie stosunki Ca : P = 0,28, Ca : Mg = 0,39 i K : Ca = 11,9 wskazują na bardzo słabe zaopatrzenie uprawianej odmiany w wapń, natomiast szerokie stosunki K : (Ca + Mg) = 3,32 i K : Mg = 4,60 świadczą o relatywnie wysokiej zawartości potasu i magnezu. Zastosowany dolistnie azot w istotny sposób wpłynął na kształtowanie stosunków pomiędzy składnikami popielnymi w bulwach. Stwierdzono, że wraz ze wzrostem pogłównej dawki N następowało zawężanie stosunku Ca : P i Ca : Mg oraz poszerzenie K : (Ca + Mg) oraz K : Ca. Zastosowane nawożenie nie miało wpływu na stosunek K : Mg.

Słowa kluczowe: makroskładniki, nawożenie mineralne, azot, magnez, solanum tuberosum, ziemniak

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CHANGES OF CALCIUM AND MAGNESIUM CONTENT IN BIOMASS OF GOAT'S RUE (*Galega orientalis* Lam.) DURING VEGETATION

ZMIANY ZAWARTOŚCI WAPNIA I MAGNEZU W BIOMASIE RUTWICY WSCHODNIEJ (*Galega orientalis* Lam.) PODCZAS WEGETACJI

Abstract: Calcium and magnesium are among macroelements, both in plant and animal feeding. Green forage, especially that obtained from legume plants, is their main source in ruminants' feed. The optimum content of those elements in fodder positively affects its quality. The aim of this study was to trace changes in calcium and magnesium content in fodder galega (*Galega orientalis*) during the vegetation period, depending on the year of cultivation and development phase. The results are based on two field experiments, conducted for the third and seventh year. Samples were taken during the harvest from 1 m^2 of the field during the following development phases: budding, start of blossoming, full bloom, end of blossoming and full ripeness. Subsequently, the samples were dried and ground. Calcium and magnesium were determined by the ICP-AES method following dry mineralisation.

Statistical calculations have revealed significant variation in calcium and magnesium content in fodder galega (*Galega orientalis*), depending on the year of cultivation and development phase. The average calcium content in dry matter of the test plant was equal to 15.57 g · kg^{-1}, while that of magnesium was 2.54 g · kg^{-1}. The largest amounts of calcium and magnesium were found in the leaves of the test plant in the third year of cultivation. Considering different development phases of fodder galega, it can be concluded that the highest level of calcium was determined at the end of the blossoming phase and the highest level of magnesium – during the full ripeness phase. The average Ca : Mg ratio was equal to 6.12 : 1.

Keywords: fodder galega (*Galega orientalis* Lam.), calcium, magnesium, year of cultivation, development phase, biomass, leaves, stem, Ca : Mg ratio

Fodder galega (*Galega orientalis* Lam.) is a perennial plant of the legume family. It originates in Caucasus, and it is currently grown in Estonia, Latvia, Finland, France,

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Canada and Poland. It has a high ability to reduce molecular nitrogen (379.7 kgN · ha^{-1}, on average) [1], owing to inoculating soil or seeds with *Rhizobium galegae* bacteria [2–5]. This makes its cultivation for fodder feasible and highly profitable. It can be used as fodder for animals in agriculture and animal feeding as green forage, hay, dried material, silage and protein concentrate [6–8]. Demand for calcium and magnesium from dicotyledonous plants is higher than from monospermous ones. Of the cultivated plants, legumes contain particularly high concentrations of calcium and magnesium. In overlimed soils, antagonism between Mg^{2+} and Ca^{2+} ions may occur. A high concentration of Mg^{2+} ions in soil may have a harmful effect on plants as the Ca/Mg balance is disturbed. To ensure proper absorption of calcium from fodders, it is necessary to maintain the proper balance between cations and anions [9]. Calcium action in animal bodies is closely linked with magnesium, which is its antagonist. There have been few reports in the foreign and Polish literature on the chemical composition of the plant [10–14]. It is important to monitor plants intended for fodder for calcium and magnesium content because any deficit or excess negatively affects the fodder quality and animal health status.

The aim of this study was to trace changes in calcium and magnesium content in the biomass of fodder galega (*Galega orientalis*) and in its leaves and stems, depending on the year of cultivation and development phase.

Materials and methods

Field experiments were conducted on soil formed from clayey sand, with pH in 1 mol KCl · dm^{-3} – 6.6. The soil contained 11.5 g · kg^{-1} of organic carbon and 0.1 g · kg^{-1} of total nitrogen. The content of available phosphorus and potassium (determined by the Egner-Riehm method) was referred to as high (80 mg · kg^{-1} P and 140 mg · kg^{-1} K) and that of magnesium (determined by the Schachtschabel method) as medium (50 mg · kg^{-1}). The total Ca content in soil on which galega was cultivated for the third year was 7.88 g · kg^{-1}, while in the seventh year it was 5.61 g · kg^{-1} . Magnesium content in the surface layer of the soil was 0.84 g · kg^{-1} under galega grown for the third year and 0.68 g · kg^{-1} in the seventh year of cultivation. Fodder galega was sown in May 1997 and 2001 at the depth of 2–3 cm in the amount of 24 kg · ha^{-1} in 12–15 cm rows. Scarified seeds were sown into soil infected with a strain of *Rhizobium galegae* bacteria. Weed control procedures were performed during the vegetation period and proper humidity level was maintained (sprinkling). Samples in the following development phases: budding, start of blossoming, full bloom, end of blossoming and full ripeness were taken from 1 m^2 of the field during the harvest of green mass of fodder galega in 2003 from both fields (3^{rd} and 7^{th} year of the experiment). The samples were dried, in some of them leaves were separated from stems and ground. Calcium and magnesium were determined in the prepared material by the ICP-AES method following dry mineralisation [15]. The results were analysed statistically with an analysis of variance and significant differences were calculated with Tukey's test at the level of significance of $p = 0.05$.

Results and discussion

Weather data for the 2003 vegetation period is shown in Table 1.

Table 1

Rainfall and air temperatures in the vegetation in the years 2003.
Reported by the measurement centre in Siedlce

May	June	July	August	September	Sum or mean
Total monthly rainfall [mm]					
37.2	26.6	26.1	4.7	24.3	118.9
Multiyear monthly rainfall [mm]					
50.0	75.0	80.0	68.0	47.3	320.3
Means monthly air temperature [°C]					
15.6	18.4	20.0	18.4	13.5	18.5
Multiyear temperature mean [°C]					
12.6	16.6	17.7	26.9	12.7	17.3

Weather conditions during that season were not favourable for field crops. A low rainfall level is particularly noteworthy. It was nearly three times lower than the multiyear average. It significantly reduced the yield and changed the calcium and magnesium content in the development phases of fodder galega (*Galega orientalis* Lam.) and changed the content of the macroelements under study in the analysed plant parts.

The average calcium content in the dry matter of fodder galega was equal to 15.57 g · kg^{-1} (Table 2) and it was significantly varied by the analysed factors and their combinations.

Table 2

The content of calcium [g · kg^{-1} d.m.] in biomass of goat's rue

Year of cultivation (A)	Development stage (B)					Mean
	Budding	Begin of flowering	Full flowering	End of flowering	Full ripeness	
Third	10.97	15.27	17.53	23.09	23.10	17.99
Seventh	10.28	9.44	10.42	18.10	17.56	13.16
Mean	10.62	12.35	13.98	20.59	20.33	15.57

$LSD_{0.05}$ for: years (A) – 0.61; development stage (B) – 1.38; interaction (AxB) – 1.36; interaction (BxA) – 1.94.

Significantly, the highest calcium content in dry matter of the test plant biomass was found in the third year of the experiment (17.99 g · kg^{-1}). Considering the effect of development phase on the Ca level in dry matter of the plant, it is clear that the highest amount of calcium was accumulated in the full ripeness phase (23.10 g · kg^{-1}). Statistical analysis has shown significant differences in calcium content between consecutive development phases and the full ripeness phase. These analyses results

were confirmed by a study by Symanowicz and Kalembasa [16], who examined the effect of phosphorus-potassium fertilisation on the yield and macroelement content in biomass of fodder galega. An increase in calcium uptake by the test plant was also affected by the weather conditions, soil pH and calcium content in soil. The results lay within the acceptable range for fodders [9].

Potential for the production of protein concentrates from leaves of fodder galega encouraged the authors to examine those parts of the plant. The analysed factors and their combinations significantly differentiated the total content of calcium in leaves of fodder galega (Table 3). A significantly higher calcium content in dry matter of leaves of the test plant was found in the third year of the experiment (26.29 g · kg^{-1}). Chemical analyses of leaves of the test plant in consecutive development phases showed a significant increase in calcium content. In the ripeness phase, it was more than twice as high as in the budding phase. The results for the budding phase and start of blossoming phase were confirmed by a study conducted by Ignaczak [7] and Symanowicz and Kalembasa [14].

Table 3

The content of calcium [g · kg^{-1} d.m.] in leaves of goat's rue

Year of cultivation (A)	Development stage (B)					Mean
	Budding	Begin of flowering	Full flowering	End of flowering	Full ripeness	
Third	16.43	19.43	26.42	34.37	34.82	26.29
Seventh	13.46	14.72	19.26	30.68	31.94	22.01
Mean	14.94	17.07	22.84	32.53	33.38	24.15

$LSD_{0.05}$ for: years (A) – 0.39; development stage (B) – 0.88; interaction (AxB) – 0.87; interaction (BxA) – 1.24.

Statistical analysis has shown significant differences in the calcium content in stems (Table 4).

Table 4

The content of calcium [g · kg^{-1} d.m.] in stems of goat's rue

Year of cultivation (A)	Development stage (B)					Mean
	Budding	Begin of flowering	Full flowering	End of flowering	Full ripeness	
Third	6.76	7.45	8.09	12.31	11.88	9.30
Seventh	5.59	3.89	4.82	6.92	6.88	5.62
Mean	6.17	5.67	6.45	9.62	9.38	7.46

$LSD_{0.05}$ for: years (A) – 0.20; development stage (B) – 0.45; interaction (AxB) – 0.64; interaction (BxA) – 0.45.

The average content of calcium in dry matter of stems of the test plant was equal to 7.46 g · kg^{-1} and it was much higher than the findings of other studies by Symanowicz

and Kalembasa [14]. Differences in calcium content in dry matter of stems in the third and seventh year of the experiment were highly significant (9.30 and 5.62 g · kg^{-1}). There was significant differentiation between development phases in calcium content. Significantly, the highest level of Ca was found in stems at the end of the blossoming phase (9.62 g · kg^{-1} of d.m.), with the level decreasing insignificantly (9.38 g · kg^{-1} d.m.) during the next development phase (full ripeness).

The average magnesium content in dry matter of fodder galega was equal to 2.54 g · kg^{-1} (Table 5) and it was significantly varied by the analysed factors and their combinations. Significantly, the highest magnesium content in dry matter of the test plant biomass was found in the third year of the experiment (2.74 g · kg^{-1}). Considering the effect of development phase on the Mg level in dry matter of the plant, it was found that the highest amount of magnesium was accumulated in the full ripeness phase (2.87 g · kg^{-1}). Statistical analysis has shown significant differences in magnesium content between consecutive development phases except for the relationship between the budding phase, start of blossoming as well as the full bloom and end of blossoming phases. The results are similar to the findings of a study of the effect of inoculation of seeds of fodder galega (*Galega orientalis* Lam.) on macroelement content [14]. Ignaczak [7] examined the quality of yield of green forage obtained from the first cut (harvested in spring during the budding phase) and determined the magnesium content to be equal to 2.6 g · kg^{-1} d.m. According to Anke [17], Falkowski et al [18], Kabata-Pendias and Pendias [19], magnesium content of about 2 g · kg^{-1} is sufficient to cover the nutritional needs of animals. Patorczyk-Pytlik [20] showed the sward of grassland to be usually of low value as fodder in terms of its mineral composition. According to the findings of that study, 59 % samples of pasture sward did not meet the feeding standards for dairy cattle in terms of magnesium content. Conversely, a study by Ignaczak [21], comparing the traditional and extensive system of using fodder galega, gave entirely different results. When the plant was used traditionally as green forage (3 cuts), the magnesium content ranged 1.07–2.01 g · kg^{-1} d.m., while in the third year of fallowing, it ranged 4.6–11.3 g · kg^{-1} d.m. A large extent of magnesium release from soil and incorporation into circulation in the cultivating of perennial plants (including fodder galega) has been indicated by the findings of studies of Raig et al [22] and Zarczynski et al [23].

Table 5

The content of magnesium [g · kg^{-1} d.m.] in biomass of goat's rue

Year of cultivation (A)	Development stage (B)					Mean
	Budding	Begin of flowering	Full flowering	End of flowering	Full ripeness	
Third	2.18	2.45	3.01	2.93	3.15	2.74
Seventh	2.40	2.09	2.26	2.36	2.60	2.34
Mean	2.29	2.27	2.63	2.64	2.87	2.54

$LSD_{0.05}$ for: years (A) – 0.07; development stage (B) – 0.16; interaction (AxB) – 0.22; interaction (BxA) – 0.15.

The analysed factors significantly differentiated the total content of magnesium in leaves of fodder galega (Table 6). The average magnesium content in dry matter of leaves was equal to 3.58 g · kg^{-1}. A significantly higher magnesium content in leaves of the test plant was found in the third year of the experiment (3.67 g · kg^{-1}). The dry matter of fodder galega harvested at the consecutive development phases was found to contain significantly more magnesium during the full bloom phase (4.12 g · kg^{-1} of d.m.). The results have confirmed the findings of earlier studies by the authors [14].

Table 6

The content of magnesium [g · kg^{-1} d.m.] in leaves of goat's rue

Year of cultivation (A)	Development stage (B)					Mean
	Budding	Begin of flowering	Full flowering	End of flowering	Full ripeness	
Third	2.83	2.87	4.35	4.24	4.05	367
Seventh	2.70	3.17	3.90	3.94	3.77	3.49
Mean	2.77	3.02	4.12	4.09	3.91	3.58

$LSD_{0.05}$ for: years (A) – 0.08; development stage (B) – 0.17; interaction (AxB) – 0.24; interaction (BxA) – 0.17.

The average content of magnesium in dry matter of stems of the test plant was equal to 1.42 g · kg^{-1} (Table 7) and it confirmed the findings of an earlier study by Symanowicz and Kalembasa [14]. Statistical calculations have revealed significant variation in magnesium content in stems of fodder galega, depending on the year of cultivation and development phase. The differences in magnesium content in dry matter of stems in the third and seventh year of the experiment were highly significant (1.61 g · kg^{-1} and 1.24 g · kg^{-1}). There is significant differentiation between each development phase with reference to magnesium content. Significantly, the highest Mg content was found in stems during the budding phase (1.63 g · kg^{-1} d.m.).

Table 7

The content of magnesium [g · kg^{-1} d.m.] in stems of goat's rue

Year of cultivation (A)	Development stage (B)					Mean
	Budding	Begin of flowering	Full flowering	End of flowering	Full ripeness	
Third	1.46	1.58	1.54	1.91	1.59	1.61
Seventh	1.81	1.00	0.90	1.29	1.18	1.24
Mean	1.63	1.29	1.22	1.60	1.38	1.42

$LSD_{0.05}$ for: years (A) – 0.05; development stage (B) – 0.11; interaction (AxB) – 0.15; interaction (BxA) – 0.11.

The average calcium/magnesium ratio in the test plant was about 6.12 : 1 (Table 8) and it lay within the range acceptable for fodders, which ranges between 3 : 1 and 6.5 : 1 [9, 24]. The Ca/Mg ratio slightly exceeded the acceptable range in the third year of the experiment. Considering individual development phases of fodder galega, it must be said that the calcium and magnesium content, determined during the budding phase, set the Ca : Mg ratio at the optimum level of 4.64 : 1. This is advantageous, as fodder galega should be harvested during the budding phase for direct feeding or for dried material. A much lower Ca : Mg ratio (2.7 : 1) was found by Ignaczak [7], who examined the quality of green forage from the first cut harvested in spring at the budding phase. A wide range of Ca : Mg ratio, calculated for fodder galega harvested during the blossoming phase, and full ripeness phase indicates the absence of a possibility of using such fodder in animal feeding [9].

Table 8

The values of molar ratio Ca : Mg in biomass of goat's rue

Year of cultivation (A)	Development stage (B)					Mean
	Budding	Begin of flowering	Full flowering	End of flowering	Full ripeness	
Third	5.03	6.23	5.82	7.88	7.33	6.56
Seventh	4.28	4.52	4.62	7.67	6.75	5.62
Mean	4.64	5.44	5.31	7.80	7.08	6.12

Table 9 shows the correlation coefficients between the content of calcium and magnesium in the plant biomass, in its leaves and stems of *Galega orientalis* Lam. in consecutive years of study. Calcium content determined in biomass of the entire plant in the consecutive development phases in the third year of the experiment was significantly positively correlated with the calcium content in leaves and stems, as determined in the third year, with calcium content in the entire plant and in leaves in the seventh year of the experiment as well as with the magnesium content in leaves in the seventh year of the experiment. The correlation coefficients indicate a significant positive correlation between the calcium content in leaves in the third year of the experiment and the calcium content in stems, as determined in the third year, with calcium content in the entire plant biomass and in leaves in the seventh year of the experiment as well as with the magnesium content in leaves in the seventh year of the experiment. Calcium content determined in stems in the third year of the experiment was significantly correlated with the calcium content in the entire plant and in leaves in the seventh year of the experiment. Calcium content in the biomass of the test plant in the seventh year was positively correlated with the calcium content in leaves and stems. Total magnesium content determined in galega biomass in the third year of the experiment was significantly correlated with the magnesium content in the leaves in the third and the seventh year of the experiment. A significant correlation of magnesium content in leaves was observed in the third and seventh year of the experiment.

Table 9

Values of the correlation coefficients between the average content of calcium and magnesium in the goat's rue parts and experimental years

Content	$Ca_{t\,III}$	$Ca_{l\,III}$	$Ca_{s\,III}$	$Ca_{t\,VII}$	$Ca_{l\,VII}$	$Ca_{s\,VII}$	$Mg_{t\,III}$	$Mg_{l\,III}$	$Mg_{s\,III}$	$Mg_{t\,VII}$	$Mg_{l\,VII}$	$Mg_{s\,VII}$
$Ca_{t\,III}$	1.00											
$Ca_{l\,III}$	0.98*	1.00										
$Ca_{s\,III}$	0.96*	0.96*	1.00									
$Ca_{t\,VII}$	0.89*	0.91*	0.98*	1.00								
$Ca_{l\,VII}$	0.96*	0.98*	0.99*	0.97*	1.00							
$Ca_{s\,VII}$	0.67	0.75	0.83	0.92*	0.84	1.00						
$Mg_{t\,III}$	0.89*	0.91*	0.76	0.67	0.82	0.49	1.00					
$Mg_{l\,III}$	0.80	0.86	0.70	0.62	0.75	0.53	0.93*	1.00				
$Mg_{s\,III}$	0.72	0.68	0.77	0.72	0.68	0.53	0.43	0.51	1.00			
$Mg_{t\,VII}$	0.44	0.55	0.58	0.69	0.65	0.86	0.41	0.36	0.04	1.00		
$Mg_{l\,VII}$	0.88*	0.88*	0.74	0.62	0.77	0.42	0.95*	0.95*	0.61	0.20	1.00	
$Mg_{s\,VII}$	–0.44	–0.35	–0.16	0.03	–0.19	0.35	–0.61	–0.48	–0.16	0.40	–0.66	1.00

* – significant at $\alpha = 0.05$; $Ca_{t\,III}$ – the content of calcium in total biomass in third year of cultivation; $Ca_{l\,III}$ – the content of calcium in leaves in third year of cultivation; $Ca_{s\,III}$ – the content of calcium in stems in third year of cultivation; $Ca_{t\,VII}$ – the content of calcium in total in seventh year of cultivation; $Ca_{l\,VII}$ – the content of calcium in leaves in seventh year of cultivation; $Ca_{s\,VII}$ – the content of calcium in stems in seventh year of cultivation; $Mg_{t\,III}$ – the content of magnesium in total biomass in third year of cultivation; $Mg_{l\,III}$ – the content of magnesium in leaves in third year of cultivation; $Mg_{s\,III}$ – the content of magnesium in stems in third year of cultivation; $Mg_{t\,VII}$ – the content of magnesium in total in seventh year of cultivation; $Mg_{l\,VII}$ – the content of magnesium in leaves in seventh year of cultivation; $Mg_{s\,VII}$ – the content of magnesium in stems in seventh year of cultivation.

Conclusions

1. The highest content of Ca and Mg was found in leaves of fodder galega (*Galega orientalis* Lam.). The content of calcium and magnesium in stems was 2–3 times lower than in leaves.

2. Significantly, the highest amounts of the elements under analysis were found in the plant in the third year of cultivation.

3. The highest content of calcium was found during the full ripeness phase and the highest content of magnesium was found during the full bloom phase.

4. A high content of calcium in biomass of fodder galega (*Galega orientalis* Lam.) set the Ca : Mg ratio at a high level.

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ZMIANY ZAWARTOŚCI WAPNIA I MAGNEZU W BIOMASIE RUTWICY WSCHODNIEJ (*Galega orientalis* Lam.) PODCZAS WEGETACJI

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Abstrakt: Wapń i magnez należą do podstawowych składników mineralnych organizmów zwierzęcych. Są one niezbędne do funkcjonowania całego organizmu i metabolizmu komórek. Podstawowym źródłem Ca i Mg w żywieniu zwierząt przeżuwających są zielonki, szczególnie roślin bobowatych. Optymalna zawartość tych składników w paszy korzystnie wpływa na jej jakość. Celem przeprowadzonych badań było prześledzenie zmian w zawartości wapnia i magnezu w biomasie rutwicy wschodniej w zależności od roku uprawy i fazy rozwojowej. Wyniki badań uzyskano na podstawie dwóch doświadczeń polowych prowadzonych trzeci i siódmy rok. Podczas zbioru pobrano próbki z 1 m^2 w następujących fazach rozwojowych: pąkowanie, początek kwitnienia, pełnia kwitnienia, koniec kwitnienia i dojrzałość pełna. Następnie próbki te wysuszono i rozdrobniono. Wapń i magnez oznaczono metodą ICP-AES, po mineralizacji „na sucho".

Obliczenia statystyczne wykazały istotne zróżnicowanie w zawartości wapnia i magnezu w biomasie rutwicy wschodniej (*Galega orientalis* Lam.) w zależności od roku uprawy i fazy rozwojowej. Średnia zawartość wapnia w suchej masie rośliny testowej wynosiła 15,57 $g \cdot kg^{-1}$ a magnezu 2,54 $g \cdot kg^{-1}$. Największe ilości wapnia i magnezu oznaczono w liściach rośliny testowej w trzecim roku uprawy. Rozpatrując poszczególne fazy rozwojowe rutwicy wschodniej, należy stwierdzić, że w fazie koniec kwitnienia oznaczono najwięcej wapnia, natomiast magnezu w fazie dojrzałości pełnej. Średni stosunek Ca : Mg ukształtował się na poziomie 6,12 : 1.

Słowa kluczowe: rutwica wschodnia (*Galega orientalis* Lam.), wapń, magnez, rok uprawy, faza rozwojowa, biomasa, liście, łodyga, stosunek Ca : Mg

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Agnieszka BARAN

ASSESSMENT OF ZINC CONTENT AND MOBILITY IN MAIZE

OCENA ZAWARTOŚCI I MOBLINOŚCI CYNKU W KUKURYDZY

Abstract: The research aimed to assess the content and mobility of zinc in maize cultivated in soils polluted with this element. The indicators of the assessment were: zinc content in maize, zinc concentration index, zinc bioaccumulation and translocation index. A two-year pot experiment was conducted parallel on soils: light and heavy one. Four levels of zinc were applied in the experiment: Zn_0 – 0 mg (control), Zn_1 – 50 mg, Zn_2 – 250 mg and Zn_3 – 750 mg · kg^{-1} soil d.m. Zinc content in the aerial parts and roots was determined after dry mineralization and dissolving the ashes in HNO_3 using atomic emission spectrometry in inductively coupled argon plasma (ICP-AES). Soil contamination with zinc significantly affected an increase in this metal contents in the aboveground and underground maize biomass. The aboveground biomass obtained on the light soil contained 2-fold (Zn_1), 5-fold (Zn_2) and 25-fold (Zn_3) bigger amounts of zinc in comparison with the treatment without zinc supplement. On the other hand, on the heavy soil the dependencies were respectively 2-fold (Zn_1), 7-fold (Zn_2) and 19-fold (Zn_3). Bigger zinc content was assessed in roots than in the aerial parts and the dependence was confirmed by low values of the translocation coefficient (TC). Greater phyto-availability and phytotoxicity of zinc was demonstrated in the light soil than in the heavy soil. The relationship was confirmed by a better zinc solubility determined by 1 mol HCl · dm^{-3} in the light soil but also by higher values of zinc bioaccumulation coefficients in this soil.

Keywords: maiz, zinc, concentartion index, bioaccumulation and translocation coefficient

Zinc, due to its many physiological functions in plants is considered as their crucial nutrient [1]. However, since it is quite common in the environment, being also a component of many compounds emitted to the natural environment and present in waste substances used in agriculture, it may accumulate in soil. Excessively high zinc concentration in soils is harmful to plants because this metal easily accumulates in vegetative and generative plant parts, which in consequence may lead to their growth inhibition, decline in yields and worsening of their quality [2–5]. This phenomenon is commonly known as phytotoxicity [6, 7]. Generally plants reveal considerable tolerance to elevated zinc content in soil whereas the extent of zinc tolerance depends in the first place on physicochemical properties of soil. Bioavailability and mobility of zinc are determined by the following soil factors: soil pH, granulometric composition, organic

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matter content, the from in which zinc occurs in soil and its solubility [8–10]. The research aimed to assess the content and mobility of zinc in maize cultivated in soils polluted with this element.

Material and methods

A two-year pot experiment was conducted parallel on soils: light and heavy one. The light soil with granulometric composition of weakly loamy sand was characterized by slightly acid reaction, whereas the heavy soil with granulometric composition of loamy silt had acid pH. Total zinc content in the light soil was 62 mg and in the heavy soil 158.35 mg · kg^{-1}. Zinc soluble in 1 mol HCl · dm^{-3} constituted 36 % of its total content in the light soil and 16 % in the heavy one. Four levels of zinc were applied in the experiment: Zn_0 – 0 mg (control), Zn_1 – 50 mg, Zn_2 – 250 mg and Zn_3 – 750 mg · kg^{-1} soil d.m. The same mineral fertilization: 0.225 gN, 0.14 gP and 0.275 gK · kg^{-1} soil d.m. was applied on all experimental treatments. Mineral salts as zinc sulphate(VI), ammonium nitrate(V), potassium dihydrophosphate(V) and potassium chloride were supplied prior to the plant sowing. The test plant was maize (*Zea mays*), "Bora" c.v. The plants were harvested at the 7–9 leaves phase, after 40 days of vegetation. The analysis of the plant material chemical composition was conducted to assess the chemical effects of the soil contamination with zinc. The indicators of the assessment were: zinc content in maize, zinc concentration index, zinc bioaccumulation and translocation index. Zinc content in the aerial parts and roots was determined after dry mineralization and dissolving the ashes in HNO_3 using *atomic emission spectrometry in inductively coupled argon plasma* (ICP-AES) on JY 238 ULTRACE apparatus (Jobin Yvon). Obtained results were elaborated statistically by means of one-way ANOVA and Tukey test. The test was applied when no equality between means was revealed. ANOVA was conducted at the significance level $\alpha = 0.01$.

Results

Zinc concentrations in maize were diversified depending on zinc dose, kinds of soil and analyzed part of test plant (Table 1).

Table 1

Content of zinc in aboveground biomass and roots of maize

Treatment		Aboveground biomass		Roots	
		[mg · kg^{-1} d.m.]			
		Light soil	Heavy soil	Light soil	Heavy soil
Zn_0	0 mg*	51.96[a*]	33.63[a]	137.54[a]	91.38[a]
Zn_1	50 mg	94.97[a]	64.10[a]	288.31[b]	155.75[b]
Zn_2	250 mg	256.29[b]	220.56[b]	999.77[c]	751.07[c]
Zn_3	750 mg	1300.75[c]	624.40[c]	2493.33[d]	1743.94[d]
LSD $_{0.01}$		*75.15*	*32.99*	*50.05*	*65.95*

* Homogenous groups according to Tukey test, $\alpha < 0{,}01$, ** mg · kg^{-1} d.m.

Soil pollution with zinc significantly affected increase in this metal content in aboveground biomass and roots (Table 1). The aerial biomass obtained on the light soil contained 2-fold (Zn_1), 5-fold (Zn_2) and 25-fold (Zn_3) higher amounts of zinc in comparison with the treatment without zinc supplement. On the other hand on the heavy soil the dependencies were 2 (Zn_1), 7 (Zn_2) and 19 (Zn_3). Irrespectively of the kind of soil, the underground biomass contained respectively 2-fold (Zn_1), 8-fold (Zn_2) and 19-fold (Zn_3) bigger amounts of this metal in comparison with the roots from the control. Higher zinc contents were assessed in the roots than in the aboveground parts. Maize roots contained between 2 and 4 times more of this element in the light soil and between 2 and 3 times more in the heavy soil in comparison with the aboveground biomass. Higher zinc concentrations in the analyzed maize parts, respectively from 16 to 108 % (aerial parts) and from 33 to 85 % (roots) were determined in the light than in the heavy soil. Zinc concentration index (C_i) was computed in the investigations (Fig. 1).

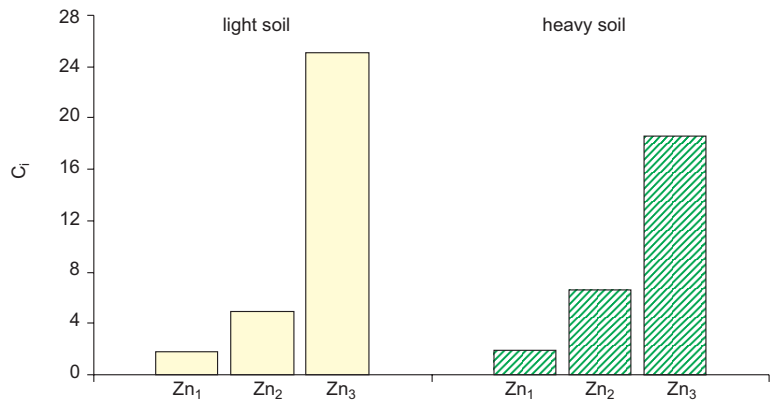

Fig. 1. Zinc concentration index (C_i) in maize

Concentration index (C_i) is an indicator of toxic elements bioaccumulation in plants [3, 10]. The parameter was calculated as a ratio of zinc content in a contaminated plant to this element content in the control plant. The value of zinc concentration index was diversified and fluctuated from 1.83 to 25.03 in the light soil and from 1.91 to 18.57 in heavy soil (Fig. 1). On treatments with Zn_1 (50 g) and Zn_2 (250 mg · kg^{-1} d.m.) the index assumed on average 14 % higher value on heavy soil than on the light one. Only on the treatment with 750 mgZn · kg^{-1} d.m. an opposite relationship was observed. Both on the light and heavy soil value of concentration index was increasing under the influence of increasing zinc doses. Growing C_i values evidence considerable zinc accumulation in maize, posing a serious hazard for animals and humans as potential plant consumers.

Bioaccumulation and translocation coefficients were computed (Table 2) to assess the extent and direction of zinc mobility in maize. Value of bioaccumulation coefficient reflected plant ability to absorb zinc from soil and informs about this metal translocation from the soil solution to the plant aboveground parts [11–14]. The index is a ratio of the

metal content in plant to its amount in soil. On the other hand, *translocation coefficient* (TC) was used to assess zinc mobility in maize [14]. This parameter was calculated as a ratio of zinc content in plant aboveground parts to its content in roots. Higher zinc accumulation in roots, in comparison with its aerial parts has been confirmed by low values of its translocation coefficients (TC) (Table 2). Moreover, with growing pollution of the light soil to 250 mgZn · kg^{-1} d.m., maize accumulated increasingly more zinc in roots whereas TC values were decreasing. On the other hand, on the treatment with 750 mgZn · kg^{-1} d.m. this metal to a greater extent moved from roots to the aboveground parts, as it has been evidenced by higher value of translocation coefficient. A different dependence was observed in the heavy soil, where the lowest dose of zinc (50 mgZn · kg^{-1} d.m.) caused a greater mobility of this metal from roots to the aerial parts in comparison with the control and treatments with higher level of zinc pollution (Table 2). At the doses of 250 and 750 mgZn · kg^{-1} d.m. of heavy soil, maize roots accumulated zinc to a greater extent than the aboveground parts, which has been confirmed by lower TC value in comparison with the control (Table 2).

Table 2

Translocation and bioaccumulation coefficient

Treatment		Translocation coefficient		Bioaccumulation coefficient			
		Aboveground biomas / Root		Root / Soil		Aboveground biomas / Soil	
		Light soil	Heavy soil	Light soil	Heavy soil	Light soil	Heavy soil
Zn_0	0 mg*	0.38	0.37	1.96	0.63	0.74	0.23
Zn_1	50 mg	0.33	0.41	2.05	0.86	0.68	0.35
Zn_2	250 mg	0.26	0.30	2.95	2.01	0.76	0.59
Zn_3	750 mg	0.52	0.35	3.37	2.10	1.76	0.75

* mg · kg^{-1} soil.

The analysis of bioaccumulation coefficient value revealed that increasing doses of zinc generally caused an increase in *bioaccumulation coefficient* (BC) value in relation to the treatment without zinc supplement (Table 2). Both in the light and heavy soil higher zinc content occurred in roots and smaller in the aboveground parts. Assessment of zinc bioaccumulation degree demonstrated its intensive accumulation in roots (BC 1–10) and medium (BC 0.1–1) in the aerial parts. Moreover, it was shown that in the light soil this parameter assumed from over 1 to 3-fold higher values in comparison with the heavy soil (Table 2). Obtained results evidence a better ability of zinc passing from the light soil to various maize parts. On the other hand, lower values of bioaccumulation coefficient (BC) on heavy soil testify a slightly poorer uptake of the analyzed element by plants in this soil. It was also corroborated by poorer zinc solubility in 1 mol HCl · dm^{-3} in heavy than in light soil, on average 57 % of its total content (in heavy soil) and 80 % (in light soil) (Fig. 2). In the light soil soluble zinc constituted between 66 and 98 %, whereas in the heavy soil between 45 and 75 % of its total content. Observed increase in zinc solubility in soils with increasing zinc dose

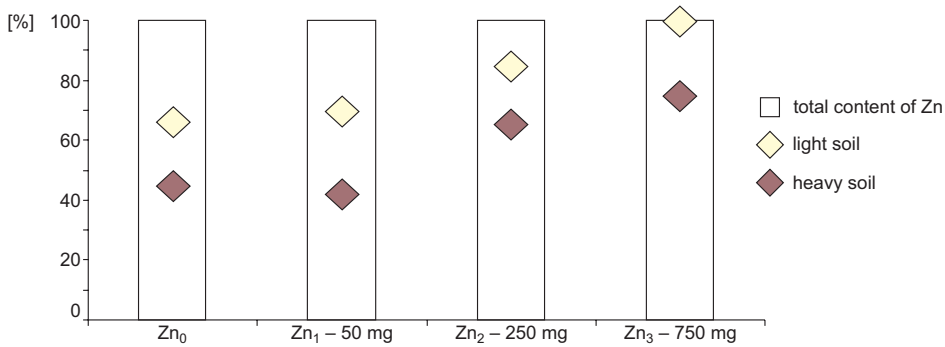

Fig. 2. The percentage soluble forms of zinc in the total content of this metal in the light and heavy soil

indicates a strict dependence between zinc content in maize and the content of its soluble forms in soil (Figs. 3 and 4), but also the fact stated by other authors that zinc uptake by plants in relation to its content in the soil solution is the dependence most approximate to the straight-line one, as compared with other metals [3]. Obtained results confirm high, significant values of the correlation coefficient between zinc content in maize and this metal concentrations in soils. In the light soil values of the correlation coefficient between zinc concentrations in the aboveground parts and roots and its content in soil were respectively 0.98 and 0.97 at $p < 0.01$, whereas in the heavy soil 0.87 and 0.84.

A very important factor at high zinc concentrations in soil is the chemical effect, *ie* zinc accumulation in the tissues of cultivated plant. In the Authors' own investigations the chemical effect was determined as an index of concentration informing about an increase in zinc content in plants in the light and heavy soil at growing doses of this metal in relation to the control. Moreover, it was demonstrated that zinc content in maize corresponded with the content of its soluble forms in soils and with its growing

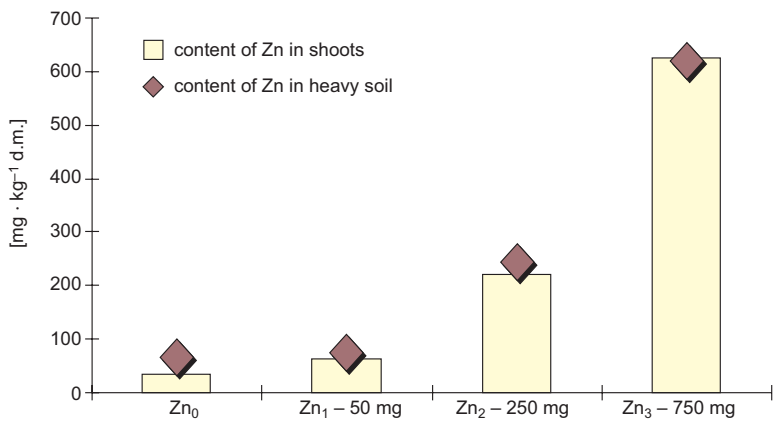

Fig. 3. Relation between content of zinc in shoots and content of zinc in heavy soil

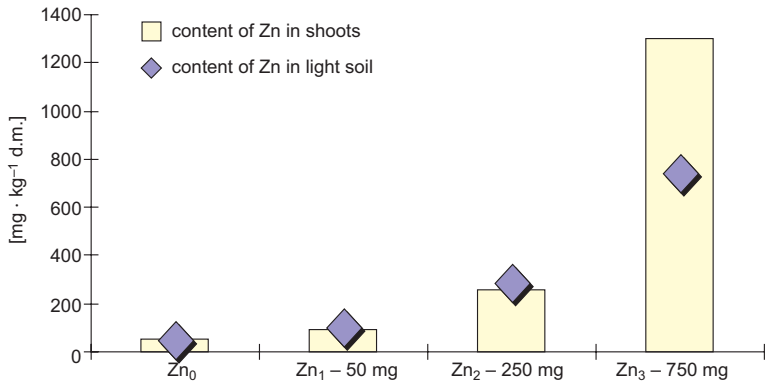

Fig. 4. Relation between content of zinc in shoots and content of zinc in light soil

concentrations in soil zinc accumulation in plants was increasing, too. The dependence has been confirmed also by high and significant correlation coefficients between zinc concentrations in maize and the content of its forms soluble in 1 mol HCl · dm^{-3} in soils. Research conducted by other authors [10, 15] showed similar dependencies by extracting zinc with 1 mol HCl · dm^{-3}. On the other hand, while analyzing the effect of soil kind on zinc content in maize it was found that its parts from light soil were characterized by higher concentrations of this element. Similar dependencies on light soils as compared with heavy soils were revealed in the research of Korzeniowska and Gembarzewski [16]. Investigations conducted by Spiak [3] demonstrated also that values of zinc bioaccumulation coefficients are growing with increasing zinc doses, whereas they diminish with growing soil heaviness, which testifies much smaller potential of zinc uptake from heavy soils than from medium or light ones. Results obtained in the presented experiment confirm this dependence because values of bioaccumulation coefficients were on average over twice higher in light than in heavy soil. Moreover, poorer zinc availability in the heavy than light soil was revealed. The content of zinc soluble in 1 mol HCl · dm^{-3} in heavy soil was on average 57 % of its total content and 80 % in the light soil. Smaller bioavailability of zinc in heavy soil has been also confirmed by lower values of linear correlation coefficients between zinc concentrations in this soil and this element content in the aerial parts and roots as compared with the light soil. Various authors differently present data concerning individual plants sensitivity to zinc stress connected with its excess in the substratum. Causes of diversified plant response to over the norm zinc contents in soils should be explained by different way of absorption and translocation of this element from roots to the aboveground parts. Dicotyledonous plants move big amounts of zinc from roots to the aboveground parts, whereas the unicotyledonous accumulate considerable quantities of zinc in roots [4, 5, 10, 17]. Moreover, greater sensitivity of the dicotyledonous (pea, sunflower, serradella, mustard or buckwheat) to zinc stress results from translocation of big amounts of zinc from roots to the aboveground parts already at the earliest development stages. Das et al [17] and Huicong et al [18] reported that plant ability to heavy metal accumulation is connected with morphological structure of roots. It was

found that plants with numerous and thin roots accumulate greater amounts of metals than plants with several thick roots [18]. Maize is characterized by a beam root system, so it may accumulate bigger quantities of heavy metals in roots. Beside specific differences concerning sensitivity to zinc, its distribution in the individual plant parts is diversified whereas the appropriate data are not unanimous. Numerous investigations revealed that zinc is primarily accumulated in roots [17, 20, 21]. Moreover, plants little sensitive to high zinc concentrations defend themselves against contamination by accumulating this element in large amounts in vacuoles of their root cells [22]. Other research demonstrated that zinc in plant aboveground parts cumulates almost in the same way as in their roots [23]. Kabata-Pendias [24] and Spiak et al [10] report that vegetative plant parts are characterized by higher zinc contents than generative ones. On the other hand, Terelak and Lipinski [25] revealed that zinc concentrations in cereal grain are twice higher than in the straw. Also Piotrowska et al [11] stated that young, physiologically active leaves contain considerably greater quantities of zinc in comparison with old leaves, which is associated with the process of this microelement reutilization. The author also demonstrated that at higher zinc concentrations in soil the excess of this metal is arrested primarily in old leaves and shoots, *ie* plant parts with lower physiological activity. In the presented experiment bigger amounts of zinc were assessed in roots than in the aerial parts. Maize roots in light and heavy soils contained on average 3 times bigger quantities of this metal than the aerial parts. Moreover, higher than in the aboveground parts concentrations of zinc in roots were evidenced by the value of zinc translocation coefficients. Considerable zinc contents in roots in comparison with maize aerial parts were also confirmed by higher values of bioaccumulation coefficients (BC) root/soil than BC aerial parts/soil.

Conclusions

1. Soil contamination with zinc significantly affected an increase in this metal contents in the aboveground and underground maize biomass. The aboveground biomass obtained on the light soil contained 2-fold (Zn_1), 5-fold (Zn_2) and 25-fold (Zn_3) bigger amounts of zinc in comparison with the treatment without zinc supplement. On the other hand, on the heavy soil the dependencies were respectively 2-fold (Zn_1), 7-fold (Zn_2) and 19-fold (Zn_3).

2. Bigger zinc content was assessed in roots than in the aerial parts and the dependence was confirmed by low values of the translocation coefficient (TC).

3. Greater phytoavailability and phytotoxicity of zinc was demonstrated in the light soil than in the heavy soil. The relationship was confirmed by a better zinc solubility determined by 1 mol HCl $\cdot$ dm^{-3} in the light soil but also by higher values of zinc bioaccumulation coefficients in this soil.

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OCENA ZAWARTOŚCI I MOBLINOŚCI CYNKU W KUKURYDZY

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Abstrakt: Celem badań była ocena zawartości i mobilności cynku w kukurydzy na glebach zanieczyszczonych tym pierwiastkiem. Jako wskaźniki tej oceny zastosowano: zawartość cynku w kukurydzy, indeks koncentracji cynku, współczynnik bioakumulacji i translokacji cynku. Dwuletnie doświadczenie wazonowe przeprowadzono równolegle na dwóch glebach glebie lekkiej i ciężkiej. W doświadczeniu zastosowano cztery poziomy cynku: Zn_0 – 0 mg (obiekt kontrolny), Zn_1 – 50 mg, Zn_2 – 250 mg, Zn_3 – 750 mg · kg^{-1} s.m. gleby. Zawartość cynku w częściach nadziemnych i korzeniach oznaczono po suchej mineralizacji i roztworzeniu popiołu w HNO_3 (1:3) metodą atomowej spektrometrii emisyjnej ze wzbudzeniem w indukcyjnie sprzężonej plazmie argonowej (ISP-AES). Zanieczyszczenie gleb cynkiem wpłynęło znacznie na zwiększenie zawartości tego metalu w biomasie nadziemnej i podziemnej kukurydzy. Uzyskana biomasa nadziemna na glebie lekkiej zawierała 2 (Zn_1), 5 (Zn_2) i 25 (Zn_3)-krotnie więcej cynku w porównaniu do obiektu bez dodatku cynku. Z kolei na glebie ciężkiej zależności te wyniosły odpowiednio 2 (Zn_1), 7 (Zn_2) i 19 (Zn_3). Większą zawartość cynku stwierdzono w korzeniach niż w częściach nadziemnych. Zależność tą potwierdzają niskie wartości współczynnika translokacji WT. Większą fitodostępność i fitotoksyczność cynku wykazano w warunkach gleby lekkiej niż ciężkiej. Zależność tą potwierdza większa rozpuszczalność cynku oznaczona 1 mol HCl · dm^{-3} w glebie lekkiej, a także wyższe wartości współczynników bioakumulacji cynku w warunkach tej gleby.

Słowa kluczowe: cynk, kukurydza, indeks koncentracji, współczynnik bioakumulacji i translokacji

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EVALUATION OF THE SOIL ENZYMES ACTIVITY AS AN INDICATOR OF THE IMPACT OF ANTHROPOGENIC POLLUTION ON THE NORWAY SPRUCE ECOSYSTEMS IN THE SILESIAN BESKID

OCENA AKTYWNOŚCI ENZYMÓW GLEBOWYCH JAKO WSKAŹNIKA WPŁYWU ZANIECZYSZCZEŃ ANTROPOGENNYCH NA FUNKCJONOWANIE EKOSYSTEMÓW LASÓW ŚWIERKOWYCH BESKIDU ŚLĄSKIEGO

Abstract: Activity of soil enzymes is considered as a good indicator of natural and anthropogenic disturbances of the functioning of the soil. Heavy metals can inhibit the activity of enzymes in varying degree, depending on soil properties such as content of clay materials, organic matter and pH of soil solution. The aim of this study was to determine the effect of physicochemical and biological properties of soils on the condition of Norway spruce stands in Silesian Beskid. In the soil samples enzymatic activity of four enzymes (alkaline and acid phosphatase, dehydrogenase, urease) and concentration of three selected heavy metals (Cd, Pb, Zn) and sulfur were determined. The analyses showed no reduced activity of investigated enzymes. Presumably, despite of low pH values of the soil, organic matter contained in the soil is able to effectively bind heavy metal ions, limiting their cycling in the environment. It can be concluded that the condition of spruce stands in Silesian Beskid is not affected by the soil contamination.

Keywords: heavy metals, soil enzymes, Norway spruce, Silesian Beskid

Introduction

Biological processes influencing soil fertility in terrestrial ecosystems are mainly based on the transformation of organic matter. Mostly they are associated with microbes and enzymes secreted by them, and the pace of their respective biogeochemical changes

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in the elements circulation [1]. Evaluation of the quality and the productivity of the soil is an important part in the study of the natural environment. In temperate forest ecosystems, dominated by ectomycorrhizal trees, acid soils accumulating large quantities of organic molecules are predominant. Forest soil fertility and productivity of the forest ecosystems depend on the activity of biochemical processes in the soils, which are catalyzed by enzymes secreted into the soil environment mainly by soil microorganisms [2–4]. Enzymes such as urease usually serve as an index of the soil fertility because their activity correlates with the organic matter content in soil, alkaline and acid phosphatase, which are closely associated with respiratory and biomass of soil organisms, and the dehydrogenase which is an excellent indicator of respiratory activity of soil microorganisms [4, 5]. The activity of soil enzymes is considered as a good bioindicator which reflects the natural and anthropogenic disturbance of the soil [6, 7]. Soil enzymes are inhibited by heavy metals to varying degrees, depending on properties of the soil, such as the contents of clay materials and organic matter, or pH value of a soil solution [8–10]. Yang et al [11, 12] reported that reducing the number of microorganisms in the soil and the inhibition of enzyme action as a result of soil pollution with heavy metals affects the soil fertility.

Forests of the Silesian Beskid Mountains are within the range of pollution impact coming from the north side from the Upper Silesian Industrial District, on the west side from the agglomeration of Ostrava (CZ), and from nearby Bielsko-Biala [13]. Other factors affecting the Silesian Beskid forest are inadequate forestry, worsening climate conditions and the gradation of insects attacking the spruces. As a result of the above-mentioned factors, there is a reduction in vitality of a Norway spruce [14, 15].

The object of the studies on the soil enzymes activity and the contamination of soil with heavy metals and sulphur was to determine the soil condition of Silesian Beskid, and estimate the possible impact of these factors on the weakening of the spruce stands in these mountains.

Material and methods

The material for the analysis was being collected from September to October 2007. Six mountain peaks of the Silesian Beskid have been investigated: Blatnia, Klimczok, Skrzyczne, Soszow, Stozek, Szyndzielnia. Soil samples for the analysis were taken from following depths: 0–10 cm, 10–20 cm and 20–30 cm [16], from five selected points in the entire area of the peak. Soil samples were mixed for each depth and each mount separately.

Soil was sifted through a sieve with a diameter of 1 mm and dried to a constant weight, upon which 10 g subsamples from each depth and each uphill were prepared. Soil subsamples were inserted into 100 cm^3 of 10 % nitric acid(V) and shaken for one hour. After that the subsamples was filtered. Concentration of three heavy metals (contamination fraction), zinc, cadmium and lead were determined on an atomic absorption spectrometer [17]. Bioavailable fraction of heavy metals in soil samples was determined with similar depth, which were first triturated in a mortar and sieved

through a sieve with a diameter of 0.25 mm. Samples were inserted in 50 cm^3 of 0.01 M $CaCl_2$ and shaken for 5 hours, then filtered.

Total sulphur content was determined nephelometrically, according to the method proposed by Ostrowska [17].

Soil pH was determined in H_2O, at a substrate to water ratio of 1:2.5. The measurements were performed by potentiometry method using a SEN 81st TIX electrode.

The content of organic matter in soil was determined by gravimetric method of weight loss during the annealing of the soil sample in a muffle furnace at 550 ^oC [17].

Activity of soil enzymes were carried out in accordance with the methodology proposed by [18]. The activity of acid and alkaline phosphatase were tested by the colorimetric method, where the activity is measured in μg of *p*-nitrophenol per 1 g fresh weight soil. Dehydrogenase activity was determined by colorimetric method, using the ability of this enzyme to transfer electrons to a synthetic acceptor, *triphenyltetrazolium chloride* (TTC) which in the oxidized form is almost colorless, but in the reduced form gives colored compound *triphenylformazan* (TPF). The activity was measured in μg of TPF on 1 g fresh weight soil. Urease activity was tested by colorimetric assay based on ammonia formed after the enzymatic hydrolysis of urea, activity is expressed in μg of N per 1 g fresh weight soil.

Statistical analysis

The results of soil chemical data and enzymes activities were tested for normal distribution (Shapiro-Wilk test) prior to statistical analysis. Statistical comparisons of the six sites were made using Tukey test. Correlation were calculated by Pearson's correlation coefficient.

Results

The amount of organic matter in the outer layer of the soil from the analyzed mountains ranged from 14.1 % in Skrzyczne to 26.6 % in Blatnia. Vertical arrangement of the organic matter content in soil indicated a high concentration of organic matter in the upper layers (0–10 cm) of the tested surface, which was particularly evident on Blatnia (41.2 %) and Szyndzielnia (40.4 %) (Table 1).

Analysis of the studied soils acidity indicated that the pH was acidic. Lowest soil pH value was noted on Klimczok (3.77), while the highest was observed on the Skrzyczne peak (4.22). In all studied sites pH increase with the lowering of soil was observed (Table 1).

The concentration of sulphur in the outer layer of the soil from the analyzed mountains ranged from 108.8 $\mu g \cdot g^{-1}$ on Skrzyczne to 176.2 $\mu g \cdot g^{-1}$ on Blatnia. Soils gathered at Skrzyczne and Stozek had greater accumulation of sulphur in the 20–30 cm level compared with level 10–20 cm (Table 1).

Vertical arrangement of heavy metals distribution indicated their accumulation in the upper layers of all studied surface. The highest concentrations of zinc, cadmium and

Table 1

List of results of investigated elements, organic matter an pH values of soil from 6 mountain peaks in Silesian Beskid

	Depth [cm]	Contamination fraction [µg/g d.m.]			Bioavailable fraction [µg/g d.m.]			S [µg/g d.m.]	Organic matter [%]	pH
		Zn	Cd	Pb	Zn	Cd	Pb			
Blatnia	0–10	51.440 ± 3.440	1.055 ± 0.009	430.500 ± 12.000	12.680 ± 0.190	0.515 ± 0.044	6.096 ± 0.749	249.150 ± 19.133	41.222 ± 0.397	3.645
	10–20	27.160 ± 2.590	0.731 ± 0.107	250.750 ± 16.950	7.382 ± 0.316	0.430 ± 0.002	3.565 ± 0.418	145.833 ± 30.612	21.826 ± 0.140	4.055
	20–30	18.28 ± 0.950	0.584 ± 0.033	155.700 ± 0.400	6.074 ± 0.104	0.369 ± 0.048	3.370 ± 0.088	133.503 ± 7.653	16.752 ± 0.245	4.200
Klimczok	0–10	22.190 ± 0.070	0.557 ± 0.054	283.700 ± 11.800	7.087 ± 0.029	0.347 ± 0.019	3.647 ± 0.090	196.003 ± 8.503	30.230 ± 0.993	3.535
	10–20	9.034 ± 0.179	0.243 ± 0.071	95.025 ± 0.665	3.813 ± 0.003	0.208 ± 0.004	0.798 ± 0.093	98.427 ± 9.141	11.523 ± 1.063	3.830
	20–30	9.130 ± 0.031	0.269 ± 0.043	58.880 ± 2.000	3.424 ± 0.067	0.221 ± 0.015	1.976 ± 1.502	87.372 ± 8.291	10.158 ± 0.592	3.940
Skrzyczne	0–10	30.075 ± 1.365	1.000 ± 0.001	223.000 ± 7.500	7.239 ± 0.082	0.430 ± 0.032	4.296 ± 0.404	166.029 ± 1.488	23.972 ± 0.192	4.015
	10–20	18.955 ± 0.725	0.767 ± 0.004	44.250 ± 0.180	4.385 ± 0.045	0.338 ± 0.004	1.339 ± 0.996	70.578 ± 2.551	9.207 ± 0.362	4.120
	20–30	16.025 ± 2.705	0.754 ± 0.054	47.890 ± 3.910	3.837 ± 0.095	0.373 ± 0.032	2.806 ± 2.197	89.923 ± 6.165	9.234 ± 0.386	4.525
Soszow	0–10	27.300 ± 2.120	0.543 ± 0.001	213.350 ± 0.350	7.357 ± 0.189	0.371 ± 0.039	3.896 ± 0.841	183.673 ± 14.456	24.277 ± 0.293	3.825
	10–20	23.495 ± 1.145	0.637 ± 0.029	119.700 ± 4.600	6.057 ± 0.063	0.354 ± 0.025	1.043 ± 0.223	153.486 ± 5.527	18.750 ± 0.590	4.060
	20–30	23.950 ± 0.830	0.739 ± 0.049	82.205 ± 3.445	5.358 ± 0.194	0.372 ± 0.014	2.975 ± 2.526	111.182 ± 4.464	15.454 ± 0.454	4.215
Stozek	0–10	24.800 ± 0.080	0.576 ± 0.001	187.200 ± 0.000	10.234 ± 1.047	0.389 ± 0.015	7.776 ± 0.718	166.241 ± 9.779	26.984 ± 0.993	3.780
	10–20	15.125 ± 1.215	0.274 ± 0.035	82.130 ± 2.140	5.806 ± 0.717	0.252 ± 0.004	2.591 ± 0.088	106.080 ± 11.692	14.843 ± 0.757	4.140
	20–30	13.615 ± 0.215	0.247 ± 0.021	67.295 ± 1.185	3.870 ± 0.437	0.232 ± 0.004	1.017 ± 0.132	111.607 ± 19.345	13.919 ± 0.888	4.205
Szyndzielnia	0–10	52.830 ± 10.200	1.241 ± 0.007	502.400 ± 24.900	13.055 ± 0.035	0.544 ± 0.026	2.323 ± 0.284	266.582 ± 8.078	40.444 ± 0.428	3.650
	10–20	24.280 ± 0.700	0.755 ± 0.111	210.450 ± 9.150	6.734 ± 0.293	0.365 ± 0.019	0.623 ± 0.597	153.699 ± 35.077	19.132 ± 1.196	3.895
	20–30	17.035 ± 0.635	0.667 ± 0.019	137.150 ± 0.650	5.935 ± 0.689	0.392 ± 0.011	0.486 ± 0.391	84.184 ± 17.857	15.153 ± 0.437	4.080

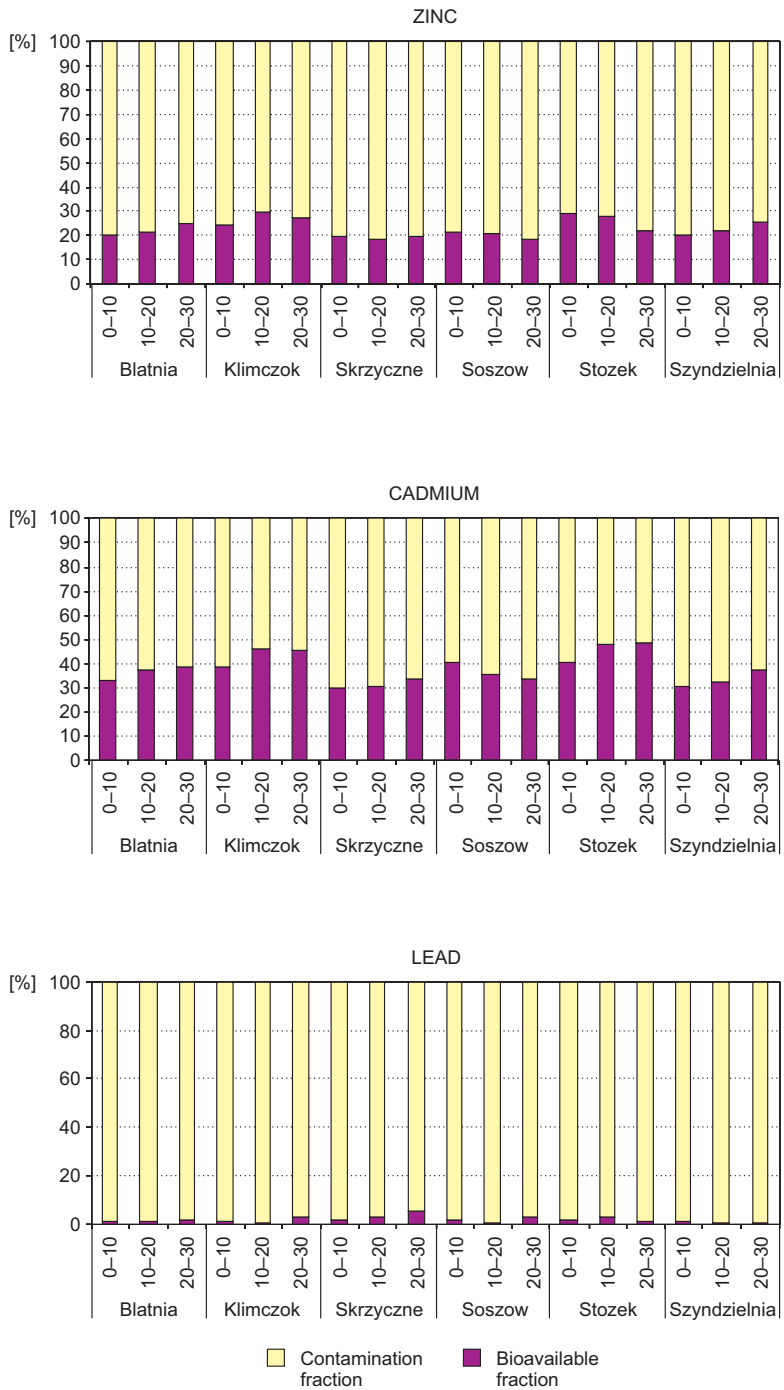

Fig. 1. Percentage of bioavailable fraction in contamination fraction of Zn, Cd and Pb

lead in the contamination fraction were noted in 0–10 cm levels on Szyndzielnia, accordingly: 52.83 μg · g^{-1}, 1.24 μg · g^{-1} and 502.40 μg · g^{-1}. Also content of investigated heavy metals in the bioavailable fraction decreased with the increasing soil depth. The highest concentration of zinc (13.05 μg · g^{-1}), cadmium (0.54 μg · g^{-1}) and lead (6.82 μg · g^{-1}) in the bioavailable fraction were recorded in Szyndzielnia (the 0–10 cm level). However, the highest average concentrations of these elements in bioavailable fraction were observed on Blatnia, for zinc it was 9.04 μg · g^{-1}, for cadmium 0.45 μg · g^{-1} and for lead 4.34 μg · g^{-1} (Table 1). Percentage of bioavailable fraction at in contamination fraction of Zn, Cd and Pb is given on Fig. 1.

The highest alkaline phosphatase activity was observed on the level of 0–10 cm on Blatnia where the concentration of *p*-nitrophenol was 309.9 μg · g^{-1}. However, the highest average activity was recorded on Skrzyczne (200.4 μg · g^{-1}). In soils gathered at Klimczok, Soszow and Skrzyczne *p*-nitrophenol concentration was higher in the 20–30 cm and was respectively: 96.6, 221.6 and 166.3 μg · g^{-1}, comparing with the concentration in the 10–20 cm level (92.2, 135.4 and 153.8 μg · g^{-1}). The highest acid phosphatase activity was determined in the level 0–10 cm from Soszow (1658.9 μg · g^{-1}), the highest average concentration of the secreted *p*-nitrophenol was

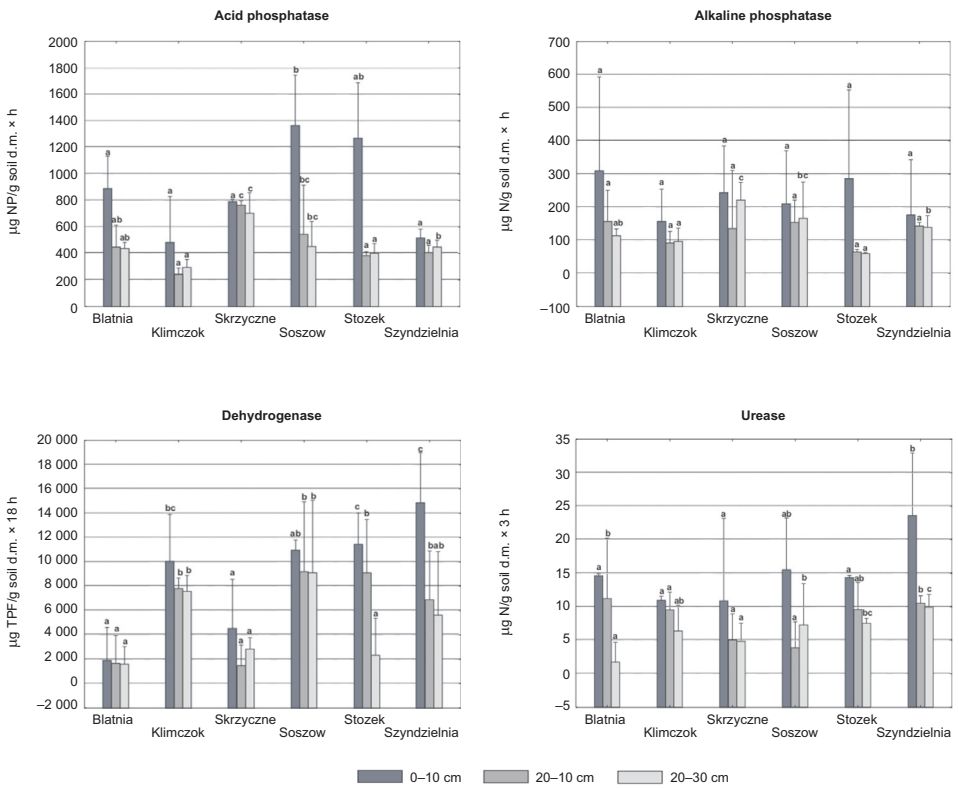

Fig. 2. Activity of investigated soil enzymes. $p < 0.05$, ANOVA Tukey test – homogenous groups marked with the same letters

Table 2

The correlation coefficient between soil enzymes and bioavailable Zn, Cd, Pb and Sulphur in soil.
Results marked by * are significant at the $p = 0.05$ level

	Alkaline phosphatase			Acid phosphatase			Dehydrogenase			Urease		
	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm
Zn bioavailable	0.24	0.30	0.08	–0.23	–0.01	0.03	0.24	–0.01	–0.13	0.59*	0.30	–0.04
Cd bioavailable	0.29	0.62*	0.65*	–0.37	0.41	0.60*	0.07	–0.40	–0.18	0.60*	0.01	–0.01
Pb bioavailable	0.38	0.12	0.35	0.40	0.02	0.27	–0.23	–0.28	–0.05	–0.28	0.23	–0.29
Sulphur	–0.10	0.41	–0.24	–0.47*	–0.27	–0.09	0.17	0.35	–0.40	0.63*	0.35	–0.38

determined also on Soszow (885.5 $\mu g \cdot g^{-1}$). In soils derived from Szyndzielnia and Klimczok *p*-nitrophenol concentration was higher in the 20–30 cm level and was respectively 295.5 and 448.4 $\mu g \cdot g^{-1}$ in relation to the concentration of the substance in level 10–20 cm where it was 241.7 and 405.7 $\mu g \cdot g^{-1}$. The interesting fact was that there were small differences in acid phosphatase activity.

Dehydrogenase activity was measured by the content of TPF produced within 16 hours of incubation. At all the sites except for Blatnia the activity of this enzyme was very high. A very high concentration of TPF was characteristic for level 0–10 cm from Szyndzielnia (14872.0 $\mu g \cdot g^{-1}$), where the average number of TPF produced was also the highest (9135.0 $\mu g \cdot g^{-1}$). Soils from Skrzyczne and Klimczok were characterized by greater activity of dehydrogenase in the level of 20–30 cm compared with level 10–20 cm.

Urease activity was measured by the content of nitrogen produced in 3 hours. The highest enzyme activity was determined on Szyndzielnia (23.6 $\mu gN \cdot g^{-1}$), where the average concentration of the produced nitrogen was also the highest (14.7 $\mu g \cdot g^{-1}$). A higher level of urease activity was observed in level 20–30 cm (7.28 $\mu g \cdot g^{-1}$) compared with level 10–20 cm (3.9 $\mu g \cdot g^{-1}$) for the Soszow soils. Results for all four enzymes activity are given in Fig. 2.

Discussion

At all the examined surfaces an accumulation of heavy metals and sulfur in the outermost layer of the soil has been stated, which gived evidence to the anthropogenic origin of these elements. The similarity in relation to the many of tested parameters showed for Szyndzielnia and Blatnia to be the most correspondent, especially in content of metal and sulphur. Higher concentrations of sulphur and heavy metals was linked to the north-west winds that bring pollution from above Bielsko-Biala. Similarity was equally often disclosed by Soszow and Stozek because of their close positions.

Heavy metals and sulphur accumulated in soils, not only modify their properties, but also severely affect the soil microorganisms and change the soil enzymatic activity. Processes such as nitrification and the pace of the organic matter decomposition are undergoing distinct inhibition [19–20].

The main factors controlling the mobility and availability of heavy metals in soil are pH and organic matter content [21, 22]. Soil pH plays a significant role in the occurrence of soluble and bioavailable forms of zinc and lead. However, according to Keller and Hammer [23] and Pueyo et al [24] the contents of bioavailable forms of cadmium in the soil is less dependent on pH. The acidity increase of the soil environment occurs among others due to the deposition of sulphur compounds [25]. At the studied localities the sulphur content was relatively low. However, statistically significant negative correlation between sulphur content in the soil and soil pH in the surface layers was found. In the layers 10–20 cm and 20–30 cm the value of the correlation was statistically insignificant. The observed dependencies indicate the influence of precipitation and accumulation of sulphur compounds on pH decrease. The factor which largely determines the enzymatic activity of the soils, is their content of

organic matter. Soil organic matter has a large absorptive surface, and many functional groups (carboxyl, thiol and phenolic) that are capable of efficient binding of heavy metals in the form of complexes [26]. The binding of various metals is different. The strongest relation can be observed between the soil organic matter and the Pb, but also strong for Cd and Zn [27]. In the investigated soils the concentration of lead in bioavailable fraction was minimal, which was indicating strong binding by organic matter. A smaller but still significant was the degree of binding of cadmium and zinc. The results also showed that despite low soil pH, the organic substance contained in the soil was able to bind heavy metals fairly effectively.

In soils contaminated by heavy metals reduction in activity of phosphatases was observed, which was confirmed by studies in the forest soil in the vicinity of the aluminum smelter and soils treated with heavy metals [4, 28, 29]. In the soils from the test sites a high activity of the acid phosphatase was reported as far as the acid phosphatase was concerned an enzyme that is associated with the amount of bacteria and fungal biomass in soil [30]. The maximum concentration of *p*-nitrophenol in this case was 1658.9 $\mu g \cdot g^{-1} \cdot h^{-1}$ in soil from Soszow. Alkaline phospahate is another enzyme which takes an active part in the decomposition of organic debris. Nowak et al [28] found decreased activity of this enzyme in soils contaminated with zinc and cadmium. In the investigated site concentration of *p*-nitrophenol in the case of alkaline phosphatase ranged from 115.3 $\mu g \cdot g^{-1} \cdot h^{-1}$ on Klimczok to 200.4 $\mu g \cdot g^{-1} \cdot h^{-1}$ on Skrzyczne. Statistical analysis did not show a negative correlation between the concentration of metals in the bioavailable fraction and the activity of both phosphatases in the 0–10 cm layer, while in the 10–20 cm layer there was a positive correlation observed for cadmium content and the activity of alkaline phosphatase. In addition, in the 20–30 cm layer a positive correlation between the concentration of cadmium and acid phosphatase activity was reported. These results suggest that soil pH for these enzymes is crucial. High activity of acid phosphatase is due to low soils pH while alkaline phosphatase shows greater activity in alkaline soils [5]. In addition, Acosta-Martinez and Tabatabai [31] postulated that the cadmium content in soil has a greater effect on the activity of alkaline phosphatase than acid phosphatase.

Total dehydrogenase activity is an indicator of the redox system and a measure of respiratory activity of microorganisms. Dehydrogenase are active only within living organisms, and after a cell death their degradation follows quickly. Therefore, the dehydrogenase activity indicates the presence of physiologically active microorganisms [3, 32]. Heavy metals have inhibitory effect on dehydrogenase activity [33–35]. Olszowska [34] found a negative correlation between dehydrogenase activity and the content of Zn, Cd and Pb in soils of pine stands located in the vicinity of the impact of lead and zinc smelter. Kieliszewska-Rokicka [4] obtained a similar result in soils from nearby aluminum smelters. In the investigated soils, dehydrogenase activity was high, and there was a lack of negative correlation between enzyme activity and the content of heavy metals in the bioavailable fraction and sulphur in each of the three examined levels.

The activity of urease, which catalyzes the hydrolysis of urea to ammonia and CO_2, is related to the pace of change in soil nitrogen. This enzyme is accumulated in the soil

in the form of complexes with organic matter and humus [36]. Nadgorska-Socha et al [37] found urease activity measured by the amount of the produced nitrogen to be around 75 $\mu gN \cdot g^{-1}$ in the soil located under the direct influence of heavy metal emitter. In the investigated soils, the urease activity was expressed as a concentration of secreted nitrogen which was at the level of 6.9 $\mu gN \cdot g^{-1}$ on Skrzyczne to 14.7 $\mu gN \cdot g^{-1}$ on Szyndzielnia. A significant decrease in the activity of this enzyme was not related to the concentration of heavy metals, as evidenced by the positive correlation between the content of bioavailable zinc and cadmium in 0–10 cm layer and lack of correlation in the other layers. Low activity of urease may be due to the sensitivity of this enzyme in the acidic soils.

The obtained results showed that low concentrations of heavy metals in the soils did not affect the activity of soil enzymes significantly. Similar conclusions were reached by Dar [38], who after adding cadmium concentration of 10 $\mu g \cdot g^{-1}$ to the soil did not report any significant changes in the activity of soil enzymes. Furthermore, it was proved that small concentrations of lead have stimulating effect on soil enzymes [39]. Also, Shah and Dubey [40] noted the increase in protease activity in the soil after adding sediment containing small amounts of cadmium (50–100 μM).

Conclusions

The studies of the soils from the site of Silesian Beskid showed no impact of anthropogenic contaminants (heavy metals and sulfur) on the activity of soil enzymes. High enzyme activity demonstrates the viability of soil microorganisms, proper circulation of biogenic elements such as phosphorus and nitrogen. Therefore, it can be concluded that the causes of Beskid spruce extinction have different backgrounds.

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Abstrakt: Aktywność enzymów glebowych uważana jest za dobry wskaźnik naturalnych i antropogennych zaburzeń w funkcjonowaniu gleby. Metale ciężkie w różnym stopniu mogą hamować działanie enzymów, w zależności od właściwości gleb, takich jak zawartość materiałów ilastych, gliny, materii organicznej czy wartości pH roztworu glebowego. Celem pracy było określenie wpływu właściwości fizykochemicznych i biologicznych gleb na kondycję drzewostanów świerkowych w Beskidzie Śląskim. Próbki glebowe zbadano pod względem aktywności enzymatycznej (fosfataza kwaśna i zasadowa, dehydrogenaza, ureaza) oraz koncentracji trzech wybranych metali ciężkich (Cd, Pb, Zn) i siarki. Analizy nie wykazały obniżonej aktywności badanych enzymów. Prawdopodobnie mimo niskich wartości pH gleby, zawarta w niej materia organiczna efektywnie wiąże metale ciężkie, ograniczając ich obieg w środowisku. Można stwierdzić, że stan drzewostanów świerkowych w Beskidzie Śląskim nie ma związku z zanieczyszczeniem gleb na tym terenie.

Słowa kluczowe: metale ciężkie, enzymy glebowe, świerk pospolity, Beskid Śląski

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SEASONAL CHANGES OF SELENIUM AND SELECTED OXIDOREDUCTASES IN SOIL UNDER DIFFERENT FERTILIZATION AND CROP ROTATION

SEZONOWE ZMIANY ZAWARTOŚCI SELENU I AKTYWNOŚCI WYBRANYCH OKSYDOREDUKTAZ W GLEBIE O ZRÓŻNICOWANYM NAWOŻENIU I ZMIANOWANIU

Abstract: The objective of the study was to evaluate effects of different doses of FYM and nitrogen on the total selenium content in soil from different crop rotation systems. The aim of the study was to determine the changes of some oxidoreductases activity and Se concentration in soil in relation to applied doses of fertilizers over vegetation period. The experiment was carried out with the crop rotation systems – depleting and enriching in organic matter. The soil was fertilized with manure under potato in the doses of 0, 20, 40, 60 and 80 Mg/ha and with nitrogen in the doses of 0, 40, 80 and 120 kgN · ha^{-1} under winter wheat. The content of total selenium in the investigated soil was in the range of 0.092 to 0.264 mg · kg^{-1}. From the comparison of the results reported in literature one can observe that the studied soil was poor in selenium. Over the investigated period manuring resulted in an increase of total selenium content in soil and for that reason the FYM application can be recommended as a source of selenium in Se-deficient soils. Fertilization with manure resulted in an increase of dehydrogenases and catalase activities in soil with increasing doses of FYM. The selenium content, as well as DHA and CAT activities demonstrated clear seasonal variations. The present studies indicated a significant relationship between activity of soil enzymes, and the organic matter content, affecting the selenium status in soil and plants.

Keywords: selenium, oxidoreductases, soil, farmyard manure, nitrogen

Selenium is an essential trace element for human and animal metabolism. Its antioxidative properties, comparable to vitamin E, are widely known [1]. However, when it is absorbed in higher concentration, it can be harmful and catalyse the oxidation of thiols and simultaneously generate superoxide [2, 3]. Many authors [4–6] have indicated a strong influence of Se on the activities of oxidoreductase enzymes, such as

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catalase, glutathione peroxidase and superoxide dismutase. The literature provides abundant information on the role Se plays in animals. However, there have been relatively few reports on the contribution of Se to biochemical processes in soil and plants [3]. According to Wyszkowska and Wyszkowski [7] soil enzymes can serve as a tool to determine biochemical soil properties by taking part and playing an important role in chemical changes of carbon, nitrogen, phosphorus and sulphur compounds. For this purpose, activity of dehydrogenases is most commonly assayed, as it is usually positively correlated with the volume of yields, which in turn may indicate, however indirectly, that the activity of those enzymes is related to soil fertility. Dehydrogenases are enzymes which catalyse the removal of hydrogen atom from different metabolites [8]. Active dehydrogenases are considered to exist in the soil as an integral part of intact cells. They conduct a board range of oxidative activities that are responsible for degradation of soil organic matter [9]. Soil dehydrogenase activity can reflect changes in the respiratory activity of a given population size in response to changes in the soil environment. Catalase is an iron porphyrin enzyme which catalyses very rapid decomposition of hydrogen peroxide to water and oxygen [8]. The enzyme is widely present in nature, which accounts for its diverse activities in soil. Catalase activity alongside with the dehydrogenase activity is used to give information on the microbial activities in soil. The objective of the study was to evaluate effects of different doses of FYM (*Farmyard Manure*) and nitrogen on the total selenium content in soil from different crop rotation systems. The aim of the study was to determine the changes of some oxidoreductases activity and Se concentration in soil in relation to applied doses of fertilizers over vegetation period.

Materials and methods

Soil samples were collected from the long-term static experiment established at the Agricultural Experimental Station at Grabow carried out since 1980 by the Department of Plant Nutrition of the Institute of Soil Science and Cultivation in Pulawy. The experiment was conducted applying the following crop rotation systems: "depleting in organic matter" (potato – winter wheat – spring barley – maize) and "enriching in organic matter" (potato – winter wheat + intercrop – spring barley + undersown and red clover + grasses) (factor I), designed in a split-plot with four replications (sub-plots). Organic fertilizer in a form of cattle manure (FYM) was applied under potato in the doses of 0, 20, 40, 60 and 80 Mg · ha^{-1} (factor II) and nitrogen at the doses of 0, 40, 80 and 120 kgN · ha^{-1} was used under winter wheat and spring barley and 0, 30, 60, 90 kgN · ha^{-1} under potato and maize (factor III). Soil samples were collected in the 22nd year of the experiment, in March, May and July 2002, from the 0–20 cm layer under winter wheat. Soil samples were air-dried and sieved through a 2 mm screen. The total selenium content was determined by the method of Watkinson [10] using a Hitachi F-2000 spectrofluorometer. Soil samples were microwave digested with concentrated nitric(V) and perchloric(VII) acids. The different forms of selenium in the samples were reduced by boiling with 10 % HCl. The selenium was complexed with 2,3-*diamino-naphtalene* (DAN) to the fluorescent compound, which was extracted with cyclohexane

and read on the spectrofluorometer at excitation and emission wavelengths of $\lambda = 376$ and 519 nm, respectively. The analytical procedures provided satisfactory values for the standard reference material CRM024-050 from the *Resource Technology Corporation* (RTC); determined value was 0.558 mg Se · kg^{-1} (certified value – 0.540 mg · kg^{-1}). The certified reference material was included in each batch of samples for quality control. *Dehydrogenases activity* (DHA) was assayed applying the method by Casida et al [11]. Soil DHA activity was estimated by reducing 2,3,5-*triphenyltetrazolium* chloride. Soil sample was mixed with $CaCO_3$ and 2,3,5-triphenyltetrazolium chloride (TTC) and incubated for 24 h at 37 ^oC. Dehydrogenase converts TTC to 2,3,5-*triphenyl-formazan* (TPF). The TPF formed was extracted with acetone, the extracts were filtered and absorption was measured at $\lambda = 485$ nm spectrophotometrically. The enzyme activities were expressed as mg *triphenyl tetrazolium formazan* (TPF) · g^{-1} · 24 h^{-1}. *Catalase activity* (CAT) was measured using the method by Johnson and Temple [12]. Soil was incubated with *hydrogen peroxide* H_2O_2 for 20 min at 20 ^oC. The remaining H_2O_2, not broken-down by catalase, was treated with potassium permanganate exposed to H_2SO_4. To eliminate a probable overestimation of enzyme activity due to chemical reduction of H_2O_2 added, a correction for autoclaved soil (0.1 MPa, 120 ^oC, 30 min) was made. The results were expressed in mg H_2O_2 consumed · g^{-1} · min^{-1}. The soil samples were analysed for granulometric composition according to *Bouyoucos-Casagrande* method, organic carbon by wet oxidation with potassium dichromate, total nitrogen following by Kjeldahl method and pH in distilled water and 1 M KCl potentiometrically.

Three-way analysis of variance (ANOVA) was used to identify significant differences ($p < 0.05$) between Se concentrations and enzymes activity in soil under study. Data analysis was carried out using Statistica 8.0 for Windows Stat.Soft. Inc.

Results and discussion

The general properties and total selenium content of the soil under study are given in Table 1. The soil, according to the FAO classification, was classified as Haplic Luvisols and demonstrated the texture of loamy sand and sandy loam; pH values measured in H_2O of soil were in the acidic and slightly acidic range 5.2–6.9. The application of manure resulted in the highest contents of organic carbon and total nitrogen in soil, especially from the plots treated with FYM at the doses of 60 Mg · ha^{-1} and 80 Mg · ha^{-1}. Total selenium content in the soil samples ranged from 0.092 mg · kg^{-1} to 0.264 mg · kg^{-1} (Table 2). Such low levels of selenium in soils indicated that plants growing on these soils are deficient in this microelement. According to Kabata-Pendias [1], the mean total selenium content in the soils worldwide is estimated as 0.44 mg · kg^{-1}, while its background contents in various soil groups range from 0.05 mg · kg^{-1} to 1.5 mg · kg^{-1}. Over the investigated period the selenium content increased with increasing doses of FYM, but nitrogen treatment affected the content of this microelement in the soil in unclear way. In soil sampled in May total selenium content increased with increasing doses of nitrogen, but in July nitrogen fertilization decreased Se concentration in soil. Analysis of variance indicated that in May and July the Se content in soil was higher from plots, where crop rotation enriching in organic matter

Table 1

General properties of soil under study

FYM doses [Mg · ha^{-1}] (factor II)	N doses [kg · ha^{-1}] (factor III)	Crop rotation (factor I)											
		A "depleting in organic matter"						B "enriching in organic matter"					
		Soil particle size fraction [%]		pH in		C_{org} [g · kg^{-1}]	N_{tot} [g · kg^{-1}]	Soil particle size fraction [%]		pH in		C_{org} [g · kg^{-1}]	N_{tot} [g · kg^{-1}]
		< 0.02 [mm]	< 0.002 [mm]	H_2O	KCl			< 0.02 [mm]	< 0.002 [mm]	H_2O	KCl		
0	0	15	5	6.8	5.7	7.62	0.826	18	6	5.8	5.1	7.87	0.889
	40	16	5	6.8	5.8	7.82	0.837	17	6	5.2	5.1	8.36	0.966
	80	14	4	6.8	5.8	8.47	0.854	19	8	6.1	5.1	8.44	0.924
	120	15	5	6.7	5.7	7.41	0.861	17	7	5.9	4.8	8.27	0.966
20	0	17	6	6.8	5.8	7.53	0.823	14	5	6.3	5.3	8.84	0.980
	40	15	4	6.5	5.7	7.30	0.893	16	8	6.4	5.2	8.52	0.956
	80	13	2	6.5	5.6	8.54	0.861	15	6	6.3	5.2	8.70	0.956
	120	12	4	6.5	5.4	8.49	0.875	18	7	6.2	5.1	9.30	0.956
40	0	13	5	6.3	5.6	8.40	0.896	19	8	6.3	5.1	9.49	0.991
	40	15	4	6.6	5.5	9.04	0.931	17	6	6.3	5.2	9.25	1.012
	80	16	4	6.6	5.6	8.13	0.886	13	5	6.2	5.1	10.44	0.942
	120	16	4	6.6	5.5	8.18	0.935	19	6	6.0	4.9	10.26	0.987
60	0	20	5	6.7	6.0	8.65	0.952	16	5	6.4	5.4	10.36	0.931
	40	16	7	6.4	5.8	8.19	0.952	16	6	6.5	5.4	10.19	0.970
	80	16	6	6.7	5.8	8.29	0.977	19	7	6.4	5.3	10.10	0.956
	120	16	6	6.7	5.9	8.39	0.991	20	6	6.4	5.3	9.76	0.921

Table 1 contd.

FYM doses [Mg · ha^{-1}] (factor II)	N doses [kg · ha^{-1}] (factor III)	Crop rotation (factor I)											
		A "depleting in organic matter"						B "enriching in organic matter"					
		Soil particle size fraction [%]		pH in		C_{org} [g · kg^{-1}]	N_{tot} [g · kg^{-1}]	Soil particle size fraction [%]		pH in		C_{org} [g · kg^{-1}]	N_{tot} [g · kg^{-1}]
		< 0.02 [mm]	< 0.002 [mm]	H_2O	KCl			< 0.02 [mm]	< 0.002 [mm]	H_2O	KCl		
80	0	17	7	6.9	5.9	8.18	1.029	15	4	6.4	5.5	10.44	0.959
	40	16	5	6.9	5.7	8.13	0.998	16	6	6.4	5.4	10.11	0.966
	80	15	4	6.7	5.9	8.11	1.015	15	5	6.2	5.3	10.31	0.980
	120	18	7	6.8	5.9	8.75	1.029	14	5	6.2	5.1	10.39	1.005

Table 2

Total selenium content in the investigated soil [mg · kg^{-1}]

FYM doses [Mg · ha^{-1}] (factor II)	N doses [kg · ha^{-1}] (factor III)	Crop rotation (factor I)					
		A "depleting in organic matter"			B "enriching in organic matter"		
		March	May	July	March	May	July
0	0	0.098	0.098	0.100	0.118	0.098	0.103
	40	0.110	0.092	0.101	0.100	0.098	0.098
	80	0.104	0.108	0.104	0.119	0.098	0.115
	120	0.104	0.096	0.095	0.101	0.102	0.108
20	0	0.135	0.172	0.176	0.152	0.171	0.119
	40	0.130	0.170	0.161	0.164	0.172	0.147
	80	0.135	0.175	0.154	0.150	0.167	0.170
	120	0.135	0.164	0.161	0.147	0.172	0.160
40	0	0.166	0.192	0.144	0.196	0.163	0.160
	40	0.172	0.143	0.174	0.177	0.166	0.159
	80	0.175	0.205	0.177	0.203	0.183	0.167
	120	0.188	0.196	0.167	0.207	0.203	0.171
60	0	0.191	0.183	0.192	0.210	0.197	0.173
	40	0.194	0.193	0.159	0.205	0.224	0.162
	80	0.191	0.197	0.196	0.203	0.163	0.165
	120	0.199	0.184	0.202	0.185	0.153	0.158
80	0	0.246	0.199	0.236	0.234	0.227	0.183
	40	0.244	0.190	0.191	0.232	0.186	0.206
	80	0.229	0.200	0.206	0.234	0.176	0.192
	120	0.229	0.190	0.189	0.231	0.173	0.197
Mean		0.169	0.167	0.164	0.178	0.165	0.156
Date of sampling		March		May		July	
Mean for crop rotation (factor I)							
A		0.169		0.167		0.164	
B		0.177		0.165		0.156	
Mean for FYM doses (factor II)							
0		0.105		0.099		0.102	
20		0.143		0.170		0.156	
40		0.185		0.181		0.166	
60		0.197		0.187		0.176	
80		0.236		0.193		0.200	
Mean for N doses (factor III)							
0		0.173		0.170		0.159	
40		0.173		0.170		0.155	
80		0.174		0.166		0.164	
120		0.173		0.158		0.161	
$LSD_{0.05}$		I – 0.002 II – 0.005 III – n.s.		I – n.s. II – 0.007 III – 0.006		I – 0.003 II – 0.007 III – 0.006	

n.s. – non significant.

was applied. Statistical analysis demonstrated a significant dependence between total selenium content and organic carbon and total nitrogen content in soil and silt and clay fractions content (Table 5), what coincides with our earlier findings [13, 14] and those reported by other authors [15, 16]. As it is shown in Fig. 1 the highest amounts of total Se content in soil under study were obtained in March, and during vegetation period its content decreased in both variants of crop rotation.

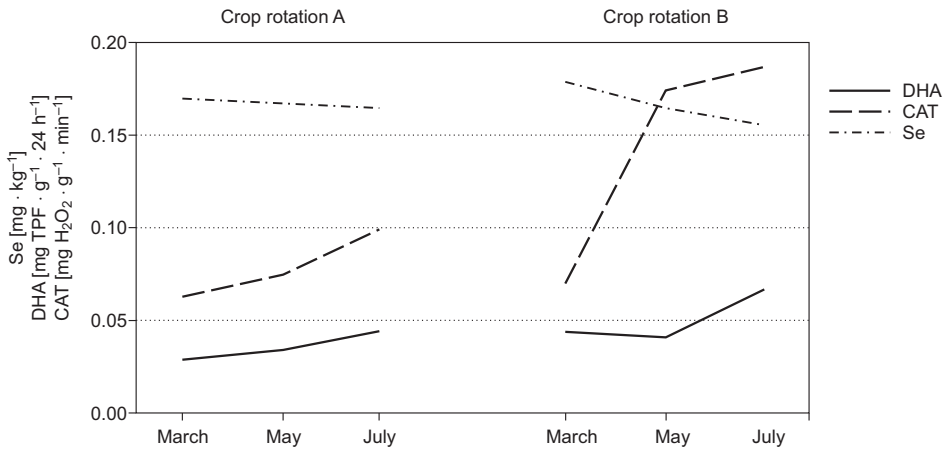

Fig. 1. Seasonal changes of selenium and enzymatic activity in soil under study

The effect of the FYM and nitrogen doses on dehydrogenases activity in soil is presented in Table 3. The organic fertilization applied significantly differentiated the activity of dehydrogenases in soil. FYM application at the doses of 60 and 80 Mg · ha^{-1} significantly increased the enzymatic activity about 47 % (mean for date of sampling), as compared with the treatment without manure. Nitrogen fertilization at the doses of 40 and 80 kg · ha^{-1} increased DHA activity from 4 to 14 % in comparison with soil without N. We found significantly higher amounts of DHA activity in soil from variant B – *"enriching in organic matter"*. Catalase activity in soil under study is presented in Table 4. Crop rotation significantly differentiated CAT activity in soil. During vegetation period higher amounts of CAT activity were obtained in soil from variant B of crop rotation (enriching in organic matter). Manure application strongly stimulated soil catalase activity with increasing doses, but nitrogen fertilization affected the enzymatic activity in unclear way. Generally, the application of nitrogen at the highest dose resulted the highest amounts of CAT activity in soil. As described by Spychaj-Fabisiak and Smolinski [17] nitrogen fertilization stimulated an increase of soil dehydrogenases activity. They concluded that the level of DHA activity in soil increased with quantity of microorganisms and the rate of their metabolism, which allowed them for turn to account the reserve of organic carbon. Koper and Piotrowska [18] noted the increase of catalase activity under mineral fertilization, but Frankenberger and Dick [19] reported that long-term mineral fertilization applying in high doses proved to inhibition of enzymatic reactions.

Table 3

Dehydrogenases (DHA) activity in the soil [mg TPF · g^{-1} · 24 h^{-1}]

FYM doses [Mg · ha^{-1}] (factor II)	N doses [kg · ha^{-1}] (factor III)	Crop rotation (factor I)					
		A "depleting in organic matter"			B "enriching in organic matter"		
		March	May	July	March	May	July
0	0	0.028	0.015	0.037	0.025	0.038	0.047
	40	0.029	0.023	0.038	0.032	0.037	0.053
	80	0.027	0.026	0.039	0.032	0.038	0.067
	120	0.026	0.031	0.038	0.027	0.023	0.060
20	0	0.031	0.035	0.032	0.031	0.027	0.063
	40	0.029	0.028	0.042	0.031	0.030	0.056
	80	0.026	0.029	0.043	0.033	0.045	0.069
	120	0.027	0.028	0.041	0.033	0.027	0.069
40	0	0.027	0.037	0.042	0.052	0.038	0.073
	40	0.028	0.030	0.041	0.041	0.037	0.079
	80	0.027	0.030	0.047	0.047	0.040	0.056
	120	0.027	0.036	0.051	0.049	0.038	0.074
60	0	0.025	0.043	0.056	0.065	0.034	0.061
	40	0.033	0.048	0.061	0.062	0.042	0.072
	80	0.035	0.040	0.047	0.062	0.036	0.082
	120	0.033	0.041	0.053	0.050	0.045	0.059
80	0	0.034	0.037	0.042	0.043	0.046	0.077
	40	0.038	0.039	0.041	0.055	0.089	0.066
	80	0.040	0.038	0.042	0.054	0.067	0.065
	120	0.034	0.038	0.047	0.048	0.064	0.066
Mean		0.030	0.034	0.044	0.044	0.042	0.066
Date of sampling		March		May		July	
Mean for crop rotation (factor I)							
A		0.030		0.033		0.044	
B		0.043		0.042		0.065	
Mean for FYM doses (factor II)							
0		0.028		0.029		0.047	
20		0.030		0.031		0.052	
40		0.037		0.036		0.058	
60		0.045		0.040		0.061	
80		0.043		0.052		0.056	
Mean for N doses (factor III)							
0		0.036		0.035		0.053	
40		0.038		0.040		0.055	
80		0.038		0.039		0.055	
120		0.035		0.036		0.056	
$LSD_{0.05}$		I – 0.001 II – 0.001 III – 0.001		I – 0.001 II – 0.001 III – 0.001		I – 0.001 II – 0.001 III – 0.001	

Table 4

Catalase (CAT) activity in soil under study [mg $H_2O_2 \cdot g^{-1} \cdot min^{-1}$]

FYM doses [Mg · ha^{-1}] (factor II)	N doses [kg · ha^{-1}] (factor III)	Crop rotation (factor I)					
		A "depleting in organic matter"			B "enriching in organic matter"		
		March	May	July	March	May	July
0	0	0.062	0.072	0.115	0.058	0.155	0.183
	40	0.058	0.068	0.106	0.068	0.117	0.191
	80	0.053	0.075	0.051	0.077	0.124	0.175
	120	0.068	0.081	0.109	0.068	0.149	0.191
20	0	0.070	0.081	0.064	0.077	0.157	0.170
	40	0.064	0.070	0.072	0.066	0.189	0.187
	80	0.053	0.066	0.066	0.070	0.191	0.191
	120	0.058	0.072	0.072	0.077	0.181	0.189
40	0	0.066	0.062	0.075	0.058	0.187	0.185
	40	0.062	0.072	0.077	0.066	0.183	0.183
	80	0.060	0.081	0.072	0.070	0.164	0.175
	120	0.060	0.064	0.153	0.077	0.170	0.170
60	0	0.068	0.064	0.077	0.077	0.189	0.195
	40	0.066	0.081	0.077	0.075	0.194	0.179
	80	0.053	0.079	0.079	0.081	0.191	0.195
	120	0.066	0.077	0.125	0.077	0.189	0.193
80	0	0.070	0.079	0.170	0.081	0.196	0.198
	40	0.072	0.068	0.175	0.079	0.175	0.193
	80	0.075	0.079	0.068	0.077	0.191	0.187
	120	0.064	0.079	0.175	0.077	0.189	0.195
Mean		0.063	0.074	0.099	0.073	0.174	0.186
Date of sampling		March		May		July	
Mean for crop rotation (factor I)							
A		0.063		0.073		0.099	
B		0.073		0.174		0.186	
Mean for FYM doses (factor II)							
0		0.064		0.105		0.140	
20		0.067		0.126		0.126	
40		0.065		0.123		0.136	
60		0.070		0.133		0.140	
80		0.074		0.132		0.170	
Mean for N doses (factor III)							
0		0.069		0.124		0.143	
40		0.068		0.122		0.144	
80		0.067		0.124		0.126	
120		0.069		0.125		0.157	
$LSD_{0.05}$		I – 0.002 II – 0.004 III – n.s.		I – 0.002 II – 0.004 III – 0.003		I – 0.006 III – 0.014 III – 0.012	

In soil under study the enzymatic activity demonstrated clear seasonal variations and considerable fluctuations depending on availability of substrate in crop rotation (Fig. 1). A highly significant dependence was found between enzymatic activity and total selenium, total nitrogen and organic carbon content in soil (Table 5).

Table 5

Simple correlation coefficients (r) between selenium content and enzymatic activity and soil properties

Examined properties	Fraction < 0.002	pH_{H_2O}	pH_{KCl}	C_{org}	N_{tot}	Se_{tot}	DHA	CAT
Fraction < 0.02	0.64*	–0.22*	–0.25*	0.29*	0.30*	0.25*	0.22*	0.21*
Fraction < 0.002		0.00	0.14	0.39*	0.40*	0.25*	0.22*	0.30*
pH_{H_2O}			–0.03	–0.04	–0.07	– 0.11	–0.04	0.05
pH_{KCl}				0.22*	0.21*	0.18	0.11	0.20*
C_{org}					0.88*	0.62*	0.62*	0.62*
N_{tot}						0.53*	0.55*	0.56*
Se_{tot}							0.59*	0.45*
DHA								0.63*

* r significant at $\alpha = 0.05$.

In general, management practices that increase inputs of organic residue, plant or animal manures, increase biological activity [20]. According to Samuel [21] addition of farmyard manure, usually increases microbial biomass and soil enzyme activities over soils that have not received any organic or inorganic amendments. However, when comparisons have been made between soils amended with farmyard manure or organic fertilizers, there have been mixed results which vary with cropping system and biological index. Thus management practices that increase incorporation of organic residue typically increase biological activity. Use of inorganic fertilizer can increase the plant biomass production which in turn increases the amount of residue returned to the soil and stimulates biological activity [22].

Conclusions

The content of total selenium in the investigated soil was in the range (0.092; 0.264) $mg \cdot kg^{-1}$. From the comparison of the results reported in literature one can observe that the studied soil was poor in selenium. Over the investigated period manuring resulted in an increase of total selenium content in soil and for that reason the FYM application can be recommended as a source of selenium in Se-deficient soils. Fertilization with manure resulted in an increase of dehydrogenases and catalase activities in soil with increasing doses of FYM. The selenium content, as well as DHA and CAT activities demonstrated clear seasonal variations. The present studies indicated a significant relationship between activity of soil enzymes, and the organic matter content, affecting the selenium status in soil and plants.

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SEZONOWE ZMIANY ZAWARTOŚCI SELENU I AKTYWNOŚCI WYBRANYCH OKSYDOREDUKTAZ W GLEBIE O ZRÓŻNICOWANYM NAWOŻENIU I ZMIANOWANIU

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Abstrakt: Celem pracy było określenie zmian zawartości selenu ogółem oraz aktywności wybranych enzymów niezbędnych w przemianach oksydoredukcyjnych w glebie w warunkach zróżnicowanego nawożenia i zmianowania. Próbki glebowe pobrano z obiektów, na których uprawiano pszenicę ozimą, trzykrotnie w 2002 roku z doświadczenia prowadzonego przez IUNG w Puławach na terenie RZD Grabów nad Wisłą, z wariantu zubożającego i wzbogacającego glebę w substancję organiczną. Nawożenie obornikiem zastosowano (jednorazowo w trakcie rotacji) pod ziemniaki w dawkach 0, 20, 40, 60, 80 Mg · ha^{-1}, natomiast azot w ilości 0, 40, 80 i 120 kgN · ha^{-1}. Wykazano, że nawożenie obornikiem w całym okresie badawczym istotnie wpływało na koncentrację selenu ogółem w glebie, która wzrastała wraz z jego dawką, niezależnie od terminu pobierania próbek glebowych i rodzaju zmianowania. Nie wykazano natomiast jednoznacznego wpływu azotu w tym zakresie. Zawartość tego pierwiastka oraz aktywność katalazy i dehydrogenaz w glebie podlegała stałym wahaniom i wykazywała zmienność sezonową. Nawożenie obornikiem wyraźnie stymulowało aktywność dehydrogenazową i katalazową gleby. Stwierdzono ścisłą zależność między aktywnością enzymatyczną gleby a zawartością w niej selenu ogółem. Uzyskane z obliczeń statystycznych wartości współczynników korelacji wykazały istotne zależności między aktywnością badanych enzymów glebowych a zawartością węgla organicznego i zawartością azotu ogółem.

Słowa kluczowe: selen, oksydoreduktazy, gleba, obornik, azot

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EFFECTS OF PETROLEUM PRODUCTS POLLUTED SOIL ON GROUND BEETLE *Harpalus rufipes*

EFEKT DZIAŁANIA PRODUKTÓW ROPOPOCHODNYCH NA CHRZĄSZCZA *Harpalus rufipes*

Abstract: The effects of soil contamination of petroleum products: unleaded petrol, diesel oil and used engine oil on ground beetle *Harpalus rufipes* De Geer were investigated. We measured development parameters (the survival and growth rate) and biochemical defense system parameters (the activity of the cytosolic fraction enzymes: superoxide dismutase, catalase, glutathione transferase, heat shock proteins HSP70, carboxyloesterase and acetylcholinesterase and microsomal fraction enzymes: cytochrome c (P450) reductase and ethoxyresorufin-O-deethylase) in the ground beetle *H. rufipes.* Animals were reared on contaminated soil (6 g of each petroleum product per kg of dry soil weight) through four week.

There was no difference in growth rate among animals from different experimental groups. The negative impact was revealed on survival rate of the ground beetles exposed to diesel oil (*ca* 30 % lower) at the end of the rearing in comparison with animals from control group. In turn, for animals exposed to other petroleum products, the inhibition of some examined enzymes was measured. The effects of each petroleum product were specific. The animals kept through four weeks on soil contaminated with diesel oil had only higher glutathione transferase activity than control ones. The decrease of catalase activity, HSP70 protein in animals exposed to used engine oil and the decrease of acetylcholinesterase, glutathione transferase activity in animals exposed to unleaded petrol were noted.

Keywords: petroleum contamination, biochemical defence, *Harpalus rufipes*

Introduction

Petroleum derivatives contamination in soil of agroecosystems may be toxic for organisms inhabited these areas [1, 2]. Petroleum products contamination is usually mixture of several substances with complicated fate in surrounding. Their composition

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depends on source, refinery process and product specificity [3]. Petroleum contaminants, among them *polycyclic aromatic hydrocarbons* (PAHs), modify properties of soil, evaporating to air spaces of soil [4]. They may also affect negatively organisms, in time, mixture type and concentration exposure dependent modes [5, 6].

Usage of the epigeic animals was effective for monitoring of soil contamination with heavy metals [7]. In case of soil contamination with petroleum derivatives, there are some informations about effects on webworms or isopods [8]. There is a scarcity of information about petroleum derivatives effect on epigeic insect. Carabidae frequency appeared to be negative affected through 4 month after soil contamination with diesel oil, engine oil and petrol (in concentration 2 dm^3/m^2) [9].

Examination of epigeic animals biochemical response may be important for early monitoring of soil contamination with petroleum derivatives and in assessment of the pollutants toxicity. The early step of PAHs metabolism in organisms are connected with microsomal enzymes activity catalyzing oxygenation reactions and esterases activity. Several of PAHs may induce or inhibit these enzymes. It is important because several of them take part in key processes connected with development, growth of animals and also with resistance to pesticides. Thus, these enzymes seem to be effective indicators of sublethal PAHs action [10, 11].

The reactions catalyzed by the microsomal enzymes may lead to production of reactive oxygen forms. It enhances an oxidative stress and affects antioxidant enzymes activity, which are sensible biomarkers of diesel hydrocarbon contamination. Enhanced oxidative stress may be also due to lipid peroxidation. Among products of lipid peroxidation, *4-hydroxynonenal* (HNE), can be metabolized by *glutathione transferases* (GST) isozymes, which as enzymes of the 2^{nd} phase of detoxification catalyze conversions of intermediates generated during microsomal reactions [12–15].

HSP proteins are also potentially valuable biomarker of petroleum contamination. The HSP proteins cellular responses may manifested non-specific mechanism of toxicity as a consequence of increased production of abnormal proteins and alteration of their functions in cells [15].

The present study examined the effects of different petroleum contaminants on beetles *H. rufipes* reared during four weeks period. The soil used in the experiment was contaminated with: unleaded petrol, used engine oil, diesel oil or uncontaminated (control). We measured: survival rate, body mass, detoxifying enzymes of cytosolic and microsomal fraction of the beetles. *Harpalus rufipes* De Geer individuals commonly inhabited different agroecosystems in Europe [16]. We suspected different sensitivity of adult beetles to petroleum derivatives, revealed by changes in performance of the adult individuals during rearing period and by variation in selected biochemical defense systems.

Materials and methods

The contaminated soil used in the experiment, was originated from cubic containers of 1 m^3 volume with drainage and system of evaporation kept in the field (Mydlniki, Krakow, Poland). The containers had an attest to use petrol substances. They were

placed into the ground and the soil inside them was up to the same level as that outside. Their walls in the upper part were perforated to allow the penetration by the field organisms. In June 2010, the soil was contaminated with petroleum products: unleaded petrol, diesel oil or used engine oil, in concentration of 6 g per kg of dry soil weight. In August 2010, the upper part of soil (ca 5 cm layer) was collected and air dried just prior experiment provided in laboratory conditions.

Animals were collected in Mydlniki area and acclimated to laboratory conditions. The animals were randomly divided to experimental groups. They were reared in cages (2 dm^3 volume) on thin layer of different contaminated soil (0.1 kg of soil in each cage) at 25 °C. The beetles were offered the larvae of houseflies as a food *ad libitum*. Every second day, water was spread on the layer of soil. The experimental groups were due to soil contamination: control group, unleaded petrol, diesel oil and used engine oil groups. Animals from each experimental group were reared in two cages (18 adult individuals of both sexes were kept in each cage).

During four weeks of rearing, we measured the gained mass of the alive beetles and recorded their number, in a week intervals. At the end of the experiment, male beetles were anaesthetized on ice, and homogenized without legs and wing cases, in 0.05 M buffer Tris-HCl, pH 7.4 with 1 mM EDTA (Ethylenediaminetetraacetic acid), 1 mM DTT, 1 mM PMSF, 1 mM PTU and 20 % sucrose. Homogenates were centrifuged at 1000 g 10 min at 4 °C. The supernatants were centrifuged at 15,000 g 10 min at 4 °C. Next supernatants were centrifuged 100,000 g 1 h at 4 ^oC. Finally obtained supernatants were used for cytosolic enzymes measurements, while microsomal enzymes were measured in suspension of pellet (in homogenization buffer contained 20 % glycerol). The enzyme assays were carried using a microplate spectrophotometer Infinite M200 and fluorescence spectrophotmeter f-7000. There was six replicates used for each biochemical measurement (for each replicate we used 2–3 animals).

CAT activity toward H_2O_2 in 0.05 M phosphate buffer (pH 7.0) was measured at $\lambda = 240$ nm [17]. Blanks were registered in the absence of H_2O_2. The enzyme assay was carried during first half of minute, within the linear range of reaction rate. Exctinction coefficient was equal to 40 $M^{-1} \cdot cm^{-1}$. One unit of the enzyme activity was defined as the decomposition of 1 nmol of H_2O_2 $min^{-1} \cdot$ mg $protein^{-1}$.

GST activity was measured as described by Yu [18] using ethanol solution of *1-chloro-2,4-dinitrobenzene* (CDNB). 1 cm^3 of the reaction mixture contained: 0.1 mM Tris-HCl buffer (pH 7.5) consisted of 1.5 mM GSH in buffer, 15 mM CDNB and 0.01 cm^3 sample. Blank values (in the absence of sample) were subtracted to yield the final absorbance values. The enzyme assay was carried during first three minutes, within the linear range of reaction rate. The measurements were done at $\lambda = 340$ nm. Enzyme activity was expressed as nmol of GSH conjugates $min^{-1} \cdot$ mg $protein^{-1}$. The extinction coefficient was equal to 9.6 $mM^{-1} \cdot dm^{-3} \cdot cm^{-1}$.

Carboxylesterase (CarE) was measured in presence of *p*-nitrophenyl acetate as a substrate. The measurement was taken at $\lambda = 400$ nm for 3 min. Results were corrected by subtracting blanks contained buffer instead of sample. To calculate CarE activity, the extinction coefficient 9.25 $mM^{-1} \cdot dm^{-3} \cdot cm^{-1}$ was used [19].

Acetylcholinesterase (AChE) activity was determined using acetylthiocholine iodide as a substrate [20]. The linear changes in absorbance were measured at λ = 412 nm through 5 min.

NADPH dependent cytochrome c (P450) reductase activity was measured in 0.3 M potassium phosphate buffer with 0.1 mM EDTA, pH 7.4 with freshly prepared cytochrome c (5 mg/cm^3) and 1 mM KCN. After addition of NADPH to the mixture, the changes in absorbance were recorded at 550 nm.

Ethoxyresorufin o-deethylase (EROD) activity was measured at excitation wavelength λ = 560 nm and emission wavelength λ = 589 nm. The measurement of the activity started after addition 7-etoxyresorufine and NADPH. The measurement of progressive increase in fluorescence lasted 40 min. The activity was defined as pmol of resorufine min^{-1} · mg protein^{-1} and calculated upon product (resorufine) curve basis. The protein concentrations were assessed using *bovine serum albumin* (BSA) as a standard [21].

To evaluate HSP70 proteins and HNE levels we used ELISA procedure. Cytosol portions contained 20 µg protein were transferred to wells of the plate and the following primery antibodies: anti-HSP 70 Cayman (No. cat. 19015) (amounts of the antibody in proportion with *physiological saline buffer* (PBS), pH 7.4 (PBS), 1:250) and Rabbit anti-HNE 11S (Alpha diagnostic) (amounts of the antibody in proportion with PBS, 1 : 250) were applied, and before evaluation of the examined products the goat anti-rabbit IgG-AP (Stressgen SAB 301) (amounts of the antibody in proportion with PBS, 1:5000) as a secondary antibody was added. Firstly, microtitre plates were coated with samples and, dependently on measurement, with HSC70 (evaluation of HSP protein level) or HNE (evaluation of HNE product level) as standards. Plates were incubated overnight at 4 °C. Active sites remaining on the plate were blocked by adding 3 % BSA solution in PBS. Afterwards, anti-HSP or anti-HNE antibodies were respectively added to wells, and the plates were incubated at 37 °C for 2 h, afterwards the secondary antibody was added. Finally, to induce the colour reaction, a solution of *p*-nitrophenyl phosphate was added to each well. In between addition of BSA as well as primary or secondary antibodies, the wells were washed three times with PBS-0.1 % Tween solution. The product of the final reaction was measured at λ = 405 nm, and amounts were determined using a standard curve of proper standards.

Data of HSP70 and HNE levels (three replicates) were analysed with the nonparametric Kruskal-Wallis test and presented as mediane and range of quartiles (25–75 %). The other data are presented as the mean ± SD of six-seven replicates in each experimental groups (each replicate was combined homogenates of two-three males). If necessary, the data were log transformed and checked for homogeneity and normality. ANOVA one-way analysis and correlation analysis were carried out to establish significant relationship between measured biochemical parameters in beetles from experimental groups. *F* values having $p < 0.05$ were considered as significant.

Results and discussion

The most affected were beetles inhabited soil contaminated with diesel oil compared with those from other experimental treatments. They started to die after two weeks of

exposure, and finally from 36 survived only 25 individuals. In others examined groups survival was not changed (Table 1). The parallel study provided that diesel oil contamination was highly toxic for other representatives of coleopteran *Pterostichus cupreus* (Kafel, unpublished). The different sensitivity of soil organisms to petroleum hydrocarbons was due to quantity and quality of contaminants mixtures [22]. The examination of *Drosophila melanogaster* performance being exposed to benzene, toluene or xylene (single or in mixture) shown significant differences between single and combinatory effects [15]. But, the possibility of different performance of different arthropod representatives was also suggested.

Table 1

Survival of the beetles of *H. rufipes* during following weeks of rearing, presented in % of alive animals, 100 % was equal to the 36 animals provided at the beginning of the experiment in each experimental group, living on soil contaminated with petroleum products: unleaded petrol, diesel oil, used engine oil and on uncontaminated soil (control)

	Control	Unleaded petrol	Diesel oil	Used engine oil
At the beginning of rearing	100	100	100	100
After one week of rearing	100	100	100	100
After two weeks of rearing	100	100	100	100
After three weeks of rearing	95	100	97	100
After four weeks of rearing	92	100	67	100

The body mass of animals did not changed significantly during rearing period, and did not differ between animals from experimental groups (Table 2).

Table 2

The body mass of beetle *H. rufipes* [mg of the alive individual] living on soil contaminated with petroleum products: unleaded petrol, diesel oil, used engine oil and on uncontaminated soil (control) in the following four weeks of rearing. There was no significant differences among the experimental groups and in the following weeks of the rearing. (LSD, $p < 0.05$)

	Control	Unleaded petrol	Diesel oil	Used engine oil
At the beginning of rearing	93 ± 20	94 ± 15	89 ± 19	87 ± 18
After one week of rearing	95 ± 24	93 ± 15	90 ± 18	84 ± 19
After two weeks of rearing	88 ± 14	91 ± 21	91 ± 18	85 ± 17
After three weeks of rearing	93 ± 13	97 ± 18	94 ± 18	86 ± 17

Looking for suitable indicators of petroleum products contamination, among microsomal enzymes activity: EROD and NADPH-dependent reductase cytochrome c we did not find any (Table 3). In the parallel study provided on *P. cupreus* males, the decrease of the microsomal enzyme activity was registered for animals exposed to petroleum contaminants when compared with the controls (Kafel, unpublished). The response of

the microsomal enzymes may depend on time of contaminants exposure. On example of *Abarenicola pacific* exposed to PAHs in sediment, the increase of the enzymes during first two weeks and the decrease after longer time of exposure was found [23]. Differences in metabolic biotransformation of PAHs between terrestrial invertebrates: isopods and springtails were presented [6]. The increasing response might be due to detoxification *via* single P450 enzyme. The responses may depend on bioavailability of PAHs. There are plenty of factors affected substances availability in soil like: pH, organic matter content, moisture, chemical properties, time of presence in the soil [24–26]. It should be also underlined the discrepancies in microsomal enzymes assessment. They might be connected with preparation of samples, as sources of group P450 enzymes activity.

Table 3

The activity of cytosolic and microsomal enzymes in the beetle *H. rufipes* living on soil contaminated with petroleum products: unleaded petrol, diesel oil, used engine oil and on uncontaminated soil (control) after four weeks of rearing presented as mean ± SD. Different letters depicts the significant difference among experimental groups (LSD, $p < 0.05$)

	Control	Unleaded petrol	Diesel oil	Used engine oil
GST [nmol · mg protein^{-1} · min^{-1}]	11.47 ± 1.75 ^{b}	8.78 ± 1.71 ^{a}	17.78 ± 2.97 ^{c}	9.05 ± 1.84 ^{ab}
CarE [nmol · mg protein^{-1} · min^{-1}]	5.45 ± 1.88 ^{a}	4.95 ± 1.74 ^{a}	6.41 ± 3.24 ^{a}	4.90 ± 0.80 ^{a}
AChE [nmol · mg protein^{-1} · min^{-1}]	1.07 ± 0.29 ^{a}	2.39 ± 0.50 ^{b}	1.58 ± 0.59 ^{ab}	1.38 ± 0.54 ^{a}
CAT [nmol · mg protein^{-1} · min^{-1}]	149.5 ± 69.9 ^{b}	125.1 ± 48.4 ^{ab}	116.9 ± 46.7 ^{ab}	89.9 ± 23.9 ^{a}
EROD [pmol · mg protein^{-1} · min^{-1}]	7.79 ± 5.11 ^{a}	5.96 ± 6.61 ^{a}	3.89 ± 2.57 ^{a}	5.37 ± 4.86 ^{a}
NADPH reductase [µmol · mg protein^{-1} · min^{-1}]	65.5 ± 11.1 ^{a}	73.1 ± 15.8 ^{a}	66.3 ± 14.0 ^{a}	71.9 ± 17.3 ^{a}

Elevation of GST activity was observed among animals exposed to diesel oil contaminations in soil. It was 1.5 times higher than in control animals (Table 3). The enhancement of GST activity under diesel oil exposure was documented for oyster *Crasostrea gigas*, but dependently on level of contamination and environment conditions. It was suggested that GST increase might be correlated with increase of lipid peroxidation [4]. But, in our case, we did not find any changes in level of products of lipid peroxidation – HNE (Fig. 2). A significant increase of some GST isozymes expression activity was shown for Atlantic code larvae (*Gadus morhua*) after crude oil exposure [27]. A correlation between GST activity and PAHs concentration in tissues of mussels was also evaluated [28].

In our experiment the animals with high GST turnover were characterized by low survival (Tables 1 and 3). Increasing energetic demands for higher enzymatic turnover may result in reduced performance of animals [29].

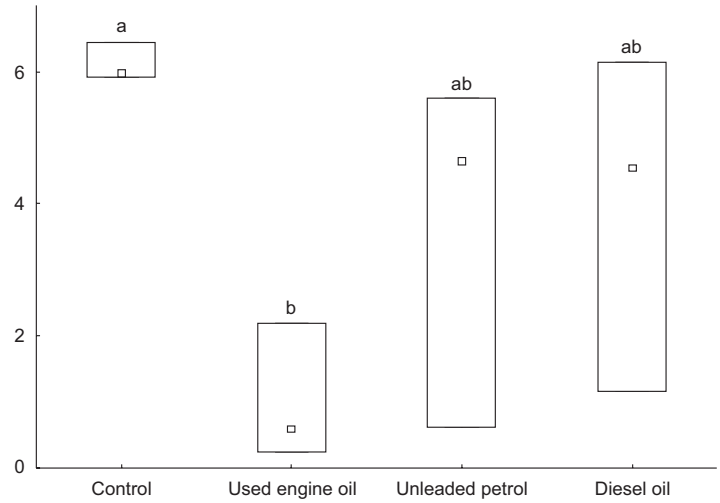

Fig. 1. The level of HSP 70 [ng · (mg · protein)$^{-1}$] in cytosolic fraction of beetle *H. rufipes* living on soil contaminated with petroleum products: unleaded petrol, diesel oil, used engine oil and on uncontaminated soil (control) after four weeks of rearing (Kruskal-Wallis, $p < 0.05$).

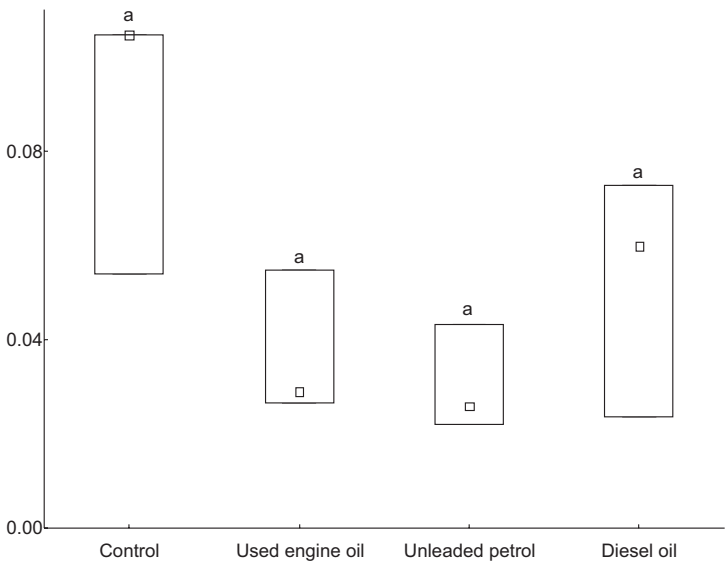

Fig. 2. The level of HNE products [ng · (mg · protein)$^{-1}$] in cytosolic fraction of beetles *H. rufipes* living on soil contaminated with petroleum products: unleaded petrol, diesel oil, used engine oil and on uncontaminated soil (control) after four weeks of rearing (Kruskal-Wallis, $p < 0.05$)

GST and AChE activity varied between animals exposed to different petroleum derivatives. The action of petrol, may depend on origin of the crude oil and processing technology. Animals from unleaded petrol group had lower activity of GST (1.3 times)

than in animals from control group. We also found an elevation of AChE activity upon unleaded petrol exposure, but not under other petroleum derivatives (Table 3). It is reported that AChE activity can serve as a good exposure biomarker but its response might be species specific [30].

The change in CAT activity was only found for animals exposed to used engine oil in soil. It was 1.7 times lower than in control group (Table 3). Generally, however, the different tendencies might be possible in response of CAT activity, that might be time exposure dependend. Such phenomenon, was presented for marine organisms [4, 10, 31].

The examined enzymes: CarE, EROD and NADPH depended reductase cytochrome c and HNE products level did not varied among different conditions of soil contamination with petroleum constituents (Table 3, Figs. 1 and 2).

Conclusions

1. Diesel oil contamination mostly affected the beetles.
2. Body mass changes are not suitable biomarker of petroleum products contamination.
3. The changes in activity of the examined enzymes were specific for particular exposure of petroleum products.
4. GST activity (however variable) seems to be the most sensitive biomarker of *H. rufipes* exposure to petroleum products.

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EFEKT DZIAŁANIA PRODUKTÓW ROPOPOCHODNYCH NA CHRZĄSZCZA *Harpalus rufipes*

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Abstrakt: W badaniach porównywano wpływ produktów naftowych (benzyna bezołowiowa, olej napędowy i zużyty olej silnikowy) na chrząszcza *Harpalus rufipes* De Geer. Mierzono parametry: rozwojowe (przeżywalność i tempo wzrostu) i parametry biochemiczne związane z funkcjonowaniem systemu obronnego (aktywność enzymów frakcji cytozolowej: dysmutazy ponadtlenkowej, katalazy, transferazy glutationowej, białek szoku cieplnego HSP70, karboksyloesterazy i acetylocholin esterazy oraz aktywność enzymów frakcji mikrosomalnej: reduktazy cytochromu c (P450) i deetylazy etoksy-rezorufiny) w ciele chrząszczy. Do badań wykorzystano zwierzęta hodowane na glebie skażonej dawką 6 g każdego produktu ropopochodnego na kg suchej masy gleby przez okres czterech tygodni.

Nie wykazano istotnych zmian w tempie wzrostu między zwierzętami z różnych grup eksperymentalnych. Ujawniono negatywny wpływ na przeżywalność chrząszczy eksponowanych na olej napędowy (ok. 30 %) pod koniec okresu doświadczalnego, w porównaniu ze zwierzętami z grupy kontrolnej. Z kolei u zwierząt eksponowanych na pozostałe produkty ropopochodne stwierdzono zahamowanie aktywności niektórych badanych enzymów. Efekty działania produktów ropopochodnych były specyficzne. Zwierzęta przetrzymywane na glebie skażonej olejem napędowym cechowała wyższa aktywność transferazy glutationowej w porównaniu do kontroli. Obniżenie aktywności katalazy i poziomu białek szoku cieplnego HSP70 zarejestrowano u zwierząt poddanych działaniu zużytego oleju silnikowego, natomiast obniżenie aktywności acetylocholinesterazy i transferazy glutationowej stwierdzono u zwierząt eksponowanych na działanie zanieczyszczeń benzyny bezołowiowej.

Słowa kluczowe: zanieczyszczenie produktami ropopochodnymi, obrona biochemiczna, *Harpalus rufipes*

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CHARACTERIZATION OF THE AEROBIC CULTIVABLE BACTERIA ISOLATED FROM SOILS: HEAVY METALS CONTAMINATED (BIALOGON, KIELCE) AND ARABLE (KLONOWSKIE RIDGE)

CHARAKTERYSTYKA TLENOWYCH BAKTERII HODOWALNYCH IZOLOWANYCH Z GLEBY SKAŻONEJ METALAMI CIĘŻKIMI (BIAŁOGON, KIELCE) I GLEBY UPRAWNEJ (PASMO KLONOWSKIE)

Abstract: The aim of this study was quantitative and enzymological analysis of aerobic bacterial microflora isolated from heavy metals contaminated soil and unpolluted arable soil. The amounts of haevy metals: zinc, copper, cadmium, lead, chromium in soil samples were determined. The commercial media and soils extracts media were used for soil microorganisms isolation. Amount of soil bacteria cultivated on commercial media were significant higher (3 to 10^6 times) in case of heavy metals contaminated soil, whereas during growth on soil extract agar more microorganisms were found in arable soil. The Gram-negative bacteria dominated among strains isolated from both soil samples. For isolated microorganisms the ureolytic and proteolytic activity, as well as the ability for nitrification and denitrification were determined. The total ureolytic and proteolytic activity of soil samples were also defined. It was revealed, that only few bacterial strains isolated from polluted soil indicated analyzed properties (27 % – urea hydrolysis, 13 % – skim milk hydrolysis, 19 % – nitrification and 27 % – denitrification) in comparison with isolates from arable soil (65 %, 35 %, 30 % and 35 % respectively). In heavy metals contaminated soil the total ureolytic activity was much lower than in unpolluted soil sample. The presence of heavy metals has inhibitory effect on appearance of microorganisms participating in nitrogen circulation.

Keywords: soil bacteria, heavy metals, biochemical properties.

Soil is environment where dwells a giant amount of microorganisms. The composition, distribution, growth and development of soil bacteria affect various factors *eg* temperature, pH, nutrients and toxic substances [1]. To these latter heavy metals are included. Small amounts of metals (zinc, copper, nickel, manganese and other) have a

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positive effect on bacterial metabolism by mediating in enzymatic reactions [2]. However, increase of their concentration result in toxic effects against microbes. On the other hand, metal ions such as mercury, cadmium or lead are harmful even at low concentrations [3]. Contamination of soil by such ions contributing in reduction of most biochemical reactions intensity and have also toxic influence on bacterial microflora amount and structure [4, 5]. It has been proven, that heavy metals decrease the activity of urease, acid and alkaline phosphatase, amidase, nitrate reductase, produced by soil bacteria [6].

The aim of this study was to quantitative and enzymologic analysis of aerobic bacterial microflora isolated from arable soil and soil contaminated by heavy metal ions.

Materials and methods

Soil samples were collected aseptically into sterile containers using Egner's stick, from the surface soil layer. Two samples were collected on November 18th, 2010: first was a brown arable soil, from the ecological area of Klonowskie Ridge (designated as G1 sample), while the second was from the outer basin of dried Bialogonski pond in Kielce (designated as G2 sample) (Fig. 1).

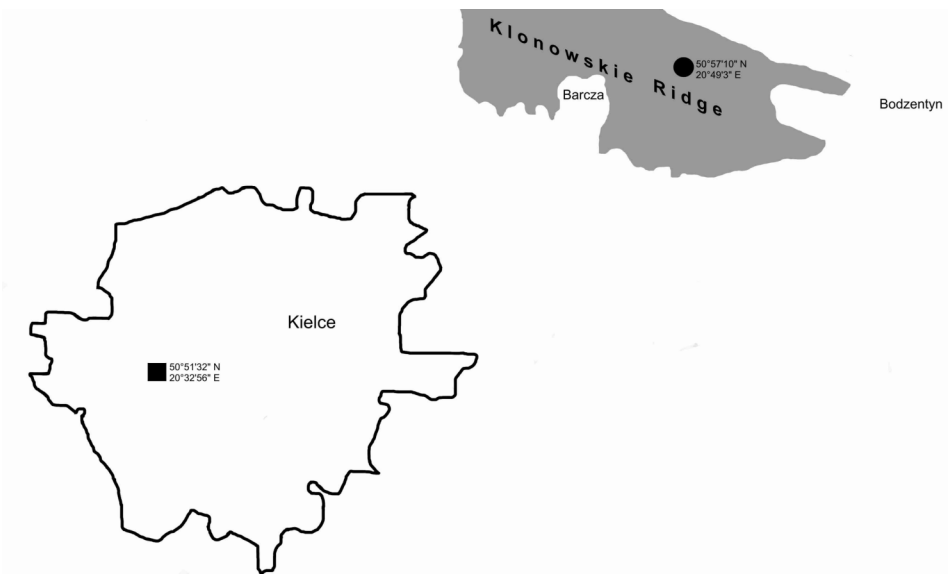

Fig. 1. Location of soil (● – G1; ■ – G2) sampling points

Chemical analysis of the soil samples – content of heavy metals: zinc, copper, cadmium, lead, chromium was determined by FAAS. The content of organic carbon and organic nitrogen were determined by titration and titration-destillation method, respectively. All analysis was performed at the Regional Agricultural-Chemical Station in Kielce.

Isolation and biochemical properties of soil bacteria – suspensions of 1 g of soil samples (G1 and G2, respectively) in 100 cm^3 of sterile Winogradsky solution were made and well stirred (20 min ok. 500 rpm). After soil particles sedimentation, solutions were serially diluted up to 10^{-6} and plated on Petri plates with media: Luria-Bertani (LB) agar, minimal M9 agar, tryptic-soy agar (TSA), KingB agar, soil extract agar (SEA). Cultures were incubated at 25 °C up to 7 days. Quantity bacteria (cfu/1g of wet soil) was determined based on the number of bacterial colonies grown on the individual media. For further studies, colonies with different macroscopic morphology were isolated. Isolated bacterial strains were stained according to Gram's method. Proteolytic activity on the medium with 2 % skimmed milk was determined, ureolytic activity – by spectrophotometric method (wavelength λ = 560 nm) on the Christensen in Maslen modification liquid medium (results were differences between culture and control absorbances), nitrification and denitrification – spectrophotometric method (wavelength λ = 520 nm) on the liquid mineral medium according to Winogradsky or medium with potassium nitrate (absorbance were calculated on the amount of nitrite ions), respectively. In spectrophotometric methods all results were related to the non-inoculated control media.

Determination of the soil samples total biochemical activity – ureolytic and proteolytic activities were defined according to previous works [7, 8].

Results and discussion

In soil sample collected from the former Bialogonski pond basin in Kielce (G2 sample), against to the arable soil from ecological area of Klonowskie Ridge (G1 sample), a higher content of heavy metals: cadmium, copper, lead, zinc and chromium was noted, where the quantity of zinc was repeatedly (almost 38 times) higher. Also content of organic carbon and nitrogen was higher in soil G2 (Table 1).

Table 1

Chemical analysis of soil samples

Amount / Soil sample	C_{org} [%]	N_{tot} [g/kg]	Cd [mg/kg]	Cu [mg/kg]	Pb [mg/kg]	Zn [mg/kg]	Cr [mg/100 g]
G1	1.56	1.46	0.190	6.45	16.2	43.5	9.42
G2	25.77	17.02	3.85	14.8	47.0	1646	22.4

This contamination has historical reason. Soil sampling station (the former Bialogonski pond basin) during the nineteenth century was the center of exploitation of copper and lead ores as well as silver melting. The earlier analysis also showed a high heavy metals (lead, cadmium, silver) contamination [9, 10].

There are evidences, that heavy metals (*eg* cadmium, chromium, copper, mercury, lead, zinc) have toxic effects on microbes [11]. In this study a quantitative analysis of aerobic bacteria isolated from soil contaminated by heavy metals (soil G2) was performed. The reference point were results obtained for arable soil from ecological

areas (soil G1). Quantity of microorganisms cultivated from two soil samples varied depending on the type of culture medium. On the commercial media, more of microorganisms was cultured from G2 soil sample, with the most abundant microbial growth on TSA and KingB was occurred. In case of soil G1, amount of microorganisms cultivated on these media was much lower. The opposite situation occurred in the case of the soil extract agar (SEA). On this medium, significantly more organisms were grown from the sample G1 (Table 2).

Table 2

The quantity of aerobic bacteria [cfu/1 g soil] cultured from G1 (arable soil) and G2 (Bialogonski pond basin soil) samples, on various microbiological media

Soil sample	Microbiological medium				
	TSA	LB	M9	KingB	SEA
G1	$2 \cdot 10^7$	$1.5 \cdot 10^7$	$2.1 \cdot 10^6$	$1 \cdot 10^7$	*
G2	$6 \cdot 10^9$	$3.5 \cdot 10^7$	$1 \cdot 10^7$	$6.5 \cdot 10^9$	$3.6 \cdot 10^8$

* – unable to count number of microorganisms.

These results may be surprising in light of earlier reports of heavy metals toxic effects on soil bacteria number and growth [12]. However, there are also studies demonstrating, that in the soils of this type numerous bacteria, including actinomycetes are present [1, 4, 13–16]. In this work, among the isolated strains, actinomycetes were also observed (based on macro- and microscopic morphology) and the percentage participation of particular morphological forms was similar to the distribution recived for G1 sample (Table 3).

Table 3

Percentage participation of bacterial strains with different microscopic morphology, isolated from various soils

Soil sample	Amount of isolate strains	Microscopic morphology [%]				
		Gram-negative	Gram-positive			
		rods	cocci	bacilli	rods	actinomycetes
G1	20	60	10	15	10	5
G2	37	48	11	11	22	8

Shentu *et al* showed a toxic effect of cadmium on the soil bacterial microflora structure [12]. In this work, the participation of individual morphological forms among the isolated bacterial strains was similar in both soil samples (heavy metals contaminated, including cadmium and uncontaminated).

These results are consistent with other work on the biodiversity of heavy metals contaminated soil microflora, where both Gram-negative as well as Gram-positive bacteria were observed [3, 4, 13]. Above this, it was demonstrated, that shortly after

contamination total amount of soil microbes rapidly decreasing. However with time, the number of isolated microorganisms growing and among them are often Gram--negative rods [4]. Contamination of G2 station was quite distant in time, so it is possible, that there was a reconstruction (at least partially) bacterial microflora size and structure.

For the 57 examined bacterial strains, their ability to participate in nitrogen cycle various stages were analyzed. Nitrification, denitrification, proteolytic and ureolytic activities were studied. The isolated bacterial strains demonstrating varied biochemical properties. The level of nitrification in analysed strains was low and does not exceed (with the exception of strain G2/18) 10 μM of nitrite(III) ions (Fig. 2A). Denitrification activity was much more diverse (Fig. 2B). Single strains possesed activity much higher than average *ie* G2/18 or G1/19 for nitrification and denitrification activities, respectively (Fig. 2).

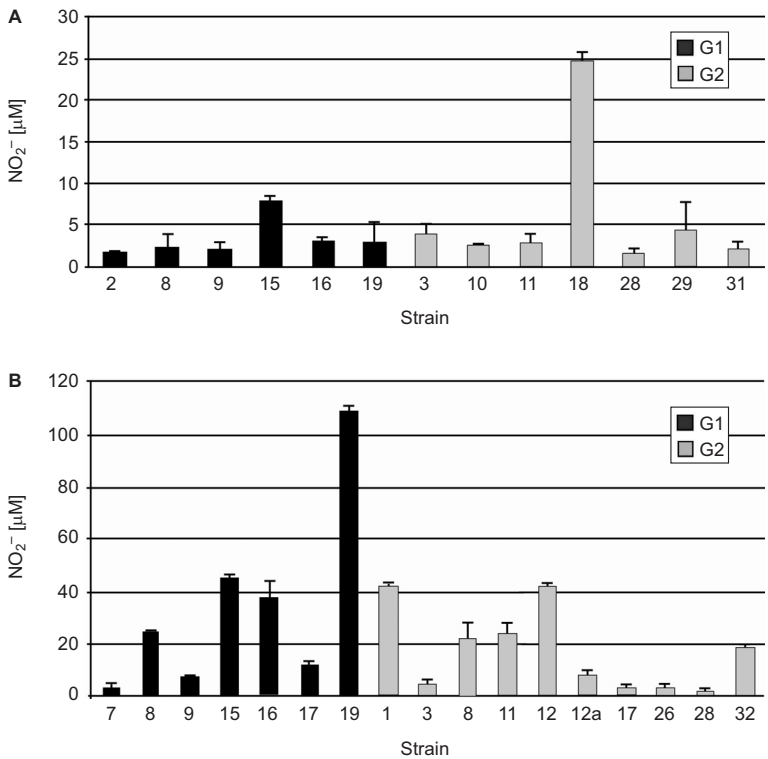

Fig. 2. Nitrification (A) and denitrification (B) of bacterial strains isolated from soil G1 and G2

Relatively large diversity were also observed in case of ureolytic and proteolytic activities, when stronger activity possesed strains isolated from arable soil (Table 4).

Analysis of the total ureolytic activity in examined soil samples showed, that soil G2 is characterized by a much lower level of this activity.

Table 4

The quantity of bacterial strains with ureolytic and/or proteolytic activities, isolated from different soils

Soil sample	Ureolytic activity				Proteolytic activity			
	–	+	++	+++	–	+	++	+++
G1	1	7	3	2	6	3	3	1
G2	8	7	1	2	13	4	1	0

– no activity; + weak activity (Δ of absorbance below 0.5 for the ureolytic activity and zone of milk hydrolysis less than 15 mm for the proteolytic activity); ++ moderate activity (Δ of absorbance from 0.5 to 1 for the ureolytic activity and zone of milk hydrolysis from 15 to 20 mm for the proteolytic activity); +++ strong activity (Δ of absorbance over 1 for the ureolytic activity and zone of milk hydrolysis over 20 mm for the proteolytic activity).

There are data, where negative effects of heavy metals on the biochemical activity of soil bacteria was observed [12]. Toxic influence of some metals (copper and lithium) on the proteolytic activity of isolated from soil *Bacillus cereus* was demonstrated [17]. Heavy metals are also nitrification inhibitors. It was shown, that chromium, nickel, copper, zinc, cadmium and lead may inhibit nitrification at each stage [18]. Nawaguo et al observed, that also urease is especially sensitive to the presence of heavy metals [19].

Conclusions

The presence of heavy metals significantly reduce biochemical activity of soil as well as bacterial strains isolated from soil. The structure of morphological forms of microorganisms isolated from contaminated soil does not differ significantly to the distribution of isolates from unpolluted soil, which may be caused by slowly (over one century) reconstruction of microflora in the area contaminated for more than 100 years.

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CHARAKTERYSTYKA TLENOWYCH BAKTERII HODOWALNYCH IZOLOWANYCH Z GLEBY SKAŻONEJ METALAMI CIĘŻKIMI (BIAŁOGON, KIELCE) I GLEBY UPRAWNEJ (PASMO KLONOWSKIE)

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Abstrakt: Celem pracy była analiza ilościowa i enzymologiczna tlenowej mikroflory bakteryjnej izolowanej z gleby zanieczyszczonej metalami ciężkimi oraz gleby uprawnej terenów czystych ekologicznie. Próbki zbadano pod względem zawartości metali ciężkich: cynku, miedzi, kadmu, ołowiu, chromu. Do izolacji drobnoustrojów z próbek glebowych zastosowano pożywki komercyjne oraz podłoża z ekstraktami glebowymi. Ilość wyhodowanych mikroorganizmów na pożywkach komercyjnych była znacznie większa (od 3 do 10^5 razy) w przypadku gleby skażonej metalami ciężkimi, natomiast na podłożu zawierającym ekstrakt glebowy obserwowano bogatszy wzrost bakterii dla gleby uprawnej. Wśród szczepów bakterii wyizolowanych z obu próbek dominowały drobnoustroje Gram-ujemne. Dla wyizolowanych mikroorganizmów określono aktywność ureolityczną, proteolityczną oraz zdolność do przeprowadzania procesów nitryfikacji i denitryfikacji. Oznaczono również całkowitą aktywność ureolityczną i proteolityczną próbek glebowych. Wykazano, że tylko nieliczne szczepy bakterii izolowanych z gleby zdegradowanej wykazywały badane właściwości (27 % – hydroliza mocznika, 13 % – hydroliza białek mleka, 19 % – nitryfikacja i 27 % – denitryfikacja) w porównaniu do izolatów z gleby uprawnej (odpowiednio: 65 %, 35 %, 30 % i 35 %). W glebie zanieczyszczonej metalami ciężkimi całkowita aktywność ureolityczna była znacznie mniejsza w porównaniu do próbki gleby czystej. Obecność metali ciężkich wpływa hamująco na występowanie drobnoustrojów uczestniczących w obiegu azotu.

Słowa kluczowe: bakterie glebowe, metale ciężkie, właściwości biochemiczne

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EXAMINATION OF DYNAMICS OF FREE MINERAL ACIDS CONTENT DURING MANUFACTURING PROCESS OF PAPR-TYPE FERTLIZERS

BADANIA DYNAMIKI ZMIAN ZAWARTOŚCI WOLNYCH KWASÓW MINERALNYCH W CZASIE CYKLU PRODUKCYJNEGO NAWOZÓW TYPU PAPR

Abstract: Partially acidulated phosphate rock (PAPR) constitute the separate group of phosphate fertilizers. Fertilizers of the PAPR type are produced as a result of the reaction of phosphate rocks with non-stoichiometric, in account of the reaction of the decomposition of fluorapatite contained in phosphate rock, amount of mineral acids. The aim of the investigations was the examination of dynamics of free mineral acids content in preparations of the PAPR type produced under laboatory scale, in respect of their application in commercial products available on the Polish market. The variable production parameters being under inestigations were: the type of mineral acid and its concentration, the degree of the PAPR stoichiometric norm (η_{PAPR}), fineness of the phosphate rock applied for the production. Examinations were consisted in determination of the amount of free mineral acids in the product both directly after the acidulation process and during curing of fertilizer after time 2, 4, 7, 10 and 14 days, respectively.

Keywords: partially acidulated phosphate rocks (PAPR), mineral acids, phosphate fertilizers, available phosphate

Recently, the importance of fertilizers containing partially acidulated phosphate rock has been increasing significantly. This is due to very high (reaching up to 1000 %) rises in prices of raw materials for phosphates manufacturing between the second half of 2007 to early 2009 [1]. The reason for this state is the fact that the annual world consumption of phosphates is about 35 million Mg (tons) while simultaneously the amount of extractable phosphate rock deposits, rich in P_2O_5 are being reduced. The largest deposits of phosphate rocks are located in Morocco, China and USA. These countries are the largest manufacturers of phosphate rocks. Poland, one of the producers of fertilizers (1.5 % of world production) is of no importance in the extraction of

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phosphate raw materials. It is also important that Poland is included among 10 EU countries of the largest (100–150 kg/ha of agricultural land) mineral fertilizers consumption [2]. The majority of Polish manufacturers of phosphate fertilizers declare the use of partially acidulated phosphate rock for production of phosphate fertilizers. The interest in the PAPR-type fertilizers (*partially acidulated phosphate rocks*) taking into consideration their application in industry appeared as a result of their versatility because phosphate rock of P_2O_5 content even below 20 % by weight can be used for their production whereas for the production of superphosphates content of about 30 % by weight is recommended [3–8]. Economic factors (low mineral acid consumption during the manufacturing process and reduced raw material costs due to the possibility of using low grade deposits) supporting use of the PAPR-type fertilizer are hard to ignore.

Fertilizers of the PAPR-type are produced as a result of the reaction of phosphate rocks with non-stoichiometric amount of mineral acids (usually H_2SO_4 or H_3PO_4) [3]. The product obtained by this method contains phosphorus in the form of water or neutral ammonium citrate-soluble (these are the forms available for plants), and soluble in mineral acids. Forms of phosphorus soluble only in mineral acids are released as a result of metabolic processes of the soil microflora [9, 10]. In the PAPR-type fertilizers the content of water-soluble forms of phosphorus should be at least 40 % of the declared content of P_2O_5 and for superphosphates this ratio should be at least 93 % [11]. Partially acidulated phosphate rocks contain depending on the degree of PAPR stoichiometric norm different content of available phosphates.

Free acids consitute phosphoric acid, which is formed during the following reaction:

$$Ca(H_2PO_4)_2 \cdot H_2O + aq = CaHPO_4 + H_3PO_4 + aq$$

Free phosphoric acid is essential in curing process, which consist in an enrichment of the product in available forms of phosphorus after the reaction in a reactor. Curing is a process which takes several weeks, but it is the most dynamic during the first several hours after the end of the process that runs in the reactor. Key elements of the manufacturing process that affect the content of free acids in the fertilizer product is an appropriate selection of the degree of PAPR stoichiometric norm and the time of the reaction in the reactor [3].

Materials and methods

The aim of this study was to analyze the dynamics of fluctuations of free acids content in obtained PAPR-type preparations in relation to other commercial products available on the Polish market. Variable production parameters were as follows:

– the type of acid used in the experiment and its concentration (sulfuric acid conc. 65 % w/w and 75 % w/w; phosphoric acid conc. 62 % w/w and 69 % w/w *ie* 45 % w/w and 50 % w/w of P_2O_5, respectively);

– degree of PAPR stoichiometric norm (η_{PAPR} = 0,3; 0,5; 0,7; 1) where η is the ratio of the actual amount of mineral acid used for dissolution of raw material to the theoretical amount of acid required for complete dissolution of the phosphate rock;

– fineness of phosphate rock used for the production process (fractions: 125–160 μm and 250–500 μm).

Tunisian phosphate rock samples with P_2O_5 content of 28.5 % w/w were taken into investigation procedure. The study consisted of the determination of amount of free acids in the product immediately after the acidulation process and during curing of fertilizer after 2, 4, 7, 10 and 14 days, respectively. Preparations manufacturing process was carried out in a Atlas Syrris batch reactor equipped with an Teflon reaction vessel with aluminum coating. The reactor provided ability to automatically control the process parameters such as temperature, stirring rate and reaction time. Weight of phosphate rock sample used for the production of each fertilizer preparation was 80 g and the amount of mineral acid introduced into the reactor, which was preliminary heated up to a temperature of 85 ^oC, was dependent on the assumed degree of PAPR stoichiometric norm. The lower acid temperature than 110 ^oC, recommended for the production of superphosphate, was decided by economic reasons [12]. Reactor operating time was 5 min. After this time the batch was unloaded and subjected to chemical analysis. Determination of the composition of obtained fertilizer products was performed using a modified "acetone method" [13]. Product sample of approximately 1 g was being weighed for appropriate test. The sample was shaken subsequently for 1 h in 25 cm^3 of organic solvents mixture, composed from acetone and 1,4-dioxane mixed in a volume ratio of 1 : 1. The sample was then filtered off using qualitative filter and washed on the filter with acetone. The filtrate was titrated with 0.1 M sodium hydroxide standard solution. Titration was performed in two stages, the first step against methyl red as indicator in order to determine the content of sulfuric acid and the first stage of dissociation of phosphoric acid, the second step of the titration was performed against phenolphthalein to determine the second stage of dissociation of phosphoric acid. As a reference point for investigated fertilizer preparations, a sample produced by using degree of stoichiometric PAPR norm $\eta_{PAPR} = 1$ was used.

Obtained preparations were evaluated for their suitability in manufacturing of fertilizer products available on the market. Commercial products of one of the Polish manufacturers were used as comparative samples. The analyzed preparations were characterized by the following contents of particular forms of phosphorus declared by the manufacturer:

– Commercial Product 1 (CP1) – 13 % w/w P_2O_5 soluble in mineral acids, 8 % w/w P_2O_5 water-soluble;

– CP2 – 10 % w/w P_2O_5 soluble in mineral acids, 8 % w/w P_2O_5 water-soluble;

– CP3 and CP4 – 10 % w/w P_2O_5 soluble in mineral acids, 2.5 % w/w P_2O_5 water-soluble. These formulations differed in the percentage of other nutrients and their use.

Results and discussion

Characteristics of the main process parameters were presented in Tables 1, 3 and 5. The free phosphoric acid (% w/w) content fluctuations during curing process of PAPR-type preparations obtained by the reaction with H_2SO_4 are shown in Tables 2, 4 and 6. Phosphate rock fineness was 125–160 μm.

Table 1

Characteristics of the main parameters of the production process of PAPR-type preparations: P1, P2, P3

	P1	P2	P3
Acid type	H_2SO_4	H_2SO_4	H_2SO_4
Conc. H_2SO_4 [% w/w]	75	75	75
η_{PAPR}	0.7	0.5	0.3

Table 2

Free H_3PO_4 content during curing process of PAPR-type preparations: P1, P2, P3

t [days]	P1 [% w/w]	P2 [% w/w]	P3 [% w/w]
0	8.83	4.64	3.45
2	7.36	0.69	0.40
4	2.70	0.69	0.46
7	2.00	0.69	0.41
10	1.74	0.72	0.39
14	1.52	0.67	0.40

An analysis of the results of investigations suggest that the degree of PAPR stoichiometric norm is the main factor determining the content of free acids. The smaller it is, the less content of free acids in obtained preparations can be observed. In addition, time required for curing of the product is being reduced with decreasing values of the degree of PAPR stoichiometric norm.

Table 3

Characteristics of the main parameters of the production process of PAPR-type preparations: P4, P5, P6

	P4	P5	P6
Acid type	H_2SO_4	H_2SO_4	H_2SO_4
Conc. H_2SO_4 [% w/w]	65	65	65
η_{PAPR}	0.7	0.5	0.3

Table 4

Free H_3PO_4 content during curing process of PAPR-type preparations: P4, P5, P6

t [days]	P4 [% w/w]	P5 [% w/w]	P6 [% w/w]
0	4.33	2.48	1.67
2	2.06	0.91	0.41
4	1.96	0.89	0.37
7	2.05	0.76	0.34
10	1.96	0.84	0.33
14	2.06	0.74	0.30

Similar dependence between value of the PAPR stoichiometric norm and dynamics of changes in free acids content occurred by using lower concentrations of sulfuric acid. In addition, the initial content of free acids was decreased for the preparations obtained by acidulation of phosphate rock with H_2SO_4 of lower concentrations.

Table 5

Characteristics of the main parameters of the production process of PAPR-type preparations: P29, P30

	P29	P30
Acid type	H_2SO_4	H_2SO_4
Conc. H_2SO_4 [% w/w]	75	65
η_{PAPR}	1	1

Table 6

Free H_3PO_4 content during curing process of PAPR-type preparations: P29, P30

t [days]	P29 [% w/w]	P30 [% w/w]
0	4.71	4.85
2	1.54	2.43
4	1.24	2.42
7	1,23	2.34
10	1.20	2.27
14	1.18	2.25

Sample of PAPR product of $\eta_{PAPR} = 1$ does not indicate dependence between the concentration of H_2SO_4 used for manufacturing process and the initial amount of free acids in the product.

Tables 7, 9 and 11 present characteristics of the main process parameters. The free phosphoric acid [% w/w] content fluctuations during curing process of PAPR-type preparations obtained by the reaction with H_2SO_4 are shown in Tables 8, 10 and 12. Phosphate rock fineness was 250–500 μm.

Table 7

Characteristics of the main parameters of the production process of PAPR-type preparations: P7, P8, P9

	P7	P8	P9
Acid type	H_2SO_4	H_2SO_4	H_2SO_4
Conc. H_2SO_4 [% w/w]	75	75	75
η_{PAPR}	0.7	0.5	0.3

Table 8

Free H_3PO_4 content during curing process of PAPR-type preparations: P7, P8, P9

t [days]	P7 [% w/w]	P8 [% w/w]	P9 [% w/w]
0	9.51	2.09	3.79
2	1.18	0.60	0.52
4	0.95	0.62	0.50
7	0.80	0.64	0.40
10	0.76	0.70	0.37
14	0.64	0.59	0.36

Alteration of the fineness of raw material had no influence on the trends observed previously. The only difference in comparison with finer size fraction is more dynamic curing process, particularly in its early stages.

Table 9

Characteristics of the main parameters of the production process of PAPR-type preparations: P10, P11, P12

	P10	P11	P12
Acid type	H_2SO_4	H_2SO_4	H_2SO_4
Conc. H_2SO_4 [% w/w]	65	65	65
η_{PAPR}	0.7	0.5	0.3

Table 10

Free H_3PO_4 content during curing process of PAPR-type preparations: P10, P11, P12

t [days]	P10 [% w/w]	P11 [% w/w]	P12 [% w/w]
0	2.50	1.85	1.30
2	1.13	0.95	0.60
4	0.89	0.73	0.55
7	1.02	0.76	0.49
10	0.98	0.78	0.43
14	1.08	0.74	0.37

For reduced concentration of applied H_2SO_4 the initial content of free acids is lower than in preparations produced in reaction with the acid of higher concentration. It was found that finer size fraction of the raw material increase the efficiency of the curing process in the initial stage.

Table 11

Characteristics of the main parameters of the production process of PAPR-type preparations: P31, P32

	P31	P32
Acid type	H_2SO_4	H_2SO_4
Conc. H_2SO_4 [% w/w]	75	65
η_{PAPR}	1	1

Table 12

Free H_3PO_4 content during curing process of PAPR-type preparations: P31, P32

t [days]	P31 [% w/w]	P32 [% w/w]
0	3.58	6.59
2	1.81	3.05
4	1.56	2.57
7	1.52	2.08
10	1.47	2.00
14	1.46	1.95

Alteration in fineness of raw material in case of PAPR of $\eta_{PAPR} = 1$ does not affect dynamics of free acids content fluctuations in obtained preparations. It was observed that increasing concentration of sulfuric acid used in acidulation process caused increase of initial content of free H_3PO_4 in obtained PAPR-type fertilizer preparations. In further stages of curing process the amount of free H_3PO_4 stabilizes at a similar level, regardless of the concentration of acid used in the production process. This dependence determines effectiveness of the curing process. This dependence was not observed for $\eta_{PAPR} = 1$. In addition, fineness of raw materials used in manufacturing process had also influence on curing of the products. More dynamic curing process in its initial phase was observed for larger fractions.

Tables 7, 13 and 15 present characteristics of the main process parameters. The free phosphoric acid [% w/w] content fluctuations during curing process of PAPR-type preparations obtained by the reaction with H_3PO_4 are shown in tables 8, 10 and 12. Phosphate rock fineness was 125–160 μm.

Table 13

Characteristics of the main parameters of the production process of PAPR-type preparations: P13, P14, P15

	P13	P14	P15
Acid type	H_3PO_4	H_3PO_4	H_3PO_4
Conc. H_3PO_4 [% w/w]	69	69	69
η_{PAPR}	0.7	0.5	0.3

Table 14

Free H_3PO_4 content during curing process of PAPR-type preparations: P13, P14, P15

t [days]	P13 [% w/w]	P14 [% w/w]	P15 [% w/w]
0	2.72	2.16	1.71
2	1.95	1.63	1.04
4	1.85	1.53	0.90
7	1.81	1.48	0.72
10	1.77	1.44	0.65
14	1.50	1.34	0.55

Studies on preparations obtained by the reaction with phosphoric acid indicate that the main factor influencing the dynamics of free acids content fluctuations was the degree of PAPR stoichiometric norm. The content of free acids in preparations was reduced together with decreasing η_{PAPR} value.

Table 15

Characteristics of the main parameters of the production process of PAPR-type preparations: P16, P17, P18

	P16	P17	P18
Acid type	H_3PO_4	H_3PO_4	H_3PO_4
Conc. H_3PO_4 [% w/w]	62	62	62
η_{PAPR}	0.7	0.5	0.3

Table 16

Free H_3PO_4 content during curing process of PAPR-type preparations: P16, P17, P18

t [days]	P16 [% w/w]	P17 [% w/w]	P18 [% w/w]
0	3.46	3.14	1.89
2	2.67	2.46	1.40
4	2.48	2.35	1.19
7	2.41	2,16	1.27
10	2.32	2.13	1.07
14	2.13	1.95	1.02

Reduction of applied phosphoric acid concentration increased concentration of free acids in obtained products. In addition, the final content of free acids was higher which effects in curing process elongation.

Table 17

Characteristics of the main parameters of the production process of PAPR-type preparations: P25, P26

	P25	P26
Acid type	H_3PO_4	H_3PO_4
Conc. H_3PO_4 [% w/w]	69	62
η_{PAPR}	1	1

Table 18

Free H_3PO_4 content during curing process of PAPR-type preparations: P25, P26

t [days]	P25 [% w/w]	P26 [% w/w]
0	3.09	5.20
2	2.16	4.21
4	2.03	4.12
7	1.84	3.82
10	1.73	3.74
14	1.70	3.71

Sample of PAPR product of η_{PAPR} = 1 represented the same dependence as the products of lower values of PAPR stoichiometric norm.

Tables 19, 21 and 23 present characteristics of the main process parameters. The free phosphoric acid [% w/w] content fluctuations during curing process of PAPR-type preparations obtained by the reaction with H_3PO_4 are shown in Tables 20, 22 and 24. Phosphate rock fineness was 250–500 μm.

Table 19

Characteristics of the main parameters of the production process of PAPR-type preparations: P19, P20, P21

	P19	P20	P21
Acid type	H_3PO_4	H_3PO_4	H_3PO_4
Conc. H_3PO_4 [% w/w]	69	69	69
η_{PAPR}	0.7	0.5	0.3

Table 20

Free H_3PO_4 content during curing process of PAPR-type preparations: P19, P20, P21

t [days]	P19 [% w/w]	P20 [% w/w]	P21 [% w/w]
0	3.19	1.69	1.18
2	2.26	1.55	1.00
4	1.88	1.47	0.85
7	1.86	1.48	0.93
10	1.75	1.43	0.83
14	1.71	1.33	0.73

Fineness alteration, unlike in the case of production using H_2SO_4, did not affect the content of free acids in the product. The dependence between degree of PAPR stoichiometric norm and dynamics of free acids content fluctuation corresponded with products manufactured from the raw material of a finer size fractions.

Table 21

Characteristics of the main parameters of the production process of PAPR-type preparations: P22, P23, P24

	P22	P23	P24
Acid type	H_3PO_4	H_3PO_4	H_3PO_4
Conc. H_3PO_4 [% w/w]	62	62	62
η_{PAPR}	0.7	0.5	0.3

Table 22

Free H_3PO_4 content during curing process of PAPR-type preparations: P22, P23, P24

t [days]	P22 [% w/w]	P23 [% w/w]	P24 [% w/w]
0	3.68	3.22	2.28
2	2.98	2.38	1.55
4	2.87	2.29	1.46
7	2.63	2.,24	1.34
10	2.48	2.22	1.39
14	2.43	2.20	1.41

The results of analyzes for products of both 250–500 μm and 160–250 μm fineness revealed correlation between increase in the content of free acids with decrease in concentrations of H_3PO_4 used for the experiment.

Table 23

Characteristics of the main parameters of the production process of PAPR-type preparations: P27, P28

	P27	P28
Acid type	H_3PO_4	H_3PO_4
Conc. H_3PO_4 [% w/w]	69	62
η_{PAPR}	1	1

Table 24

Free H_3PO_4 content during curing process of PAPR-type preparations: P27, P28

t [days]	P27 [% w/w]	P28 [% w/w]
0	5.57	6.20
2	3.55	4.04
4	3.22	3.68
7	2.86	3.37
10	2.56	3.17
14	2.48	3.06

Products acidulated with H_3PO_4 demonstrated opposite dependence than products dissolved with H_2SO_4. Decrease in concentration of acid used for acidulation process caused an increase in free acids content. Unlike products acidulated with H_2SO_4, these preparations are not influenced by fineness of phosphate raw material in relation to dynamics of free acids content fluctuations.

Commercial fertilizer preparations were characterized by the following content of free phosphoric acid: CP1 = 0.18 % w/w; CP2 = 0.16 % w/w; CP3 = 0.22 % w/w; CP4 = 0.17 % w/w. The content of free acids in tested commercial products was much lower than in preparations obtained during the research. Minimal amount of free sulfuric acid in the products acidulated with H_2SO_4 were observed, what can be explained by the accuracy of the analytical method.

Conclusions

The results of tests performed under laboratory conditions indicate that the curing process carried out for production of PAPR-type fertilizers acidulated with H_2SO_4 gives more satisfactory results by using higher acid concentration. Use of lower degree of PAPR stoichiometric norm may cause local acidulation, resulting in an insufficient product homogeneity. Use of efficient stirring during acidulation process could be the possible solution for this problem. The content of free phosphoric acid in laboratory--produced preparations is higher than in tested commercial products, what may cause the physical properties instability. Future research should attempt to correlate the content of various forms of phosphates with free acids content in obtained PAPR-type fertilizer preparations.

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BADANIA DYNAMIKI ZMIAN ZAWARTOŚCI WOLNYCH KWASÓW MINERALNYCH W CZASIE CYKLU PRODUKCYJNEGO NAWOZÓW TYPU PAPR

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Abstrakt: Fosforyty częściowo rozłożone (*partially acidulated phosphate rock* – PAPR) stanowią odrębną grupę nawozów fosforowych. Nawozy typu PAPR powstają w wyniku reakcji fosforytów z niestechiometryczną, względem reakcji rozkładu fluoroapatytu zawartego w fosforycie, ilością kwasu mineralnego. Celem badań była analiza dynamiki zmian zawartości wolnych kwasów w otrzymanych preparatach typu PAPR. Parametrami produkcyjnymi, które ulegały modyfikacji, były: rodzaj stosowanego kwasu i jego stężenie, stopień normy stechiometrycznej PAPR (η_{PAPR}), uziarnienie fosforytu użytego do produkcji. Badania polegały na oznaczeniu ilości wolnych kwasów w produkcie zarówno bezpośrednio po zakończeniu procesu produkcji, jak i podczas dojrzewania nawozu odpowiednio po czasie 2, 4, 7, 10 i 14 dób.

Słowa kluczowe: fosforyty częściowo rozłożone (PAPR), wolne kwasy, nawozy fosforowe, fosforany przyswajalne

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SONOCHEMICAL METHODS OF REMOVING THE ORGANIC IMPURITIES FROM WATER

METODY SONOCHEMICZNE USUWANIA ZANIECZYSZCZEŃ ORGANICZNYCH Z WODY

Abstract: Surface water is characterized by changeability of composition/content and organic intensity of both natural and anthropogenic impurities. As a result of chemical changes developed in the process of oxidation and disinfection, impurities and organic admixtures may become harbingers of damaging by-products of these processes (OBP/DBP). Thus, removing organic impurities from water is a crucial problem in water technology, which predominates in the research subject matter. The decrease of water organic contamination can be obtained by the following methods: coagulation, adsorption, oxidation and membrane processes. The use of different solutions in this field has been researched recently as well. The present research is aimed at so-called 'hybrid methods' combining chemical methods with physical factors. For instance, the processes of profound oxidation, in which ultraviolet or ultrasounds are used as hydroxyl radical initiators.

The literature based research presented in the first part of the article indicates that the unconventional ultrasound method was described on the basis of the effects obtained with the use of prepared water (most frequently with commercial humic acids preparation). Due to the composition of humic substances in surface water (where fulvic acids are predominant), the verification of these effects in natural water environment is justified. In the following part of the article we presented the research results concerning the effects of the use of ultrasound field with the high intensity and constant frequency of 24 kHz. The substrate for the research was surface water. The laboratory tests were conducted at changing sonification time and vibration amplitude. Changes in water organic impurity were controlled mainly by the TOC index analyses. What was researched as well, was the influence of water pH on the removal of organic impurities measured by the TOC index. In order to characterize it the experiments were conducted at natural water pH, alkaline and acid reaction. At the beginning of the experiment and after the use of the researched sonochemical method we marked the chosen parameters: TOC, DOC, oxygen consumption, UV_{254}, and pH. On the basis of these parameters we evaluated the effects of the process depending on the changing ultrasound parameters.

Keywords: water treatment, organic impurities, ultrasounds

Nowadays, water is exposed to rapid contamination, and it is presently hard to find water of natural physicochemical composition. It is obvious that contaminants find their way to surface water as a result of human activity, but the concentration of natural components is determined by natural conditions. However, in water subjected to

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treatment any undesirable substance that is to be removed in the technological process is considered a contaminant, regardless its origin. The reduction of the organic contamination of water is achieved by the following methods: coagulation, adsorption, oxidation, and membrane processes [1].

Humus substances (HS) are present in almost all natural waters and may account for 60–80 % of the overall mass of *natural organic matter* (NOM) occurring in water and bottoms [2]. Their contents depend on the type of soil, from which they have been washed out, water contact time, the chemical composition and pH of water. The predominant substances dissolved in surface water are fulvic acids, constituting up to 87 % HS, their sodium or/and potassium salts; *humic acids* (HA) (2 % HS) and hymatomelanic acids are less frequent. In ground water, HA are prevalent. HS present in water can be a source of an undesirable smell and impart a colour (ranging from light yellow to dark brown) to the water, being characteristic of a specific group of acids. In addition, HS form complex compounds with heavy metals, iron, aluminium, and pesticides occurring in natural water, to maintain these pollutants in a dissolved form. Previously, only the water colour and smell justified the removal of those compounds from water being treated, as humus substances naturally occurring in quantities below 100 g/m^3 are not hazardous to health. However, organic compounds present in water subjected to oxidation and disinfection processes are responsible for the formation of undesirable halogenated hydrocarbons referred to as *oxidation/disinfection by-products* (OBP/DBP). They exhibit adverse health properties, and even mutagenic or carcinogenic effects [3].

In Poland, gaseous chlorine is still most frequently used in disinfection processes; chlorine dioxide and ozone are less frequently used, and ultraviolet radiation is employed sporadically [4]. The chlorination of water (Cl_2 treatment) leads to a reaction of substitution in organic matter molecules, as a result of which toxic chlorinated organic compounds, such as trihalomethanes (with their precursors being mainly HS), halogenoacetic acids, halogenoketones, or trichlorophenols, form. To reduce the risk of the chlorination reaction occurring, gaseous chlorine is substituted with chlorine dioxide with higher oxidation potential. The by-products of oxidation with ClO_2 may include chlorates(V), and chlorites(III), which are also categorized into DBPs. The application of ozone intended to eliminate the formation of chlorinated organic compounds causes the oxidation of inorganic compounds and oxidation of organic compounds into carbon dioxide and water. However, sometimes it results in harmful intermediate products, such as ketones, aldehydes, or carboxylic acids. Pre-oxidation processes result in a reduction of water colour, but this is not always indicative of a sufficient removal of organic matter [5]. Therefore, new possibilities for removing biodegradation-resistant organic substances are being searched for.

The need for minimizing the amount of reagents used in the water treatment process and reducing the *total organic carbon* (TOC) content to the lowest possible level has initiated searches for organic contaminant removal methods that would be more effective, while not leading to the formation of OBP/DBP and reducing the risk to the natural environment. Hence, AOP (*Advanced Oxidation Process*) methods are increasingly popular and of great interest. These are methods that combine the chemical

oxidation process with physical factors. The following can be employed in various combinations: ultrasounds, ultraviolet radiation, and additions of catalysts (TiO_2, MnO_2, Fe^{2+} and Fe^{3+}). The application of these methods results in the formation of hydroxyl radicals ($OH^{\cdot}$) of a very high oxidation-reduction potential of 2.8 V. Moreover, hydroxyl radicals are characterized by the ability to non-selectively react with many organic compounds, whereby they are sufficiently effective in removal of organic compounds by transforming them into carbon dioxide, water and inorganic compounds. It is important to note that even with an incomplete oxidation of organic matter simpler forms with smaller molecular masses are obtained, which are susceptible to bio-degradation [6, 7].

Literature review

Owing to the ultrasonic cavitation phenomenon, the *ultrasonic* (US) field can also independently initiate the formation of hydroxyl radicals, hydrogen dioxide, and ozone. Free radicals generated become the main source of so-called sonochemical reactions. Their intensity, with additional combination of ultrasounds (UD) with chemical reagents or a gaseous phase (air, argon) introduced, may be increased even further. By reacting with the molecules of various substances (dissolved or suspended in water), hydroxyl radicals cause the chemical decomposition, reduction or destabilization of those molecules. The effect of sonochemical methods on the degradation of numerous organic compounds has been confirmed by some studies; however, model substances, such as carbon tetrachloride (CCl_4) or phenol were mostly used in those studies. Studies on the application of an unconventional ultrasonic method for the removal of organic water admixtures are described mainly based on the effects obtained for water solutions prepared using commercially available humic acid (HA) preparations. However, HAs do not represent the surface water environment, where *fulvic* acids (FA) are predominant. Example results of those studies are presented below.

Naddeo et al [8] studies the effectiveness of ultrasonic removal of natural organic matter (NOM). The studies showed that the degree of NOM removal was dependent on the duration and intensity of sonification. Solutions, each of a volume of 300 cm^3, were prepared using a commercial HA preparation supplied by Aldrich. Sonification was conducted using a VCX-750 disintegrator by Sonics & Materials, equipped with a 1.3 cm-diameter sonotrode (with a frequency of 20 kHz, and a field intensity from to 42 W/cm^2). The NOM content was measured as TOC and absorbance (at λ = 220–620 nm). The authors noted a reduction in HA concentration (measured as TOC) at a level from 24.5 % to 34.9 % after 20 minutes of sonification and at a field intensity of 42 W/cm^2. The results of absorbance measurements indicated that a reduction in organic matter contents had resulted, whereas the values of the TOC index did not show the same effectiveness. The increase in the absorption of UV_{254} radiation and the increase in the turbidity of solutions subjected to sonification are explained by the authors by an aggregation of split fragments of humic acids. They also point out that the TOC measurement is not affected by these phenomena, as intermediate forms of organic compounds are also subjected to analysis. Owing to this, the determination of the TOC index is more universal.

Y.-S. Ma and J.-G. Lin [9] investigated the effect of different methods using ultrasounds on HA removal from water. For this purpose, they compared the effectiveness of three systems: ultrasounds, O_2 and ultrasounds/O_2. An ultrasonic generator with the following parameters was employed: frequency, 20 kHz; power, up to 160 W; and intensity, 126.5 W/cm^2. The parameters representing the effectiveness of a specific system were: oxidation-reduction potential, BOD_5 (*Biochemical Oxygen Demand, 5-day*), and TOC. The substrate for the tests were solutions prepared from a HA preparation (HO161, supplied by Tokyo Chemicals) and deionized water. The initial TOC concentration was 18.6 ± 0.4 mgC/dm^3. The coupled ultrasound/O_2 process turned out to be the most effective in HA mineralization. After 120 minutes of sonification the TOC was reduced by 52.6 %. Not combined, these methods only showed a TOC reduction in the case of US (26.5 %) and O_2 (27.4 %) alone. The authors showed that the hybrid process (US/O_2) increases TOC removability and reduces chlorine demand.

Chemat et al [10] state that an alternative for conventional oxidation methods is the application of a high-intensity (>10 W/cm^2) US field in combination with the use of a commercial oxidizer, that is H_2O_2. In the researchers' assumption, this system was supposed to provide an increase in the degradation and mineralization of organic compounds. A field of an intensity of 20 kHz and a power of 50 W (20 W in the centre) was used. The determinations of TOC and UV_{254} absorbance, both correlating with the HS contents, were made. The reduction in the value of absorbance indicated a complete removal of synthetic HAs after 60 minutes of sonification, which was not confirmed by the TOC analysis (a removal of a mere 40 %). The authors explain this by the accuracy of the TOC assay that detects also intermediate products. They claim that after 180 minutes of sonification the reduction of TOC was at a level of 90 %. At the subsequent stage of experiment, the effectiveness of an oxidizer aided by a magnetic stirrer (50 rpm) and an oxidizer aided by ultrasounds was compared. The mineralization of organic compounds in the first model was 25 %, while for the same contact time of the oxidizer, but US aided, a mineralization degree of 90 % was achieved. The aim of the investigation was also to determine the effect of pH and H_2O_2 concentration on the HA degradation. The best results (50 % relative to absorbance at λ = 254 nm) were obtained for pH = 3. The analysis of the effect of oxidizer dose (0, 50, 100, 200, 300, 500) mg/dm^3 for a solution of 100 mg/dm^3 HA showed that the best process effectiveness had been achieved for an H_2O_2 concentration of 200 mg/dm^3.

Results of studies on the removal of THMs (Trihalomethanes), compounds formed as a result of the chlorination of *eg* humus substances, from water solutions are also known. In an experiment described by H. Shemer and N. Narkis [11], an ultrasonic processor (20 kHz, field intensity 3.75 W/cm^2) was employed. The authors made a summary of THM removal effectiveness as a function of sonification time. Within 180 minutes of US field operation, 100 % $CHCl_3$, 80 % $CHBr_3$ and 60 % CHI_3 were removed sonochemically.

Thus, the literature review indicates that the effectiveness of the unconventional ultrasonic method described in the hitherto existing studies on prepared waters not always can be taken into account in the water treatment technology. From the point of

view of the composition of humus substances, particularly those contained in surface water (where fulvic acids are predominant), it is justifiable to verify these effects in a natural water environment.

Experimental

The aim of the experimental studies carried out was to assess the effectiveness of reducing the contents of organic and inorganic compounds in surface water by sonochemical methods with the use of a high-intensity ultrasonic field. Natural water was taken from the water reservoir at Poraj near Czestochowa, Poland. Samples, each of a volume of 500 cm^3, were subjected to sonification. An Hielscher UP400S disintegrator with the following parameters was used:

– vibration frequency: 24 kHz,
– effective power: 400 W (300 W in water),
– H22 sonotrode diameter: 22 mm (S = 3.8 cm^2),
– maximum ultrasonic field intensity: 85 W/cm^2 (for the selected sonotrode and a maximum amplitude of A = 60 μm).

The effect of the vibration amplitude and sonification time, as basic ultrasound parameters determining the level of field intensity and energy input, were assessed. To this end, the following combinations of ultrasonic parameters was examined: a constant vibration amplitude and the sonification time variable in the range from 2 to 8 minutes, and a constant sonification time with a different vibration amplitude of 18, 36, and 54 μm, respectively. As each of the individual humus substance fractions dissolves in its specific environment, the experiment was conducted at the pH of natural water (pH = 7.98), at an alkaline reaction (pH ~ 9), and at an acid reaction (pH ~ 3). The reaction of water was changed by adding solutions of HCl (1:1) and 30 % NaOH to it. The examinations of water samples at the above-mentioned pH values were also aimed at determining the effectiveness of the oxidizing effect of radicals on organic substances, depending on the reaction of a sample. At the beginning of the experiment and upon the application of the methods under examination, the assays and measurements of TOC, DOC (Dissolved Organic Carbon), oxygen consumption, UV_{254} and pH were made. On their basis, the effects of a process were assessed, depending on the variable ultrasonic parameters. The basic assay used for the assessment of change in the contents of organic compounds in the water was the TOC index. The determination of TOC and DOC were made according to the standard PN-EN 1484:1999 using a Multi N/C 2000 analyzer (the DOC index was determined after the sample had been filtered through a 0.45 μm mesh filter). Absorbance was measured with an Helios α Spectrometer in 5 cm-long optical cells. Oxygen consumption (permanganate value) was determined according to the standard PN-EN ISO 8467:2001.

Analysis of investigation results

The examined water was characterized by the following parameters: TOC = 10.27–14.69 mgC/dm^3; DOC = 8.01–11.97 mgC/dm^3; oxygen consumption: 9.79–10.4 mgO_2/dm^3;

pH = 0.92–8.55; turbidity: 8.31–4.9 NTU. These indicate an increased organic contamination of water.

Two combinations of ultrasonic field parameters were applied. The first of them consisted in the variation of the amplitude with a selected fixed sonification time. The effect of ultrasonic parameters visible in the examination results (Table 1) indicates that the vibration amplitude has a major importance for the process effects. Increasing the vibration amplitude enables an increase in ultrasonic field intensity to be obtained, which for A = 54 μm amounted to approx. 75 W/cm^2. For the highest amplitude value and a sonification time of 3 minutes, the most favourable reduction of the TOC index (by 6.64 mg C/dm^3) was noted.

Table 1

Effect of amplitude and sonification time
on the selected indicators of water (natural water pH) [12]

Index	Before UD* natural water pH	After UD					
		3 min			18 μm		
		18 μm	36 μm	54 μm	2 min	5 min	8 min
pH	7.92	7.78	7.74	7.71	7.8	7.73	7.7
TOC [mgC/dm^3]	14.69	11.14	10.11	8.05	9.56	10.62	11.02
DOC [mgC/dm^3]	11.97	—	—	7.88	—	—	10.72
Oxygen consumption [mgO$_2$/dm^3]	10.4	9.15	8.32	7.71	8.74	8.32	7.9
Absorbance UV$_{254}$	1.01	1.19	1.08	1.02	1.1	1.09	1.31

* UD – ultrasounds.

For these parameters, the effectiveness of TOC reduction was 45 % (Fig. 1A), whereas for DOC it was lower, amounting to 35 %. As a result of the sonification process, dissolved fractions, accounting for more than 80 % TOC for crude water,

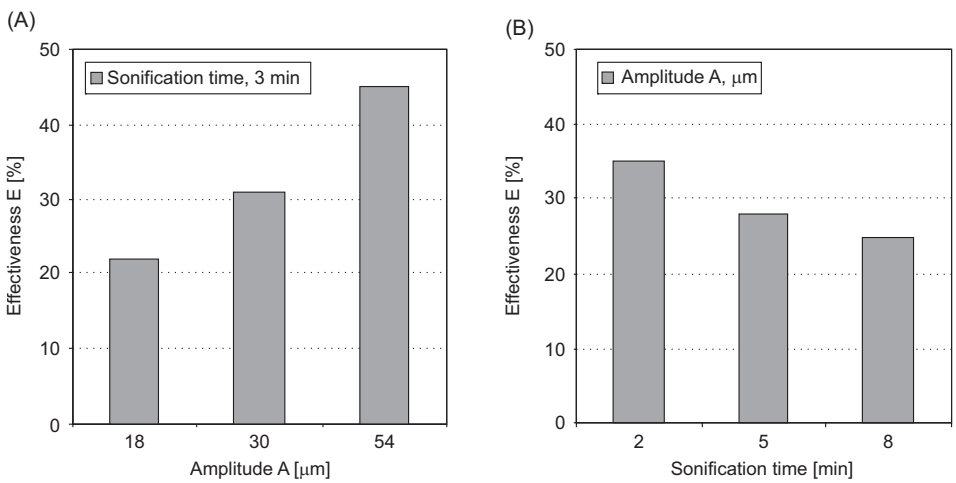

Fig. 1. The effectiveness of the TOC decreasing at the changeable vibration amplitude (t = 3 min) (A) and at the changeable sonification time (A = 18 μm) (B)

increased their share in TOC even further. The effectiveness of sonochemical organic matter removal is confirmed also by the decrease in oxygen consumption. This indicator decreased with increasing vibration amplitude in the range examined. The most favourable effect was noted for the sample sonificated at the highest field intensity (for A = 54 μm) – a reduction in oxygen consumption by more than three units. The pH values of the water tested (with pH = 7.92) slightly decreased upon sonification and remained at a comparable level for the amplitude values examined. However, no effect of decreasing water contaminant concentration was confirmed in the UV_{254} absorbance determination results. The lack of relationship between the TOC determinations and the UV_{254} absorbance values found by other researchers, as indicated in the literature review, suggests different relationships to exist in the results of sonification of HA-prepared and natural water samples. In the second combination of parameters, fixed vibration amplitude of 18 μm and a variable sonification time were applied. Lengthening the sonification time decreased the effectiveness of the organic compound removal process, as measured by the TOC index. The best effectiveness equal to 35 % (a reduction in TOC by 5.13 mgC/dm^3) was obtained for the shortest sonification time of 2 minutes (Fig. 1B). The reduction of the DOC index (with a lower efficiency compared with TOC) increased again the share of dissolved carbon in the determination of TOC. Similarly as in the first parameter combination, a reduction in the oxygen consumption of sonificated water samples was noted with lengthening time. The decrease of oxygen demand in this experiment might indicate that the organic contamination was reduced by means of sonochemical oxidation. Increasing the sonification time, similarly as the amplitude, slightly decreased the pH of water. Favourable results of TOC reduction in natural water tested were obtained after a sonification time shorter than stated in the results for HA-prepared water samples, as presented in the literature review.

Further tests were aimed at the determination of the effect of the reaction of water sonificated (pH ~ 3 and pH ~ 9) on the variation of organic contaminant contents, as measured both directly as TOC and indirectly as oxygen consumption and UV absorbance. The results of tests for water samples with acid reaction (Table 2) do not confirm the favourable effect found at the natural water pH. Upon the application of ultrasounds the TOC index decreased only slightly. Its values remained, however, at

Table 2

Effect of amplitude and sonification time on the selected indicators of water (acid reaction)

Index	Before UD acid reaction	After UD					
		3 min			18 μm		
		18 μm	36 μm	54 μm	2 min	5 min	8 min
pH	3.01	2.99	2.97	2.96	3.0	2.99	2.98
TOC [mgC/dm^3]	10.27	9.68	9.80	10.0	10.22	9.87	9.90
DOC [mgC/dm^3]	6.94	—	—	6.55	—	—	6.69
Oxygen consumption [mgO_2/dm^3]	8.98	8.57	8.16	7.75	8.57	8.16	8.16
Absorbance UV^5_{254}	0.82	0.94	0.95	0.96	0.94	0.94	0.95

a comparable level, without any distinct relationships with time and amplitude. Water samples with a corrected pH value of pH ~ 3 were characterized by a smaller share of dissolved organic carbon in TOC concentration. This is associated with the solubility of humus substances, which increases with increasing water basicity.

In the subsequent phase of the experiment, the effect of basic water reaction on the effectiveness of sonochemical water HS removal was examined. For this purpose, surface water was alkalized to pH ~ 9. Similarly as for the natural and acidified water samples, the pH of the water sample was slightly decreased by ultrasonic processes. However, no favourable effects of organic water contamination removal was noted under these conditions, as indicated by the values of the TOC and DOC indices and UV_{254} absorbance (Table 3). At the same time, a reduction in water oxygen consumption was confirmed in the basic medium, irrespective of the ultrasonic parameter combination used. This might suggest an oxidation of inorganic compounds that are also covered by this indicator.

Table 3

Effect of amplitude and sonification time on the selected indicators of water (alkaline reaction)

Index	Before UD alkaline reaction	After UD					
		3 min			18 μm		
		18 μm	36 μm	54 μm	2 min	5 min	8 min
pH	9.04	9.00	8.98	8.91	9.00	8.98	8.91
TOC [mgC/dm^3]	11.73	14.54	14.66	14.23	13.59	14.01	14.10
DOC [mgC/dm^3]	8.08	—	—	9.40	—	—	10.36
Oxygen consumption [mgO_2/dm^3]	12.24	11.83	11.42	11.02	11.42	10.61	10.2
Absorbance UV^5_{254}	0.94	1.03	1.06	1.11	1.05	1.05	1.07

The analysis of the results obtained at different water pH values may lead to the conclusion that the form of occurrence of compounds prevailing in the composition of humus substances is crucial to the effectiveness of the process under examination. Water reaction influences also the intensity of radical reactions that provide a basic mechanism of sonochemical oxidation of organic contaminants. In the surface water examined, the process effects are largely associated with the susceptibility of fulvic acids and its compound to ultrasounds. The solubility of HS increases with increasing pH, which has an effect on the sonochemical oxidation results. At acid and neutral reactions, HAs can occur mainly in the form of colloids. It is only in a basic medium that they pass into dissolved forms. However, FAs, being prevalent in surface water, undergo dissociation already at a lower pH. Therefore, no decrease in organic contamination indices was noted at acid reaction, whereas favourable effects were observed at a slightly alkaline reaction of natural water of pH = 7.92. The increase of the amplitude (intensity) intensifies these effects in the water examined (Fig. 2A). A significant TOC reduction took place already within the first 2 minutes of sonification at an amplitude of 18 μm (Fig. 2B). On the other hand, no organic contaminant removal by the ultrasonic method occurred in a strongly basic medium (pH ~ 9). The basicity of water inhibits the processes of oxidation of the radical type. The acceptors of $OH^{\bullet}$

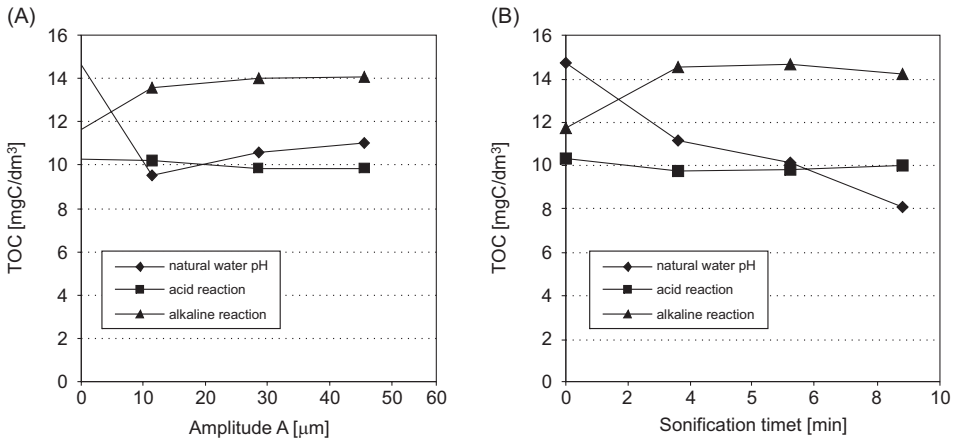

Fig. 2. The influence of the vibration amplitude (t = 3 min) (A) and the sonification time (A = 18 μm) (B) on the TOC index changes depending on the water pH

radicals are then mainly bicarbonate and carbonate ions (that determine the water basicity).

Summary

Organic contaminants, as measured by the TOC index, were removed from natural water to a different extent, depending on the ultrasonic field parameters and pH values applied. The effect of ultrasonic field intensity, as defined by the vibration amplitude, on process effectiveness was confirmed for water investigated. The maximum effectiveness of the ultrasonic method, *ie* 45 %, was achieved for the highest amplitude of 54 μm and short exposure time of 3 minutes. The effect of removing water contaminants in the sonochemical oxidation processes was indicated by a reduction in the oxygen consumption index. The effect of water pH on the process effectiveness remains disputable. From the preliminary investigation results provided herein it can be inferred that the medium of natural water pH is most advantageous. However, this does not resolve the question of validity of the obtained effect to other natural waters, *ie* those with a different pH. For establishing the pH range advantageous from the point of view of ultrasonic field application, investigation will be continued for surface waters with a different pH. To determine preliminary relationships, water samples prepared with fulvic acids, which are prevalent in the surface water medium, will be used. Further experiments will be aimed also at the determination of the effectiveness of the H_2O_2 and US hybrid process in the removal of organic water contamination.

Acknowledgements

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METODY SONOCHEMICZNE USUWANIA ZANIECZYSZCZEŃ ORGANICZNYCH

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Politechnika Częstochowska

Abstrakt: Wody powierzchniowe charakteryzują się zmiennością składu i stężeń organicznych zanieczyszczeń zarówno naturalnych, jak i antropogennych. Zanieczyszczenia i domieszki organiczne, na skutek przemian chemicznych wywołanych utlenianiem i dezynfekcją, mogą stać się prekursorami szkodliwych ubocznych produktów tych procesów (UPU/UPD). Usuwanie zanieczyszczeń organicznych jest więc istotnym problemem technologii wody, który dominuje w tematyce badawczej. Zmniejszenie organicznego zanieczyszczenia wody uzyskuje się metodą: koagulacji, adsorpcji, utleniania i procesów membranowych. Prowadzone są także badania nad możliwością zastosowania w tym celu także innych rozwiązań. Są one skierowane na tzw. metody hybrydowe, łączące metody chemiczne z czynnikami fizycznymi. Przykładem mogą być procesy pogłębionego utleniania, w których jako inicjatory rodników hydroksylowych wykorzystuje się promieniowanie ultrafioletowe lub ultradźwięki.

Badania literaturowe, stanowiące pierwszej część artykułu, wskazują, że niekonwencjonalna metoda ultradźwiękowa jest opisana na podstawie efektów uzyskanych dla wód preparowanych (najczęściej komercyjnymi preparatami kwasów humusowych). Z punktu widzenia składu substancji humusowych w wodach powierzchniowych (gdzie dominują kwasy fulwowe), uzasadniona jest weryfikacja tych efektów w środowisku wód naturalnych. W drugiej części artykułu przedstawiono wyniki badań dotyczące efektów stosowania pola ultradźwiękowego o dużym natężeniu i stałej częstotliwości 20 kHz. Substratem badań była woda powierzchniowa rzeczna pobrana z dwóch źródeł. Testy laboratoryjne prowadzono przy zmiennym czasie nadźwiękowiania i różnej amplitudzie drgań. Kontrolę zmian zanieczyszczenia organicznego wody zapewniały głównie analizy wskaźnika OWO. Na początku eksperymentu i po zastosowaniu badanych metod sonochemicznych, wykonywano oznaczenia wybranych parametrów: OWO (TOC), RWO (DOC), utlenialności, UV_{254} i pH. Na ich podstawie oceniano efekty procesu w zależności od zmiennych parametrów ultradźwiękowych.

Słowa kluczowe: uzdatnianie wody, zanieczyszczenia organiczne, ultradźwięki

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EFFECTIVENESS OF WATER TREATMENT BY MEANS OF INTEGRATED PHOTOCATALYSIS AND ULTRAFILTRATION PROCESSES

EFEKTYWNOŚĆ OCZYSZCZANIA WÓD W ZINTEGROWANYM PROCESIE FOTOKATALIZA–ULTRAFILTRACJA

Abstract: In water treatment, photocatalysis is an advanced oxidation method which is becoming an alternative to classical processes. Its application results in the total degradation of contaminants present in water which are decomposed to carbon dioxide and water. The results of the study presenting the dependence of a catalyst dose, exposure time and feed water quality on the effectiveness of *natural organic matter* (NOM) photodegradation are discussed in this article. The research involved the treatment of surface water taken from a lake in Chelm Slaski area (southern Poland) and synthetic water which contained fulvic and humic acids. Photocatalysis was carried out using three catalyst (TiO_2) doses of 0.25, 0.5, 0.75 g/dm^3 and an exposure time of 30–180 min. It has been found that it exhibited high effectiveness in removing NOM which increased with prolonged exposure time and depended on a catalyst dose and composition of treated waters. The choice of combined photocatalysis and ultrafiltration was fully justified, because ultrafiltration enabled the separation of catalyst particles from clean water.

Keywords: NOM, fulvic and humic acids, photocatalysis, ultrafiltration, water treatment

Introduction

The increasing contamination of waters and shrinking resources of drinking water necessitate a search for and then implementation of new and more effective methods for treating waters. The conventional techniques (coagulation, sedimentation, filtration, adsorption on activated carbon) do not yield a complete removal of contaminants, but merely pass them to another phase, while the application of modern advanced oxidation techniques (photocatalysis) may lead to a complete degradation of contaminants in

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water, decomposing them to carbon dioxide and water. Photocatalytic oxidation using the TiO_2 semiconductor is especially popular, because the anatase TiO_2 catalyst has a lot of advantages. It displays a fast reaction time, high photochemical stability and reactivity, stability in a water system over a wide pH range, and low toxicity to the environment. Photocatalysis can be supplemented with microfiltration and ultrafiltration. During photocatalysis, which uses a suspended catalyst in a solution, a membrane can be an effective barrier for catalyst particles, enabling their recovery and return to the photoreactor [1, 2].

The study was aimed at assessing the effectiveness of combined photocatalysis (photooxidation process) and ultrafiltration (membrane process) to remove *natural organic matter* (NOM) from waters.

Methods

The tests were carried out on a bench scale, using synthetic water which contained *fulvic acids* (FA) produced by Beijing Multigrass Formulation Co. Ltd., *humic acids* (HA) manufactured by Sigma-Aldrich and surface water collected from a lake near Chelm Slaski (southern Poland). The characteristics of the waters are given in Table 1.

Table 1

Characteristics of waters

Parameter	Synthetic water	Surface water
pH	5.52	5.50
Conductivity [mS/cm]	0. 562	0.261
Turbidity [NTU]	2.49	1.60
Absorbance $UV_{254\ nm}$ [l/m]	48.9	29.30
TOC [gC/m^3]	12.45	13.46
DOC* [gC/m^3]	10.01	12.70
Colour* [gPt/m^3]	75	33
Alkalinity [$gCaCO_3/m^3$]	10	10
NO_3^- [g/m^3]	—	18.63
SO_4^{2+} [g/m^3]	—	98.23
Cl^- [g/m^3]	—	309.1
SUVA** [$m^3/gC \cdot m$]	4.88	2.31

* Samples filtered through a 0.45-μm filter; ** specific absorbance in ultraviolet radiation UV_{254}/DOC.

The integrated photocatalysis – ultrafiltration system (Fig. 1) covered sequential photocatalysis followed by ultrafiltration. NOM photodegradation was carried out at 25±2 ^oC in a Heraeus reactor with a 150 W multi-wave UV lamp. The P25 titanium dioxide TiO_2 produced by Degussa was used as the photocatalyst. The photocatalytic oxidation of natural organic matter from the waters was conducted over a time range of

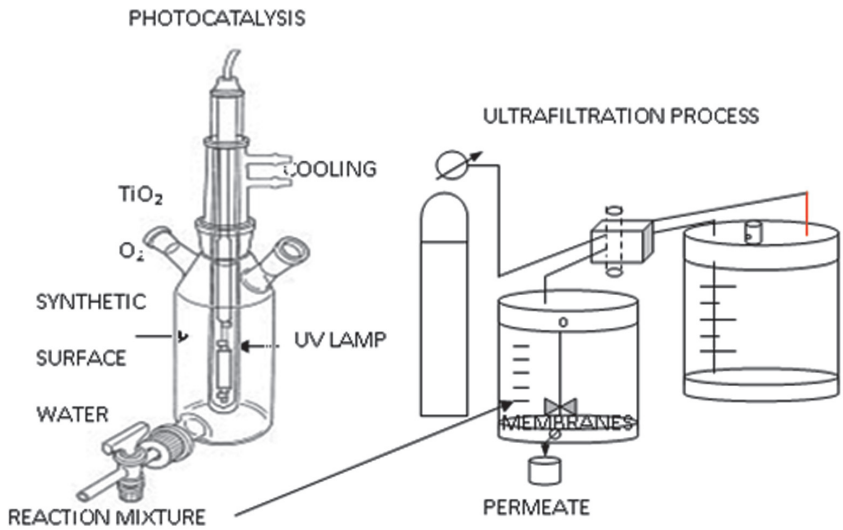

Fig. 1. Scheme integrated photocatalysis – ultrafiltration system

30–180 min with intervals after every 30 min. The ultrafiltration of the reaction mixture was carried out in the dead-end mode, using a polyethersulfone UF membrane (for synthetic water) and polyvinylidene fluoride (UF) membrane (for surface water), cut-off of 30 kDa. The membrane filtration was conducted at a transmembrane pressure of 0.1 MPa, using the Millipore CDS-10 System described in paper [3]. The tests examined the effect of TiO_2 catalyst dose (0.25; 0.50; 0.75 g/dm^3) and exposure time (30–180 min) on the effectiveness of NOM photodegradation. The effectiveness of the processes was determined measuring *dissolved organic carbon* (DOC) with a HiperTOC analyzer produced by Thermo Electron Corporation, absorbance at a wavelength of 254 nm with a Cecil UV-VIS CE 1021 spectrohpotometer and colour with a Merck Nova 400 photometer.

Results and discussion

Synthetic water

The effectiveness of photocatalytic oxidation is affected by a number of operational parameters [2, 4–6], the most important being a catalyst dose, exposure time, quality, temperature, pH and the concentration of dissolved oxygen in water. Table 2 shows the results of natural organic matter removal from synthetic water (humic and fulvic acids) by photocatalysis and the integrated system of photocatalysis – ultrafiltration. The tests examined the effect of a catalyst dose and exposure time on the photocatalytic oxidation of organic matter in water.

Table 2

The effectiveness of natural organic matter removal from water by photocatalysis and integrated photocatalysis-ultrafiltration system (*synthetic water*)

Exposure time [min]	Reduction in value [%]								
	Catalyst dose [$gTiO_2/dm^3$]								
	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75
	Colour			DOC			UV_{254}		
30	87.5	—	92.9	50.8	62.1	76.8	83.6	93.3	98.1
60	93.8	—	92.9	70.5	86.8	95.4	97.3	99.1	99.2
UV 60 – UF*	—	—	—	72.5	93.6	95.2	97.7	98.8	98.7
90	100	—	92.9	92.2	89.6	100	99.8	99.5	99.4
120	100	—	100	94.1	93.1	100	99.3	99.8	100
UV 120 – UF*		—		95.1	95.5	100	99.1	99.5	100
150	100	—	100	97.2	91.2	100	99.8	98.8	100
180	100	—	100	97.8	95.3	100	100	99.8	100
UV 180 – UF*		—		98.4	96.1	100	100	100	100

* UV exposure + ultrafiltration.

Surface water

Like synthetic water in Table 2, Table 3 shows the results of natural organic matter removal from surface water by photocatalysis and integrated photocatalysis-ultrafiltration system.

Table 3

The effectiveness of natural organic matter removal from water by photocatalysis and integrated photocatalysis-ultrafiltration system (*surface water*)

Exposure time [min]	Reduction in value [%]								
	Catalyst dose [$gTiO_2/dm^3$]								
	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75
	Colour			DOC			UV_{254}		
30	28.4	60.6	54.5	40.1	48.2	41.5	77.4	83.7	79.0
60	55.3	72.7	69.7	53.5	65.9	57.5	86.3	89.9	90.6
UV 60 – UF*	68.4	72.7	72.7	56.7	68.0	67.9	86.3	98.4	91.9
90	47.4	69.7	66.6	64.1	67.8	67.2	91.1	94.8	89.1
120	50.0	66.6	66.6	71.4	69.3	83.1	91.1	96.7	96.8
UV 120 – UF*	68.4	72.7	70.4	71.8	79.2	90.4	93.5	98.7	97.3
150	55.3	72.7	69.7	69.8	76.7	81.2	92.5	99.7	91.6
180	68.4	72.7	66.6	70.5	83.2	89.6	95.6	99.6	94.4
UV 180 – UF*	73.7	84.8	78.8	73.1	88.0	94.5	95.2	99.9	98.3

* UV exposure + ultrafiltration.

The photocatalytic oxidation of natural organic matter carried out in the synthetic and surface water revealed an effect of water matrix (physical and chemical composition) on the effectiveness of photooxidation. In the synthetic water, a reduction in DOC at a level of 51–77 % and absorbance of 84–98 % (depending on a catalyst dose) were observed as early as 30 minutes after the photocatalysis had started. As to the surface water, DOC and absorbance reduction were lower and reached 40–48 % and 77–84 %, respectively. The complete removal of colour after a 90-minute exposure was obtained for the synthetic water while in the surface water, it was 70 % maximum and did not exceed the standard for drinking water of 15 mgPt/dm^3 [7]. That was probably connected with the quality of treated water. Water quality definitely affects the efficiency of advanced photocatalytic processes with TiO_2. The tested waters had identical alkalinity but different SUVA parameter which is an indicator of the qualitative composition of water. Waters that contain considerable amounts of hydrophobic, aromatic, macromolecular humic organic matter exhibit a SUVA value of ≥ 4 m^2/gC. On the other hand, the hydrophilic, low-molecular and non-humic matter exhibit SUVA ≤ 2 m^2/gC. The $SUVA_{254}$ values over a range of 2–4 m^2/gC indicate the presence of a mixture of hydrophilic and hydrophobic matter in water [8]. SUVA for the synthetic water, which displayed higher photocatalysis effectiveness than the surface water, was 4.88. This proves that humic matter is considerably degraded during photocatalysis. The exposure to UV radiation in the presence of titanium dioxide results in the breaking of the aromatic rings of humic and fulvic acids and their partial mineralization.

The higher effectiveness of photocatylsis for the synthetic water than surface water is connected to the fact that natural water is composed of inorganic ions which can affect the efficiency of photocatalysis. The authors of paper [6] found that the use of a suspended catalyst in the presence of inorganic ions in water can cause the deactivation of the photocatalyst surface. At certain concentrations, some ions (Cu^{2+}, Al^{3+}, PO_4^{3-}) reduce the effectiveness of the photocatalytic reaction, while others (Ca^{2+}, Mg^{2+}) do not affect its efficiency. The literature [6] also reports that the activity of a catalyst surface can be inhibited by the presence of NO_3^-, Cl^-, SO_4^{2-}, HCO_3^- ions in water. Such inhibition mechanisms normally involve competition in the adsorption on the surface of active places and photons on the surface of particles, "sweeping" of radicals and "electron holes", and direct reaction with the photocatalyst. The composition of the tested surface water included nitrate, sulfate and choride ions which probably decreased the effectiveness of photocatalysis compared with the process carried out in the synthetic water.

The increasing catalyst dose increased the effectiveness of photocatalysis, however, one should remember that if the dose is too high, it may cause screening *ie* preventing UV radiation from reaching the deeper layers of a solution. Thus, the rate of a photocatalytic reaction depends on a catalyst dose and exposure time to UV radiation. The course of photocatalytic oxidation of natural organic matter can be described calculating constant reaction rates (k, 1/min) from the following Langmuir-Hinshelwood equation [9–11]:

$$r = \frac{dC}{dt} k \left(\frac{KC}{1 + KC} \right) \tag{1}$$

Assuming that the degradation of organic matter by photocatalysis is a first-order reaction, then the constant of the reaction rate can be calculated as follows:

$$\ln\left(\frac{C_0}{C_1} \right) = kt \tag{2}$$

where: k – rate of organic matter oxidation following the "L-H" model [mg/dm^3 · min];
K – equilibrium constant of organic matter adsorption;
C_0/C_t – concentration of organic matter at time t = 0 and after time t.

Table 4 shows the reaction rate constants (k) and half-lives of the contaminants in the synthetic and surface waters. An analysis of the reaction rate constants proved that the oxidation of natural organic matter in the synthetic water was much faster than that in the surface water. The reaction rate constant correlated with the half-life of NOM. In the surface water, the half-life for organic matter was much longer than in the synthetic water.

Table 4

Rate constants of NOM photocatalytic degradation calculated for different catalyst doses

Catalyst dose [g/dm^3]	Reaction rate constants k [min^{-1}]		Half-life [min]	
	Synthetic water	Surface water	Synthetic water	Surface water
0.25	0.023	0.009	30.1	77.0
0.50	0.022	0.010	31.5	69.3
0.75	0.050	0.013	13.9	53.3

Effect of catalyst particles on ultrafiltration membrane fouling

The research into water treatment was carried out in the integrated system of photocalysis and ultrafiltration. The latter was aimed at separating catalyst particles form clean water. The reaction mixture underwent ultrafiltration after 60, 120 and 180 minutes of exposure to UV radiation. Table 5 shows the relative permeabilities of UF membranes (α) which indicate the intensity of membrane fouling.

Table 5

Relative permeabililty (α) of membranes

Catalyst dose [$gTiO_2/dm^3$]	Coefficient, α					
	PES membrane Synthetic water			PVDF membrane Surface water		
	Exposure time [min]					
	60	120	180	60	120	180
0.25	0.97	0.97	0.91	0.90	0.91	0.94
0.50	0.80	0.86	0.86	0.73	0.85	0.89
0.75	0.84	0.85	0.91	0.80	0.86	0.94

The results proved that both the polyethersulfone and polyvinylidene fluoride membranes retained 100 % of the catalyst particles which resulted in clean and clear water. The intensity of membrane fouling increased with increasing concentration of the photocatalyst and reduction in exposure time. The relative permeabilities for the membranes were similar, which points to the similar intensity of fouling. Ultrafiltration after a 60-minute exposure produced the lowest α coefficients. Probably, the reaction mixture after 60 minutes of exposure contained the highest load of undegraded contaminants which could have accumulated in the membrane pores causing its lower efficiency. The highest relative permeability was observed for the catalyst dose of 0.25 g/dm^3 over a range of 0.91–0.97 for the PES membrane and 0.90–0.94 for the PVDF one, while the lowest α coefficients were found for higher catalyst doses ranging from 0.80 to 0.91 for PES and from 0.73 to 0.94 for PVDF membranes. The application of the higher catalyst doses resulted in the formation of a thick layer of filter cake, composed of catalyst particles, which covered the membrane surface thus increasing the resistance of the filter layer.

Conclusions

The results revealed high effectiveness of NOM removal by photocatalysis, being higher for the synthetic (Table 2) than surface water (Table 3). This was also confirmed by the higher reaction rate constants and shorter half-lives for the synthetic water than the surface water (Table 4). That is connected with the diversified composition of natural waters in which some contaminants may inhibit photocatalysis. The application of ultrafiltration enabled a 100 % retention (recovery) of the catalyst and increase in the effectiveness of water treatment.

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EFEKTYWNOŚĆ OCZYSZCZANIA WÓD W ZINTEGROWANYM PROCESIE FOTOKATALIZA-ULTRAFILTRACJA

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Politechnika Śląska

Abstract: Alternatywną metodą uzdatniania wody w stosunku do metod klasycznych jest zaawansowana technika utleniania – fotokataliza, w której dochodzi do degradacji zanieczyszczeń, w efekcie otrzymuje się ditlenek węgla i wodę. W pracy omówiono wyniki badań wpływu dawki katalizatora, czasu ekspozycji oraz jakości wody na efektywność fotodegradacji *naturalnych substancji organicznych* (NOM). W badaniach oczyszczano wodę modelową zawierającą kwasy fulwowe i humusowe oraz wodę powierzchniową z jeziora na terenie Chełmu Śląskiego. W procesie fotokatalizy zastosowano trzy dawki katalizatora (TiO_2) 0,25; 0,5 i 0,75 g/dm^3 oraz czas naświetlania 30–180 minut. Stwierdzono wysoką efektywność usuwania NOM w procesie fotokatalizy, która rosła z wydłużaniem czasu naświetlania oraz zależała od dawki katalizatora i składu oczyszczanych wód. Połączenie procesu fotokatalizy z ultrafiltracją było w pełni uzasadnione, ponieważ ultrafiltracja pozwoliła oddzielić cząstki katalizatora od czystej wody.

Słowa kluczowe: NOM, kwasy fulwowe i humusowe, fotokataliza, ultrafiltracja, oczyszczanie wód

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REMOVAL OF ZEARALENONE FROM WATER BY MEANS OF OZONATION AND INTEGRATED SYSTEM OF OZONATION/NANOFILTRATION

USUWANIE ZEARALENONU Z WODY W PROCESIE OZONOWANIA ORAZ W UKŁADZIE OZONOWANIE/NANOFILTRACJA

Abstract: Results of the study on the effectiveness of zearalenone removal *via* ozonation and integrated ozonation/nanofiltration water treatment are presented in the paper. The influence of ozone dose, contact time, pH and water properties on ozonation performance was investigated. The impact of nanofiltration membrane type on the integrated system effectiveness was tested. The study shows that application of integrated system of ozonation and nanofiltration is advantageous according to the effectiveness of zearalenone and other water contaminants removal as well as membrane capacity. The best performance of the system was observed for cellulose acetate nanofiltration membrane.

Keywords: zearalenone, organic micropollutants, ozonation, nanofiltration, water treatment

Ozone was found to be a very strong oxidizer already at the beginning of XIX century. The redox potential of ozone in the acidic environment is equal to 2.07 V while in basic to 1.27 V. Theoretically, it assures the amount of energy sufficient to oxidize organic and inorganic compounds present in water. Ozone is usually applied for removal of color, taste and smell of water as well as for its disinfection [1].

The elimination of organic micropollutants by means of ozonation is also discussed in the literature [2–4]. The effectiveness of the process depends on ozone dose, contact time, pH and water properties [1–4]. The ozone dose sufficient for total oxidation of organic compounds (determined as *dissolved organic carbon* DOC) is quite high and equal to 8 mgO_3/mg DOC [1]. The decrease of required ozone dose can be obtained by integration of ozonation with other unit operations *eg* activated carbon adsorption [5]. The application of membrane processes is also possible, and additionally the polishing of water is performed [6, 7].

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It was shown in [7] that combination of eg ozonation and nanofiltration had significantly limited the formation of disinfection by-products as the amount of dissolved organic compounds in water was decreased.

The effectiveness of ozonation and integrated water treatment system *ie* ozonation/nanofiltration for removal of zearalenone was investigated. Zearalenone is a compound from mycotoxins group of estrogenic properties which are produced by fungi of *Fusarium* type [8]. Nowadays, it is found to be present in surface water as a result of environment pollution [8, 9].

Materials and methods

Simulated solutions prepared on two water matrices *ie* deionized water and tap water with and without addition of humic acids and constant zearalenone concentration equal to 500 μg/dm^3 were used in the study (Table 1). *Humic acids* (HA) and zearalenone standards were supplied by Sigma-Aldrich. The content of humic acids in water was determined *via* absorbance measurements (at wavelength λ = 254 nm) using UV VIS Cecil 1000 spectrophotometer by Jena AG, while inorganic substances concentration *via* conductivity measurements with laboratory multiparameter analyzer inoLab® 740 by WTW. The determination of zearalenone concentration was made by solid phase extraction SPE and HPLC analysis. Supelclean™ ENVI-18 tubes (volume – 6 cm^3, phase – 1.0 g) by Supelco were used. The tube phase was firstly conditioned with acetonitrile (5 cm^3) and next washed with distilled water (5 cm^3). The separated compound was washed out with acetonitrile (4 cm^3). Quantitative and qualitative analyses of zearalenone in obtained extract were performed by means of HPLC (UV detector, wavelength λ = 235 nm). Microsorb 100 C18 column of length 25 cm, diameter – 4.6 mm and granulation – 5 μm. Methanol by POCH was used as a mobile phase.

Table 1

Physico-chemical characteristics of the waters

Waters	pH	Conductivity [μS/cm]	Absorbance, UV_{254} [1/cm]
Deionized water	5.4 and 7.0*	5.180	0.000
Tap water	7.0	1064	0.004
Tap water with HA [15 mg/dm^3]	7.0	1112	0.170

* Correction of water pH was made by addition of 0.1 M/dm^3 HCl or 0.2 M/dm^3 NaOH.

Ozonation process was carried out under 20 ^{o}C in cylindrical reactor of volume 1000 cm^3 in which treated solution was constantly mixed with the use of magnetic stirrer. Ozone was generated in Ozoner FM 500 (by WRC Multiozon, Poland) and introduced to reactor *via* ceramic diffuser. The concentration of ozone was determined by iodometric method. In order to remove ozone from post-reaction mixtures 24 mM/dm^3

Na_2SO_3 (analytical grade, P.P.H. Stanlab) was added. Next, samples were filtered through 0.45 μm membrane made from cellulose acetate by Millipore.

Two commercial nanofiltration membranes differ in the polymer material were used in the study ie composite NF-270 membrane with polyamide skin layer by Dow Filmtec (USA) and cellulose membrane CK by Osmonics Inc. (USA). The characteristic of applied membranes is shown in Table 2.

Table 2

Characteristic of the membrane

Membrane	Molecular weight cut-off [Da]	Deionized water flux (J_w)[a], 10^{-6} [$m^3/m^2 \cdot s$]	Removal of salts[b] [%]	
			$MgSO_4$	NaCl
NF-270	200	70.6	92.1	41
CK	150–300	7.60	96.8	75

[a] Determined in this work at ΔP = 2.0 MPa; [b] determined in experiment during filtration of $MgSO_4$ or NaCl solution (1000 mg/dm^3) at ΔP = 2.0 MPa.

The transport and separation parameters of the nanofiltration membranes were assessed using the equations given in Table 3. The determination of nanofiltration effectiveness was based on the measurements of both membrane efficiency (J_v and α) – equations (1 and 2) and selectivity (R) – equation (3).

Table 3

Equations used to evaluate membrane properties and removal efficiencies

Parameter, unit	Equations	Number
Volumetric permeate flux (deionized water) J_v (J_w) [$m^3/m^2 \cdot s$]	$J_v(J_w) = \frac{V}{F \cdot t}$	1
Relative permeability of the membrane α [-]	$\alpha = \frac{J_v}{J_w}$	2
Removal degree R [%]	$R = \left(1 - \frac{C_p}{C_f}\right) \cdot 100$	3

V – volume [dm^3], F – membrane area [m^2], t – filtration time [s], C – concentrations [μg/dm^3], p – permeate, f – feed.

The process was carried out under transmembrane pressure 2.0 MPa in steel membrane cell (volume – 350 cm^3, membrane area 38.5 cm^2) enabling dead-end process configuration.

The study determining effectiveness of zearalenone removal from water using integrated system ozonation/nanofiltration comprised of water treatment in ozonation process after which nanofiltration was performed. In the part of study discussing

ozonation, the influence of ozone dose, contact time, pH and water matrix properties on the degree of zearalenone removal was investigated.

Results

Ozonation

The degree of zearalenone removal depended on ozone dose and as the dose increased the degree of compound removal also increased (Fig. 1). Moreover, the elongation of ozone contact time with treated water also improved the effectiveness of zearalenone removal (Fig. 2).

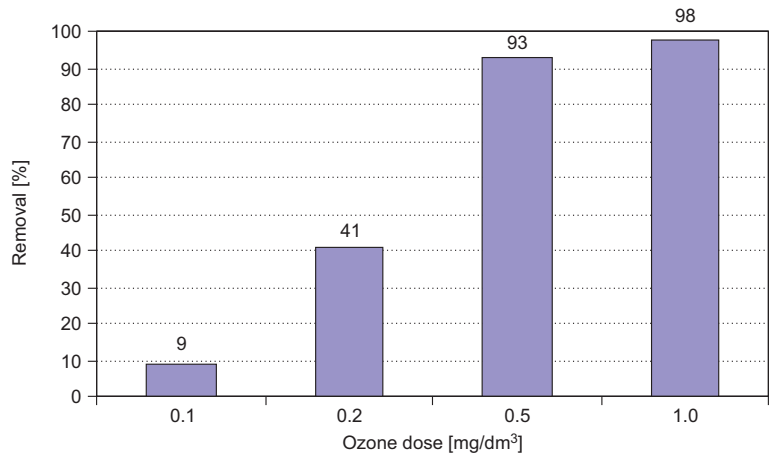

Fig. 1. The influence of ozone dose on degree of zearalenone removal (deionized water, contact time 1 min, pH = 5.3)

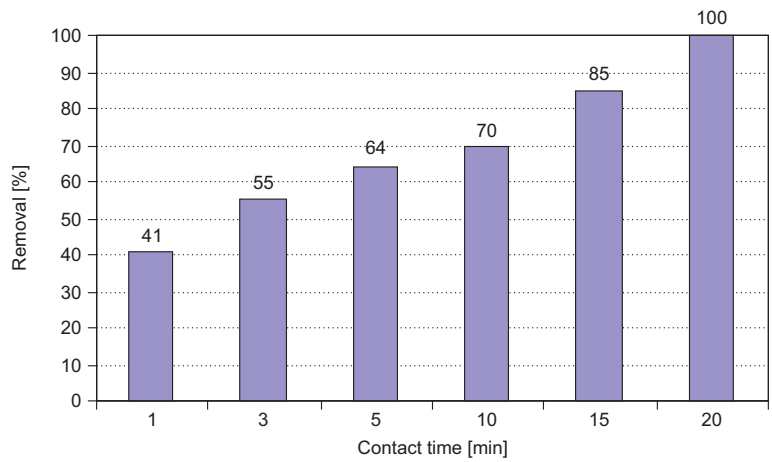

Fig. 2. The influence of contact time on effectiveness of zearalenone removal (deionized water, ozone dose 0.2 mg/dm^3, pH = 5.3)

It was found that the increase of water pH resulted in decrease of zearalenone removal degree (Fig. 3). It proved the greater reactivity of molecular ozone (direct oxidation) in comparison with free radicals $OH^{\bullet}$ formed during ozonation [1]. The lower process effectiveness was also observed in cases when except from zearalenone also other compounds ie inorganic and high-molecular weight organic substances were present in water (tap water with and without humic acids addition). The phenomenon is probably caused by decrease of ozone concentration reacting with low-molecular weight zearalenone, which is used for oxidation of inorganic and organic compounds present in water.

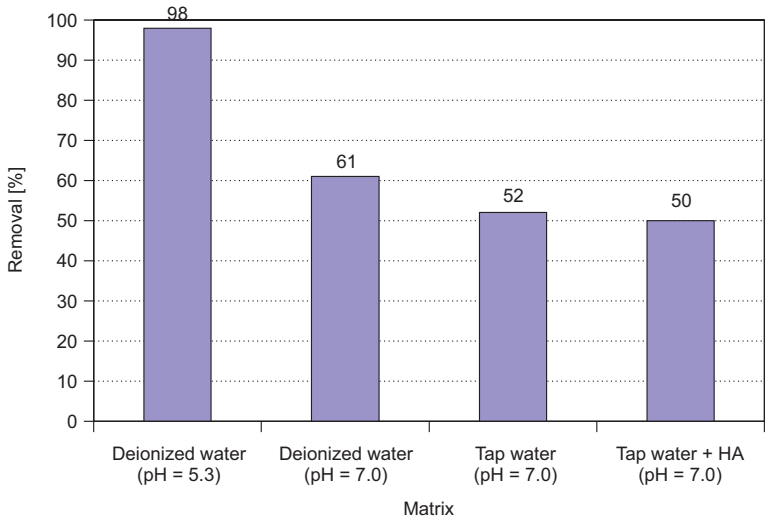

Fig. 3. The influence of water properties on effectiveness of zearalenone removal (ozone dose 1 mg/dm^3, contact time 1 min)

Integrated system ozonation-nanofiltration

Nanofiltration was considered as a method of polishing of water treated *via* ozonation (tap water + HA, ozone dose 1 mg/dm^3 and contact time 1 min). It was found that introduction of nanofiltration to water treatment system improved not only removal of zearalenone, but also other water contaminants (decrease of conductivity and absorbance). However, the effectiveness of the integrated system performance depended on the type of nanofiltration membrane applied (Fig. 4). The highest efficiency of contaminants removal was observed in case of cellulose membrane CK for which the removal rates of conductivity, zearalenone and absorbance were equal to 65, 97 and 100 %, respectively. The membrane characterized also with lower affinity to fouling what was determined by means of membrane relative permeability α (Fig. 5). The phenomenon is probably connected with properties of membrane material including contact angle of the membrane [10–13].

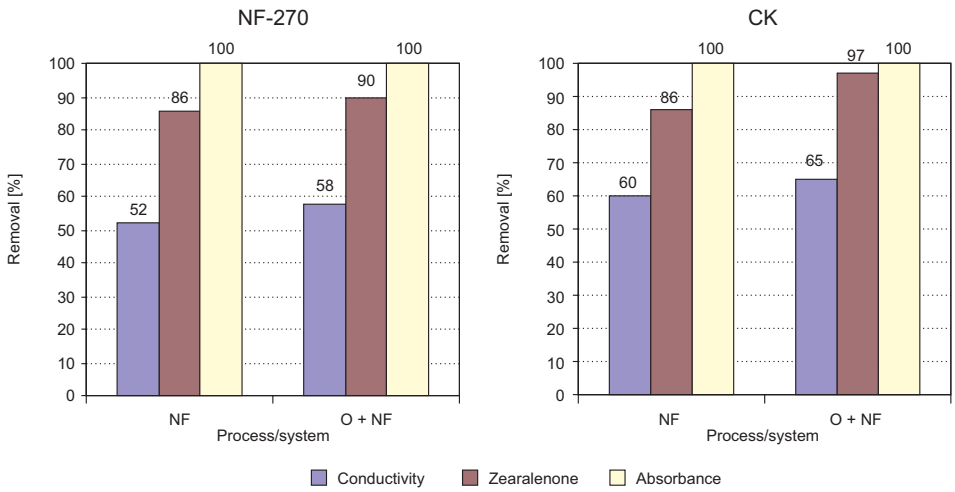

Fig. 4. The effectiveness of nanofiltration performed as a unit process or as a part of integrated system with ozonation

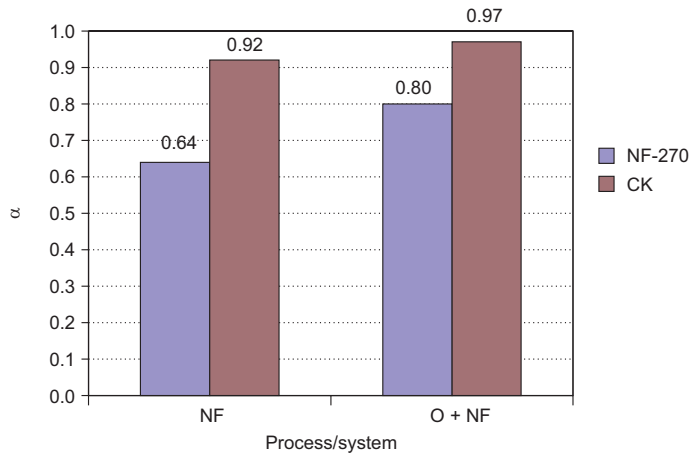

Fig. 5. Relative permeability of the membrane α

Conclusions

The study allow to conclude that the effectiveness of removal of zearalenone in ozonation process depends on ozone dose and contact time. The lower degree of compound removal was observed in case when inorganic and high-molecular weight organic substances (humic acids) were present in water or pH of water increased.

The application of nanofiltration after ozonation (integrated system) improves zearalenone removal in comparison with single ozonation treatment. The total removal of high-molecular weight compound and sufficient decrease of inorganic substances

concentration are also obtained for integrated system. The combined solution also improves the capacity of applied membrane.

The higher effectiveness of the integrated system was observed in the combination with cellulose acetate membrane CK, which additionally revealed the lower affinity to fouling.

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USUWANIE ZEARALENONU Z WODY W PROCESIE OZONOWANIA ORAZ W UKŁADZIE OZONOWANIE/NANOFILTRACJA

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Abstrakt: Zaprezentowano wyniki badań dotyczące efektywności usuwania zearalenonu w procesie ozonowania i w zintegrowanym układzie oczyszczania wody ozonowanie/nanofiltracja. W trakcie ozonowania badano wpływ dawki ozonu, czasu kontaktu, pH i rodzaju wody na stopień usunięcia zearalenonu. Badano również wpływ rodzaju membrany nanofiltracyjnej na efektywność pracy układu zintegrowanego. Wyniki wskazują, że zastosowanie układu zintegrowanego kojarzącego ozonowanie z nanofiltracją jest korzystne pod względem efektywności usuwania zearalenonu oraz innych wskaźników zanieczyszczenia wody, jak również biorąc pod uwagę wydajność membrany. Wyższą efektywność pracy układu obserwowano w przypadku zastosowania membrany nanofiltracyjnej z octanu celulozy.

Słowa kluczowe: zearalenon, mikrozanieczyszczenia organiczne, ozonowanie, nanofiltracja, oczyszczanie wody

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EMPIRICAL MODEL FOR ESTIMATING THE ACTUAL LNAPL THICKNESS BASED ON THE HYDRAULIC CONDUCTIVITY

MODEL EMPIRYCZNY DO WYZNACZANIA RZECZYWISTEJ MIĄŻSZOŚCI LNAPL KORZYSTAJĄCY ZE WSPÓŁCZYNNIKA FILTRACJI

Abstract: The actual thickness of lighter-than-water non-aqueous phase liquid (LNAPL) on the groundwater table is always different from the apparent thickness (measured in the monitoring well). There are several methods developed for estimating the actual LNAPL thickness on the base of the apparent thickness, but the results obtained with different formulas are inconsistent and (in many cases) very imprecise. The obtained results of laboratory investigations indicate that the appropriate model for estimating the actual thickness of light non-aqueous phase liquid should include the properties of soil and LNAPL. The investigations confirmed that the hydraulic conductivity is very important parameter in the case of homogeneous soils.

On the base of the results the empirical model was developed. This model includes the hydraulic conductivity of soil and the density and dynamical viscosity of LNAPL. The results of the verification of developed model indicate that the calculated values corresponded in many cases with the values obtained during laboratory investigations.

Keywords: LNAPL, actual thickness, apparent thickness, hydraulic conductivity, empirical model

The main sources of soil and groundwater contamination with *lighter-than-water non-aqueous phase liquids* (LNAPL) are surface spills from cisterns and leakages from underground storage tanks and pipelines. If the layer of LNAPL floats on the groundwater table the initial remediation step should be its recovery [1–3]. A proper design of recovery requires an assessment of the contamination plume volume on the base of the LNAPL thickness measured in specified points of the contaminated area [1]. Unfortunately, the thickness of LNAPL on the groundwater table (the actual thickness) is different from the thickness observed in the well (the apparent thickness) [4–6] and this difference depends on the properties of soil, and the properties and amounts of the free product floating on the groundwater table [7–9]. The results obtained with different

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formulas for estimating the actual thickness are inconsistent, and in many cases inaccurate [4, 5, 10, 11]. The results of laboratory investigations indicate that the appropriate model for estimating the actual LNAPL thickness should include the properties of both: soil and LNAPL [7, 10].

This paper presents the developed empirical model for estimating the actual LNAPL thickness on the groundwater table. The model is based on the properties of soil (hydraulic conductivity) and properties of LNAPL (dynamical viscosity).

Materials and methods

Laboratory experiments were conducted in Plexiglas columns with filter-tubes as monitoring wells (Fig. 1) with use of six model soils and six types of LNAPL. The properties of soils and LNAPLs are given in Tables 1 and 2.

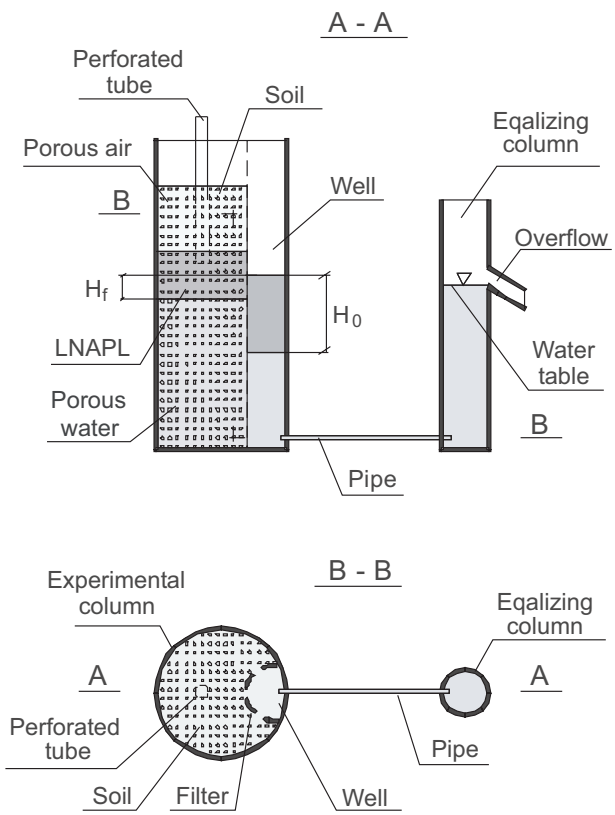

Fig. 1. The experimental columns with filter-tubes connected with equalizing columns

The experimental columns were packed with the soil samples (soils 1–6) and filled with tap water until the water table reached the assumed elevation. After 3–4 days 50 cm^3

Table 1

Properties of soils used in the experiments

Soil	Type of soil	Soil grain size [mm]	Medium soil grain size [mm]	Hydraulic conductivity at 10 °C k [m/d]
1	fine sand	0.1–0.25	0.175	$1.0 \cdot 10^1$
2	medium sand	0.25–0.315	0.2825	$2.3 \cdot 10^1$
3	medium sand	0.315–0.5	0.4075	$7.1 \cdot 10^1$
4	coarse sand	0.5–0.63	0.565	$1.1 \cdot 10^2$
5	coarse sand	0.63–0.8	0.715	$1.5 \cdot 10^2$
6	coarse sand	0.8–1.0	0.9	$2.3 \cdot 10^2$

Table 2

Properties of LNAPLs used in the experiments (at temperature of 20 °C)

LNAPL	Notation	Type of LNAPL	Density ρ_o [kg/m^3]	Dynamic viscosity η_o [kg/m · s]
LNAPL 1	L1	Petroleum	820	$1.8 \cdot 10^{-3}$
LNAPL 2	L2	Rape oil	918	0.072
LNAPL 3	L3	Mineral oil “Lotos”	880	0.30
LNAPL 4	L4	Semi-synthetic oil “Orlen”	872	0.22
LNAPL 5	L5	Synthetic oil “Lotos”	855	0.18
LNAPL 6	L6	Synthetic oil “Orlen”	871	0.19

of diverse LNAPLs, coloured with the pigment – Sudan III, were injected above the capillary fringe zone. After subsequent 3–4 days, the apparent and actual LNAPL thicknesses were measured in the well and in soil. This procedure was repeated at least 10 times. The actual thickness was the distance between LNAPL-water interface in the soil and air-LNAPL interface in the well (without the capillary fringe of LNAPL in the soil). The columns were hydraulically connected with equalizing columns which aim was to keep the water table constant during experiments. The styropore covers protected the top of columns.

Results and discussion

On the base of the experiments for each composition of soil and LNAPL the graphs and trend lines were plotted which present the relationships between apparent and actual thickness [9]. Results and high values of the determination coefficients (R^2) for linear functions show that these relationships have the linear character. Then were fixed the values of apparent thicknesses corresponding to the actual thicknesses of 5, 10 and 15 cm (for each composition of soil and LNAPL). On the base of these results the graphs

were plotted which show the influence of the hydraulic conductivity of soil in 10 °C (k) on the difference between apparent and actual thicknesses ($\Delta H = H_0 - H_f$) for three selected actual thicknesses: 5, 10 and 15 cm (Fig. 2). The results indicate that the difference between apparent and actual thicknesses increases with the decrease of the hydraulic conductivity of soil. This relationship has the logarithmic character. It can be confirmed by high values of the determination coefficients derived for logarithmic functions. These high values suggest the functional relationship.

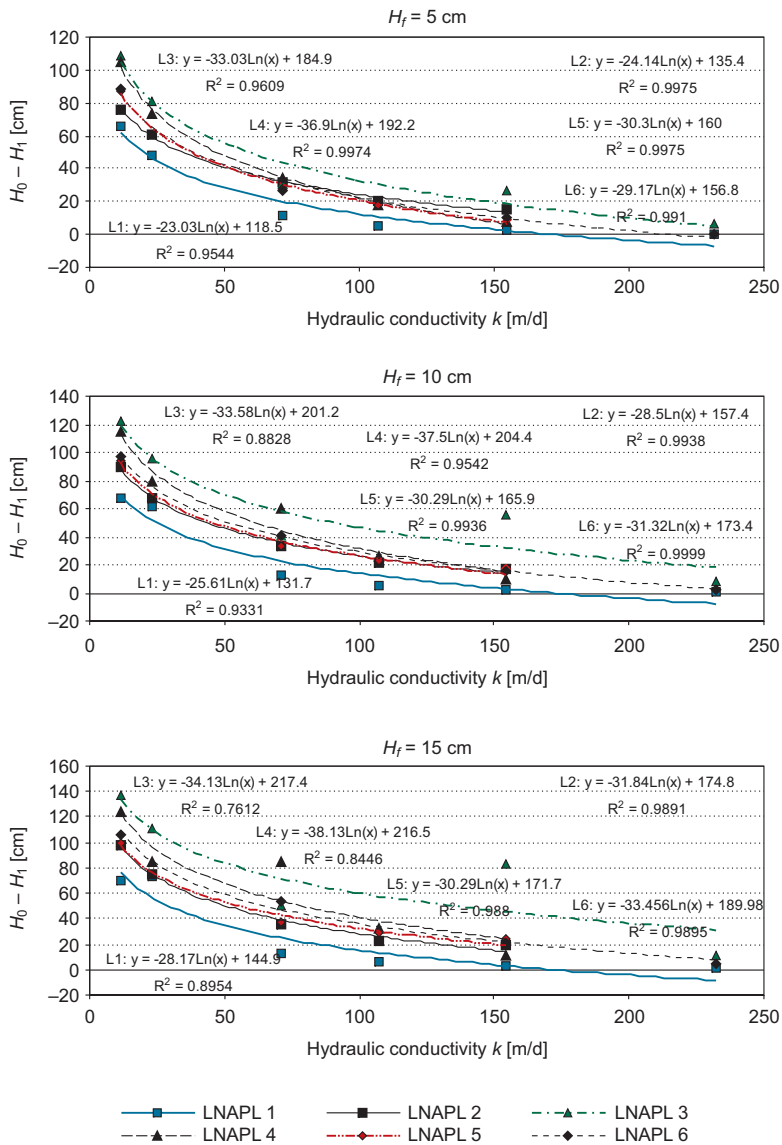

Fig. 2. The influence of the hydraulic conductivity on the difference between apparent and actual thickness

On the base of the results presented in the Fig. 2 the empirical model was developed that describes the relationship between apparent and actual LNAPL thicknesses (Equation 1). Model is based on the hydraulic conductivity and the dynamic viscosity of LNAPL. The actual LNAPL thickness can be derived from equation:

$$H_f = H_0 - (\omega\eta_o + \varphi)\ln k - \chi\eta_o - \xi \tag{1}$$

where: H_f – actual LNAPL thickness [cm],
H_0 – apparent LNAPL thickness [cm],
k – hydraulic conductivity in 10 ^{o}C [m/d],
η_o – dynamic viscosity of LNAPL [kg · m^{-1} · s^{-1}],
ω, φ, χ and ξ – factors depending on actual LNAPL thickness [-].

Factors ω, φ, χ and ξ can be calculated from the equations 2–5:

$$\omega = 1.989\,H_{fe} - 52.006 \tag{2}$$

$$\varphi = -0.6445\,H_{fe} - 19.457 \tag{3}$$

$$\chi = -0.878\,H_{fe} + 247.29 \tag{4}$$

$$\xi = 2.932\,H_{fe} + 104.29 \tag{5}$$

where: H_{fe} – initial estimated actual LNAPL thickness [cm].

Use of proposed model requires initial estimation of actual thickness (H_{fe}) to determine the proper values of factors ω, φ, χ and ξ. The calculation should be repeated until the calculated actual thickness is equal to the initial estimated value.

Figure 3 presents the verification of the model. The relationships between apparent and actual thicknesses obtained from the model (the curves) were compared with the experimental data (point graphs). The results indicate that the values calculated from the developed model corresponded in many cases to the experimental data. In the case of soil 1 the best results were obtained for LNAPLs 1, 2 and 6. In the case of soil 2 the model curves are similar to the experimental graphs for all LNAPLs. The best fits are also reached for the compositions: soil 3 – LNAPL 4; soil 4 – LNAPLs 2, 4, 5; soil 5 – LNAPLs 1, 5, 6 and for soil 6 – LNAPL 6. Only for a few compositions of soils and LNAPLs the results derived from the developed model differ from the experimental data. The most unfavorable results were obtained for the compositions: LNAPL 1 – soils 3, 4, 6 and LNAPL 4 – soil 5. The relationships between apparent and actual thicknesses drawn on the base of the proposed model have the linear character. Further studies aimed at the improvement of the model should include the check of the importance of the hydraulic conductivity in the case of the heterogeneous soils. The experiments should also include the examination of the influence of other parameters *eg* the equivalent diameter and the coefficient of uniformity.

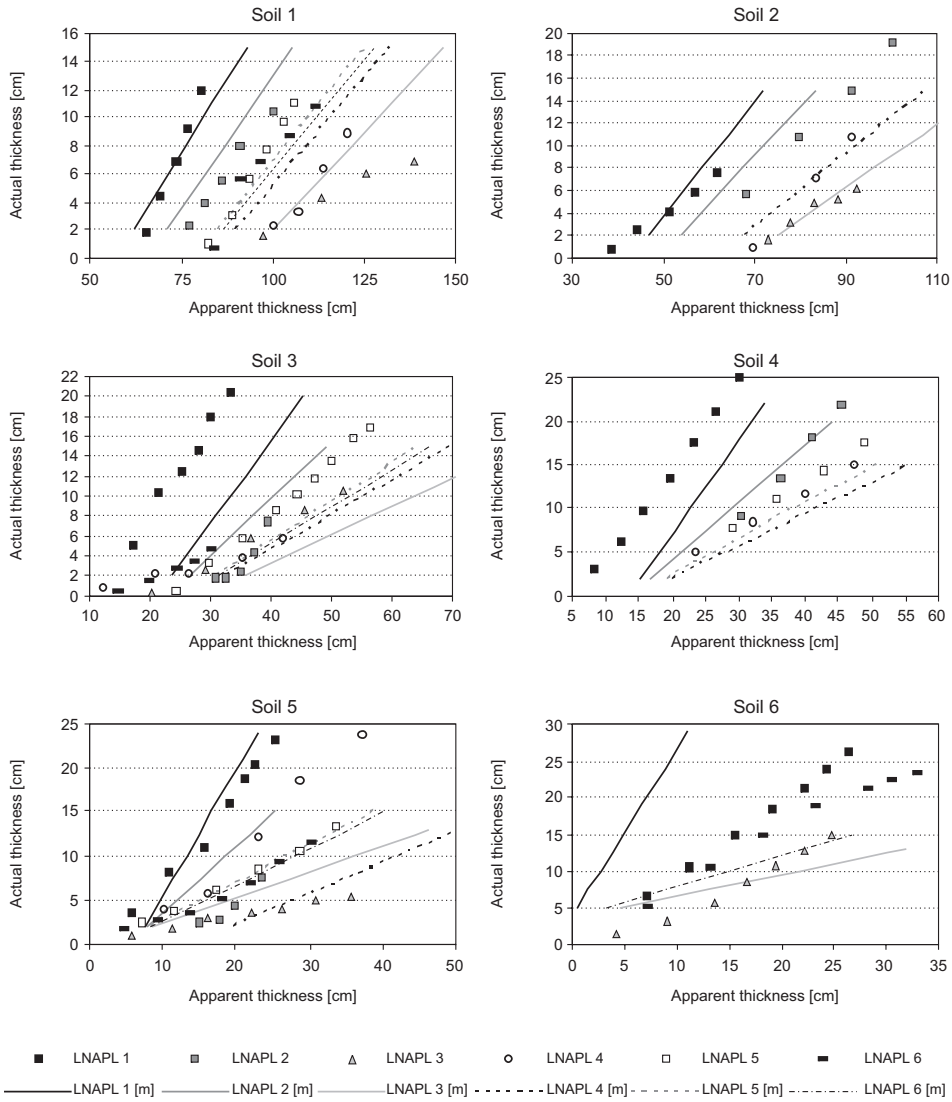

Fig. 3. The verification of the developed model on the base of laboratory investigations

Conclusions

1. The difference between apparent and actual thicknesses increases with the decrease of the hydraulic conductivity of soil. This relationship has a logarithmic character.

2. The appropriate model for estimating the actual LNAPL thickness on the groundwater table should include the properties of both: soil and LNAPL.

3. The values of actual LNAPL thickness calculated from the developed model are in many cases consistent with the results of the laboratory investigations. The best results were obtained in the case of the fine-grained soils.

4. Further studies aimed at the improvement of the proposed empirical model should include the laboratory experiments with the use of heterogeneous soils to check the importance of the hydraulic conductivity in this case and to study the influence of other parameters of soils *eg* the equivalent diameter and the coefficient of uniformity.

Acknowledgements

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MODEL EMPIRYCZNY DO WYZNACZANIA RZECZYWISTEJ MIĄŻSZOŚCI LNAPL KORZYSTAJĄCY ZE WSPÓŁCZYNNIKA FILTRACJI

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Abstrakt: Rzeczywista miąższość lekkiej cieczy organicznej (LNAPL) na zwierciadle wody podziemnej zawsze różni się od miąższości zmierzonej w studni (tzw. miąższości pozornej), a różnica między nimi jest

zależna od właściwości gruntu i LNAPL oraz od ilości cieczy organicznej na zwierciadle wody. Metody stosowane obecnie do ustalania rzeczywistej miąższości LNAPL na podstawie zmierzonej miąższości pozornej pozwalają na uzyskiwanie wyników bardzo rozbieżnych i w większości przypadków nieprecyzyjnych. Metody te są bardzo uproszczone, uwzględniają zbyt małą liczbę parametrów (jedynie właściwości gruntów lub jedynie właściwości LNAPL). Poza tym, poprawne ustalenie wartości niektórych parametrów uwzględnionych w metodach jest bardzo trudne, zarówno w warunkach laboratoryjnych, jak i terenowych.

Na podstawie uzyskanych wyników badań laboratoryjnych ustalono, że poprawnie opracowany model obliczania rzeczywistej miąższości LNAPL powinien uwzględniać zarówno właściwości gruntu, jak i LNAPL. Ustalono, że w przypadku gruntów jednorodnych, bardzo równomiernie uziarnionych, jednym z ważniejszych parametrów jest współczynnik filtracji. Na podstawie analizy kluczowych parametrów wpływających na zależność między miąższością pozorną i rzeczywistą opracowano model empiryczny uwzględniający współczynnik filtracji gruntu oraz współczynnik lepkości dynamicznej LNAPL. Weryfikacja modelu potwierdziła, że w większości przypadków jego zastosowanie pozwoliło na uzyskanie wyników zbliżonych do ustalonych w warunkach laboratoryjnych.

Słowa kluczowe: LNAPL, miąższość rzeczywista, miąższość pozorna, współczynnik filtracji, model empiryczny

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Wojciech PĘCZUŁA[1]

METHODS APPLIED IN CYANOBACTERIAL BLOOM CONTROL IN SHALLOW LAKES AND RESERVOIRS

METODY STOSOWANE W KONTROLI ZAKWITÓW CYJANOBAKTERII W PŁYTKICH ZBIORNIKACH WODNYCH

Abstract: The eutrophication of freshwaters – including shallow lakes – has become a global problem in the 21st century. Cyanobacterial blooms belong to the most frequent effects of this phenomenon. Although the problem, as well as methods for control it, are known since the beginning of the last century, in the last 25 years we can observe an increasing number of publications concerning methods of bloom control. The paper gives a review of different methods (chemical, physical and biological) applied in cyanobacterial bloom control in shallow lake ecosystems, taking into account not only the effectiveness of the methods but also their impact on other water biocenoses

Keywords: cyanobacteria, algal bloom control, lake restoration, phosphorus inactivation, biomanipulation, barley straw

Introduction

The Water Framework Directive (2000/60/EU) has obliged the Member States of the European Union to improve the quality of theirs water resources, and to achieve a good ecological status of both surface and ground waters by 2015 [1]. This requirement is a difficult challenge for lake managers and local authorities: not only to maintain a good status of water but, in the case of degradation, to also make an effort to restore it.

Overfertilisation is the main factor leading to the degradation of lakes and reservoirs in Europe. The phenomenon is so common that, one could say, the eutrophication of waters has become a global problem [2]. Overfertilisation (phosphorus and nitrogen enrichment) of the water body results in phytoplankton (cyanobacterial) domination (hypereutrophy, a turbid water state) which in turn leads to the ecosystem maltfunctioning, and as a consequence, to the exclusion of the economical and social functions of lakes and reservoirs [3]. Cyanobacterial blooms which have occurred recently in many

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recreational and water supply reservoirs in Poland have brought the owners many problems [4–7].

The risk of eutrophication and the occurrence of cyanobacterial blooms in the lake depends on many factors, such as: the lake basin morphometry, the thermal regime or the dominance of macrophytes [8]. Shallow lakes (which constitute the most common type of lakes in Europe) can show a great resistance to degradation because of the dense macrophyte vegetation which often covers the bottom and which plays a buffering role by maintaining a clear water state [3, 9]. On the other hand, the lack of thermal stratification, which leads to a quick turnover of nutrients during the summer, is one of the disadvantages of the shallow lake ecosystem. But the restoration of shallow lakes is often easier than restoring a deep one, because of the smaller volume of water they have, and because of the positive role of macrophytes which could support the return to a clear water state [10–11].

Cyanobacterial bloom control is a problem that has often been described in scientific papers, particularly in the last 25 years. It could be connected with an increase in restoration programmes in Europe which have been undertaken to fight the blue-green algae or, on the other hand, with the progress of shallow lake ecology, which has greatly expanded our knowledge about the functioning of these ecosystems. A review of the methods for algal bloom control in lakes has been given by, amongst others, Sosnowska [12], Kajak [13] and Klapper [14]. One can also find chapters concerning this problem in books by Cooke et al [15] and McComas [16]. Some of these papers are out of date, while the others are too extensive or focused on one aspect (*eg* technical) of the problem, and do not provide the review of the broad spectrum of methods from hydrological ones to biological ones.

The aim of this paper is to review the various methods applied in cyanobacterial bloom control in shallow lake ecosystems. The analysis was carried out on the basis of several dozen case studies on mainly European lakes in the last 25 years, taking into account not only the effectiveness of the methods but also their impact on other water biocenoses. As a conclusion, a strategy for the effective and environmentally safe control of cyanobacterial blooms is presented.

Reduction of external phosphorus loading

Any measure which is undertaken to eliminate cyanobacterial blooms has a chance of succeeding only in the case of a reduction in the phosphorus external loading into the lake [11, 15]. Lake Washington in the USA remains the model study of this method. In the late sixties the lake was isolated from nutrient rich streams which caused a rapid improvement in the water quality and, consequently, a decline in blue-green blooms several years later [17]. However, a reduction in external phosphorus loading does not always lead to an improvement in the water quality – which was shown by the example of Alderfen Broad in Great Britain (area 7 ha, mean depth 0.8 m). The lake was isolated from several canals which were enriched by nutrients from wastewater treatment plants. Positive changes were noted four years later: water transparency had increased, phytoplankton biomass had declined and macrophytes had covered the bottom. After

a short period of recovery, however, the water quality declined again because of strong phosphorus loading from sediments [18]. Jeppesen et al. [19] analysed examples of external loading reduction in thirty five north temperate lakes. In the majority of cases, the water quality of the lakes improved after reduction (a decline in the concentration of TP, TN and chlorophyll *a*, an increase in transparency, a change in the zooplankton structure). But a positive change in the phytoplankton structure and a decline in cyanobacterial dominance was observed only in deep lakes.

A reduction of nutrient external loading into surface waters could be achieved through many measures established in catchments. In Denmark, where most lakes are shallow and rapidly flushed, a lot of programmes for the reduction of nutrients were performed during the last twenty five years. They included such measures as the modernisation of sewage treatment plants, an increased use of phosphate-free detergents, an increased storage capacity for animal manure, regulations on agriculture practices (the establishment of 2 m wide cultivation-free riparian buffer strips along streams; the use of green cover during winter), increased afforestation etc. As a result of these activities a 73 % reduction in the total phosphorus in surface waters has been achieved [9].

Reduction of internal phosphorus loading

The reduction of external phosphorus loading performed as a measure to control cyanobacterial blooms is indispensable, but often insufficient. Blue-greens will also develop if they have an internal source of phosphorus coming from lake sediments. This internal load could be as big as the one delivered from the lake catchment [20]. The phenomenon that phosphorus is supplied mainly from internal sources in overfertilised lakes is more a rule than an exception [15]. Therefore, the process of lake recovery after reducing the external loading of nutrients could be delayed by 10–15 years because of internal loading from sediments [19].

Sediment removal is one of the methods of internal loading reduction. The aim of this measure is to get rid of both phosphorus deposited in the sediments, and of cyanobacterial cells and spores, which could be the inoculum of further blooms [21]. The most simple way to achieve this is by lowering the water level in the reservoir and then removing the dried sediments. Sediments could also be removed by dredging, which is more expensive because of the great amount of wet matter which would have to be stored or utilised [14]. The destruction of bottom fauna during sediment removal is one of the environmental costs of this method. The future restoration of benthos could last up to several years [15]. Sediments have been removed *eg* in lake Cockshoot Broad in Great Britain (mean depth 1 m, area 3.3 ha). After being isolated from the nutrient rich river Bure, the top 70 cm layer of sediments was pumped out. Postive changes were noted immediately after the operation: a decline in the concentration of chlorophyll *a* and phosphorus, an increase in filter feeding cladocerans and the recolonisation of the bottom by macrophytes. Unfortunately, the positive effects only lasted several years. The further strong development of planktivorous fish caused a decrease in the zooplankton population and the return of algal blooms [18]. Similar effects were achieved after the restoration of lake Braband in Denmark (area 150 ha, mean depth 0.8 m),

where 500 000 m^3 of nutrient-rich sediments were pumped out. There was no improvement in the water quality because of the dense population of planktivorous fish inside the lake, and the high external nutrient loading [22].

A cheaper technique is sediment capping. In this method a kind of barrier which prevents phosphorus returning to the water column is created. The role of this barrier can be played by sand with an admixture of materials, which can inactivate the phosphorus, such as: zeolits, calcium compounds and clay materials [23–24]. The main problem of this techniqe is the stability of this layer, which can be affected both by the mixing and the activity of benthic organisms [14, 20]. Sediment capping is still tested, mainly in laboratory studies. In the Paldang dam reservoir in Korea (volume 0.9 km^3, max depth 20 m) sediment capping by gypsum was experimentally applied *in situ*. As a result a high reduction (52 %) of chlorophyll *a* concentration was achieved [25].

Phosphorus bonding by sediments could be improved by the oxydation of its upper layer, using a method called RIPLOX. It is achieved by adding some chemicals (*eg* calcium nitrate and ferric chloride) to the sediments, which results in an increase of redox potential. Such conditions support the process of denitrification, which favours phosphorus bonding [26]. This method was applied *eg* in lake Lyng in Denmark (area 10 ha, mean depth 2.4 m). During the two following summer seasons calcium nitrate in doses of 8–10 g N/m^2 were added to the sediments. Preliminary results showed that the method was successful [22, 27]. RIPLOX was also used in the Old Danube lake in Vienna (area 158 ha, mean depth 2.3 m) which is an oxbow lake by the river Danube. Calcium nitrate was applied in doses of 16–240 g N/m^2 which resulted in an improvement in water quality, *eg* a change in the domination in the phytoplankton structure from cyanobacteria to greens and diatoms [28].

Recently, one of the most wide used methods of internal phosphorus reduction is its precipitation and immobilisation. This technique consists of adding of coagulants which form the flocs and, consequently bond the phosphorus and, therefore, makes it unavailable for algae. Additionally, such a suspension caps the sediments and prevents the phosphorus from returning to the water column [15]. The precipitation of phosphorus is the most commonly used method in reservoirs with a slow water exchange rate [14]. The following chemicals are used as coagulants: compounds of aluminium, iron or calcium, as well as clay materials.

Aluminium sulphate was used to reduce cyanobacterial blooms in several cases, *eg* in Green Lake (area 105 ha, mean depth 3.9 m) in Seattle (USA). A substantial improvement of water quality (including an increase in transparency from 1.9 to 6.1 m) had been noted after dosage of 8.6 mg Al/dm^3, but the positive effects lasted only a few years [29]. Similar effects were noted in lake Courtille in France (area 22 ha, mean depth 2.5 m) where 1.5 mg Al/dm^3 was used and in lake Sonderby in Denmark (area 8 ha, mean depth 2.8 m). In both cases, after the water had improved its quality (2 months – 3 years), cyanobacterial blooms returned again [11, 30, 31].

Iron(III) chloride is another coagulant used in phosphorus inactivation. It was applied (mixed with an upper layer of sediments) in lake Groot Vogelenzang in the Netherlands (area 18 ha, mean depth 1.75 m). The decrease of chlorophyll *a*, phosphorus and seston

concentration was noted three weeks after the treatment, although because of a high external loading of nutrients the water quality collapsed after several months [32].

Phosphorus can be immobilised through the addition of calcium compounds. Calcium treatment was applied in two north Canadian lakes: Lofty (area 70 ha, mean depth 2.9 m) and North Halfmoon (area 77 ha, mean depth 3.2 m) in doses of 74 and 107 mg $Ca(OH)_2/dm^3$, respectively. As a result, a 16–27 % decrease in internal phosphorus from sediments was noted, but the effectiveness of the treatment was as short as one year. Moreover, there was no decline in the phytoplankton biomass, and a considerable negative effect on the macrophyte biomass was observed [33–34]. Multiple doses of lime were added to two other Canadian lakes: Halfmoon (area 41 ha, mean depth 4.7 m) and Figure Eight (area 37 ha, mean depth 3.0 m) in the amount of 5–78 mg/dm^3. The concentrations of chlorophyll *a* and total phosphorus decreased, as well as the biomass of macrophytes. No negative effects on zooplankton and benthic macroinvertebrates were noted [35].

An example of the clay material which is used in phosphorus inactivation is modified bentonite known as "Phoslock". The use of this preparation was tested *eg* in the river Canning in Australia, where a 1 mm layer of bentonite was created on the bottom sediments of the river. The effects (a very considerable decrease in phosphate concentrations) could be seen immediately after the treatment, but there is a lack of data about the long term results of the treatment [36]. The decrease in phosphate concentrations in the case of two estuaries: Swan-Canning River and Vasse (Australia) was also a result of Phoslock treatment. The triplicate dosage of the preparation created 0.5–1 mm layer on the surface of bottom sediments. Despite phosphate reduction, a decrease in the phytoplankton biomass was observed only in one of these cases [37].

Biological control

It has been argued that a reduction in both external and internal phosphorus sources is the best way for a long term reduction of cyanobacterial blooms in the lake [15]. But the domination of blue-greens in the lake is determined not only by the availability of nutrients, but also by the proper structure of food webs in the ecosystem, *eg* by the appropriate zooplankton phytoplankton ratio. There are a lot of cases where, after a reduction in nutrient loading, cyanobacterial blooms are present, mainly because of a dense stock of planktivorous fish in the lake. The fish suppress the zooplankton population, so the factor which can effectively control the phytoplankton population is lacking [9].

Biomanipulation is the most often used method in the biological control of algal blooms. The method consists of reducing the pressure of planktivorous fish on crustacean plankton which feed on phytoplankton [38]. The desired result can be achieved by stocking the predatory fish or/and by removing the planktivorous fish. As a result the population of planktonic crustaceans increases, generating increased feeding on phytoplankton, which in turn suppresses its population [39]. Benthivirous fish removal is also one of the biomanipulation methods. These fish contribute a lot to the increase in resuspension while feeding in lake sediments, thus increasing the amount of

phosphorus in the water column. Therefore, the reduction of the benthivorous fish population can result in a decrease in the phytoplankton biomass in the lake [11]. However, biomanipulation has some disadvantages. One of them is that highly qualified limnologists should be involved. This method is best for smaller reservoirs, because of the requirement to control the entire fish population [15]. Biomanipulation will fail in lakes that are strongly enriched with nutrients – with concentration of total phosphorus greater than 100 $\mu g/dm^3$ [9]. In the majority of cases the effects of biomanipulation are not long lasting – after approximately ten years cyanobacterial domination often returns [11].

In Denmark biomanipulation (zooplankton- and bethi-vorous fish removal with additional piscivorous fish stocking) was carried out in a total of forty two lakes. Fish reduction ranged from 10 % to 80 % of the fish stock, which means 100–870 kg/ha of the lake. In many cases positive effects were achieved directly after the measure was taken: in most lakes there was observed an increase in the water transparency, but it was not connected with a decrease in the phosphorus concentration and macrophyte reappearance [11]. An example of a temporarily successful fish reduction is lake Vaeng (area 15 ha, mean depth 1.2 m). Despite prior external nutrient reduction, the biomass of blue-greens remained high. Thus, the removal of 50 % of the population of zooplankton- and benthi-vorous fish was carried out. An increase in water transparency, a decrease in the phytoplankton biomass (including cyanobacteria) and the recolonisation of the bottom by macrophytes was observed as a result of the biomanipulation. As a consequence, these changes resulted in the decrease of nutrient concentrations [9]. Unfortunately, over the following years some negative changes in the fish structure and macrophyte cover have occurred. Thus water quality has returned to the pre-treatment state [11].

In the Netherlands eighteen lakes were biomanipulated in the period from 1987 to 1995. In many cases a significant improvement in water quality was achieved [10] but in the long term perspective (> 10 years), a permanent positive change (the disappearance of cyanophyte blooms) occurred in only two of lakes [11].

Another idea for biological treatment which could be used in cyanobacterial bloom control is the use of phytoplanktivorous fish like silver carp (*Hypophthalmichthys molitrix*) or bighead carp (*H. nobilis*). Besides the fact that both species are alien to waters in *eg* Europe, the method has a lot of disadvantages: fish graze on crustacean plankton and can cause ichthyoeutrophication [40, 41]. Despite this, both species of carps are sometimes used to graze on cyanophyte biomass, especially in Asian countries. Xie i Liu [42], for example, have observed the positive role of high silver carp and bighead carp stock on the decline of cyanobacterial blooms in lake Donghu (area 3200 ha, mean depth 2.2 m) in China.

Aquascaping is a biological method which could support cyanobacterial bloom control [16]. The method involves the re-establishment of macrophytes in the lake, or activities within the lake which can favour this process. Macrophytes interact with phytoplankton *via* several mechanisms, like: the competition for nutrients and light, sediment stabilisation and the limitation of resuspension, the creation of refuges for zooplankton and the secretion of chemicals suppressing algal growth [43].

Hydrological, physical and mechanical methods

Cyanobacterial booms could be also limited by lake flushing or by the dilution of lake water. The aim of these measures is both the reduction of nutrient concentrations and the increase of the water exchange rate, which should lead to intensification of seston sedimentation [15]. The main condition which should be met is that the water used for dilution or flushing should be nutrient-poor. It would generate extra costs if this water had to be purified before use [32]. This technique has been applied since the beginning of 20th century, *eg* in lake Rotsee in Switzerland. The lake was flushed in 1920 by the waters of the river Ruess, but the results were poor, mainly because of the high nutrient content in the water used [15]. There are some examples of lake flushing from the Netherlands, *eg* in the hypereutrophic lake Veluwe (area 3240 ha, mean depth 1.3 m), where nutrient-poor water from polders was used. Phosphorus concentrations decreased and cyanobacterial blooms vanished for one year after the measure was taken but there were no further positive changes, mainly because of the high internal nutrient loading [32].

Cyanobacterial biomass can be also suppressed using ultrasonic radiation [44, 45]. An example of this is lake Senba in Japan (area 32 ha, mean depth 1 m) where a special device was constructed and was operated together with an air pump which, mixed the water column below the system. After two years of the experiment the water quality improved but there is a lack of data about the long term effects of the treatment [45]. Mechanical techniques can also be applied for the same purpose. The mechanical removing of cyanobacterial biomass is a low effect technique, because it is applied mainly to surface algal scums [14, 16]. But Shen et al [46] showed that it could be effective even on a whole lake. In lake Dianchi (area 30900 ha, mean depth 5.4 m) in China, about 400 Mg (tons) of dry cyanobacterial biomass was mechanically removed during a one and a half year period. The process consisted in the shaking, centrifugation, condensation and dehydratation of the biomass. The authors did not provide any data about the long term effects of the technique used.

Chemical methods

The use of many kinds of chemicals, known as algicides, was one of the first methods of algal control [15–16]. Algicide treatment – applied directly to the water body – has a lot of different types of disadvantages: negative effects on other water organisms, short term operation, the release of toxins, and oxygen depletion, which could appear as a consequence of mass cyanobacterial killing [15].

Copper sulphate is the most popular algicide which was formerly used. Because of environmental concerns, this chemical is not widely applied now. Hanson and Stefan [47] analysed the short and long term effects of copper use in cynaobacterial bloom control in five shallow lakes in the vicinity of Fairmont in the USA (the lakes were: George, Sisseton, Budd, Hall, Amber; the area 34–224 ha). The lakes were treated with a dose of 1647 kg $CuSO_4$/ha over a period of more than 50 years. Although the treatment was usually effective in killing cyanobacteria, other effects occurred: an

increase in the dead algal biomass lead to oxygen depletion and an increase in the release of phosphorus from the sediments, which consequently resulted in the reoccurence of algal blooms. Very short lasting effects of copper application were also observed in lake Courtille in France (area 22 ha, mean depth 2.5 m), where a dose of 63 μg/dm^3 Cu^{2+} was applied. The decrease in the chlorophyll *a* concentration and the decline of the blue-green *Microcystis* sp. population were noted directly after the treatment, but the positive effects lasted only two months [48].

Currently, the most popular chemical method in algal control is the use of barley straw. Barley straw is not an algicide itself, but the inhibiting action of this material is connected with its decomposition in lake water, when some algicides (*eg* phenolics, carboxylic acids) are released [49, 50]. The use of this material is cheap, environmentally safe and user-friendly, although it has some disadvantages, such as a lack of influence on some cyanobacterial species [51–53] or even stimulating effects on some bloom-forming species like *Planktothrix aghardii* and *Microcystis aeuruginosa* [54].

Barley straw was applied *eg* in two reservoirs suffering from cyanobacterial dominance: Middle Linacre and Bottom Linacre in Great Britain. While the first one was used as a control, the second one was treated in the spring with 3.5 mln Mg (tons) of barley straw placed in floating bales. After three months positive changes in the phytoplankton structure and its abundance were noted in the treated reservoir [49]. The effect of the long term application of barely straw on phytoplankton was presented by Barett et al [55]. Barley straw was exposed over several seasons in a tap water reservoir in Aberdeen in Great Britain. As a result, 25–50 % decrease in phytoplankton abundance was observed, but unfortunately it was not connected with a decline in cyanobacterial dominance.

Several attempts at limiting cyanobacterial growth are known in which other organic materials were tested. The influence of wheat straw (with negative results) and rice straw (with positive results) was analysed [56, 57], as well as the influence of oak leaves and bark [58], and of mandarin skin and banana peel [59]. However all these reports come from laboratory studies, so the efficiency of these methods applied in a lake or reservoir is unknown.

Conclusions

An extensive analysis of the literature concerning current methods for cyanobacterial bloom control in shallow lakes leads to some conclusions. They are enumerated below and could act as a kind of guide for water managers and local governments having problems with blue-greens in water bodies.

1. A cheap, easy and safe method of cyanobacterial bloom control in shallow lakes is still lacking.

2. Current methods, in the majority of cases, are effective only in the short term (< 10 years).

3. The use of several methods together (but in a specific sequence) appears to be the most effective approach (*eg* isolation from external sources of phosphorus – internal phosphorus inactivation – biomanipulation).

4. A precise analysis of lake or reservoir functioning is needed before undertaking any treatment. The analysis should cover such characteristics of the water body like: the water and nutrient budget, the thermal regime, the structure and chemical composition of sediments as well as the structure of biocenoses and food webs. Good diagnosis will help to choose the best and most suitable method.

5. The permanent withdrawal of cyanoabcterial blooms in any shallow lake without a reduction in external and internal sources of phosphorus is impossible.

6. Although the application of algicides is effective, it is short lasting. Barley straw exposition is the cheapest and the most user-friendly method, but its real effectiveness is still unknown.

7. The knowledge about the effects of most methods on water biocenosis is still poor, therefore any given method should be tested in the laboratory or in enclosures before being applied to the whole lake.

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METODY STOSOWANE W KONTROLI ZAKWITÓW CYJANOBAKTERII W PŁYTKICH ZBIORNIKACH WODNYCH

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Abstrakt: Jednym z globalnych problemów XXI w. jest eutrofizacja wód śródlądowych, w tym płytkich jezior, której częstym efektem są zakwity cyjanobakterii planktonowych. Problem zakwitów sinic znany jest

co najmniej od początku poprzedniego stulecia, podobnie jak i sposoby walki z tym negatywnym zjawiskiem, jednak to w ciągu ostatnich 25 lat, wraz z rozwojem ekologii płytkich jezior, daje się zauważyć wzrost liczby publikacji na temat nowych metod kontroli zakwitów. W artykule przedstawiono przegląd różnorodnych (chemicznych, fizycznych i biologicznych), aktualnie stosowanych metod ograniczania nadmiernego rozwoju cyjanobakterii w płytkich zbiornikach wodnych uwzględniając zarówno ich skuteczność, jak i wpływ na inne biocenozy wodne.

Słowa kluczowe: sinice, zakwity wód, rekultywacja zbiorników wodnych, inaktywacja fosforu, biomanipulacja, słoma jęczmienna

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TREATMENT OF ALIMENTARY INDUSTRY WASTEWATER IN SEQUENCING BATCH REACTORS SYSTEM

OCZYSZCZANIE ŚCIEKÓW PRZEMYSŁU SPOŻYWCZEGO W SYSTEMIE SEKWENCYJNYCH REAKTORÓW PORCJOWYCH

Abstract: This paper presents experiments conducted by various researchers on the purification of food industry wastewater in sequential batch reactors (SBR). The literature review covers such issues as 1) biological purification in SBR of wastewater containing molasses with a chemical oxygen demand (COD) of the order of 100,000 mgO_2/dm^3, 2) purification of wastewater from fish freezing in a hybrid system, 3) a SBR with granulated sludge and 4) membrane filtration or purification of abattoir wastewater with granulated sludge. The purification of dairy wastewater in the SBR system has also attracted considerable interest; the literature review therefore covers various methods of wastewater treatment, for example, the membrane SBR system and the anaerobic-aerobic (AF-SBR) system, applied on a very small production scale. This paper also presents the results of experiments conducted by these authors, involving purification of model dairy wastewater in the SBR system with the use of aids, such as pre-aeration of raw wastewater, introducing an additional anoxic phase or using natural zeolites. The degree of ammonium nitrogen removal achieved in the experiments, was high and equalled 97.5 %; the value for total phosphorus was 93 % and for total organic carbon – 99 % under the most favourable conditions.

Keywords: SBR, alimentary industry wastewater, dairy wastewater

The history of activated sludge is relatively short. As late as the end of the 19th century, wastewater treatment was understood as filtration, chemical precipitation or combinations thereof. Activated sludge systems originated in the USA and England. Studies of biological wastewater treatment started in simple experiments that involved pumping air

into tanks containing wastewater and hoping that the oxygen would oxidise the impurities. The experiments failed because the amount of microorganisms in the tanks was insufficient. According to Dupre and Dibbin (1884), *"aeration affected the wastewater to a small extent"*. In 1893, Mather and Platt showed the sludge accumulated at the bottom of an aerated tank to have a better ability to purify

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wastewater. From then on, intensive studies of wastewater aeration continued. In 1914, Ardern and Lockett presented "activated sludge" formed in wastewater using the "fill and decant" system. Unfortunately, in the 1920's sequential systems were transformed into continuous systems for several reasons: no simple devices for controlling the device operation time were available; as a result, all the process phases were changed manually, which required the constant presence of an operator; aerating stones in a sequential system were clogged more frequently than in a flow-through one and wastewater treatment in a sequential system was energy-consuming. The system was reintroduced after five decades, when computers had appeared on the market and effective aeration methods had been developed. In the meantime, studies had been conducted by Ardern and Lockett, Hoover and Porges, and others. In the 1950's, Hoover and Porges again presented a periodical system for dairy wastewater treatment and in the 1960's, one for purification of municipal wastewater. When Irvine called his periodical system SBR, he did not expect it to have great capabilities, which flow-through systems could not hope to reach. Since that time, SBR has been used to purify both industrial and community wastewater [1].

Molasses-containing wastewater has very high *chemical* (COD) and *biological oxygen demand* (BOD) of 100,000 mgO_2/dm^3 and 23,000 mgO_2/dm^3, respectively, high concentration of nitrogen of ca 2,300 mg/dm^3 and intensive colour. After pretreatment in anaerobic lagoons, molasses-containing wastewater is purified in SBR reactors [2]. 7.5 dm^3 of wastewater with COD of 1150 mgO_2/dm^3 and a nitrogen content of 1850 mg/dm^3 were put into 6 reactors with a capacity of 7.5 dm^3 each. The wastewater has still an intensive colour. Reactors worked in a 24-hour cycle, with the filling phase lasting one hour, the aeration phase 18 hours, sedimentation *ca* four hours, and decantation and holding time one hour. Acetic bacteria BP103 were added to the wastewater in order to remove its colour as they have the best ability to adsorb the molasses colour agent [3]. Apart from live bacteria cultures, autoclave-sterilised bacteria cells were put into the reactor; their adsorption ability was twice as high as that of live cells. Before wastewater was put into the SBR reactors, glucose was added to improve the purification effectiveness. The highest effectiveness of the reactors' operation was observed when glucose concentration was 30 g/dm^3. Under such conditions, over 50 % of colour was removed, as was 65 % of COD, 83 % of BOD and 32 % of Kjeldahl nitrogen. It was shown that dead bacteria cells could be reused after being washed with NaOH 0.1 mol/dm^3.

Another application of sequential batch reactors is the purification of abattoir wastewater. Canadian researchers treated wastewater in an SBR reactor using granulated sludge [4]. Initially, the sludge was used in anaerobic UASB reactors; now it is also used in aerobic ones. Creation of granules and increasing their diameter and density is favoured by short sedimentation time, high organic load (2.5 to 15 $kg \cdot m^3/d$), and short *hydraulic retention time* (HRT). The wastewater from the beef processing plant had the following composition: COD – 7685 mgO_2/dm^3, Kjeldahl nitrogen – 1057 mg/dm^3, total phosphorus – 217 mg/dm^3, suspension – 1742 mg/dm^3, volatile suspension – 1520 mg/dm^3. The reactor with an active capacity of 6 dm^3 and total volume of 8 dm^3 operated in the following process phase system: dynamic filling – 120 min, aeration –

220 min and decantation – 15 min. Sedimentation lasted 60 min in the first cycle, 30 min in cycles 2–5, 15 min in cycles 6–9, 5 min in cycles 10–13 and 2 min from cycle 14 onwards. Under stable experimental conditions, the sludge's age was equal to 20 days and the hydraulic retention time to three days. The granulated sludge was very effective in removing organic impurities. During the system's operation from day 16 to 76, average COD removal was equal 98.6 %, average nitrogen removal – 97.4 %, that of phosphorus – 98 % and volatile suspension – 97.2 %. These are very good results, considering the fact that the system was not optimised.

SBR reactors with flocculent and granulated sludge in combination with membranes were used by researchers [5] to purify fish freezing wastewater. The SBR reactors' operation mode were as follows: 3 min of aerobic feeding, 147 min of reaction, 1 min of settling, 3 min of withdrawal and 26 min of dead time in granular SBR. 60 min of anoxic feeding, 90 min of reaction, 20 min of settling and 10 min of withdrawal in flocculent SBR. The wastewater from the reactors was transferred to filtration chambers with membranes where they were constantly aerated to control membrane clogging. COD concentration in raw wastewater was about 700–1000 mgO_2/dm^3, that of nitrogen – 110–180 mgN/dm^3 and the mean concentration of phosphorus – 110 mgP/dm^3. COD removal in both systems exceeded 90 %, with the concentration in the effluent below 50 mgO_2/dm^3. Maximum nitrogen removal in the reactors did not exceed 60 %. However, the nitrification mechanism, which took place only in the reactor, was different. Nitrification took place during aeration in filtration chambers, which played the role of second stage reactors. In these reactors, reactors were fine purified. A membrane that filters wastewater purified in the reactor with granulated sludge is more effective than a wastewater-filtering membrane that filters effluent wastewater from a reactor with a classic activated sludge. The pressure that was to be applied was lower by 50 % than that applied in a system with a fine-purifying membrane in a reactor with classic activated sludge.

Some experiments were conducted on a small scale on dairy wastewater purification in the AF-SBR system [6]. Wastewater from a milk quality control laboratory was pumped onto an anaerobic filter with a capacity of 12 m^3, and subsequently to the SBR reactor with a capacity of 28 m^3. The wastewater was additionally contaminated with chemicals, such as sodium azide or chloramine used to fix the wastewater before the analyses. Fats were not removed before the wastewater got into the AF reactor. Research in the system was conducted over two years. COD in the effluent from the AF reactor was reduced by over 90 % (from the initial value of 222 000 mgO_2/dm^3), whereas COD concentration in the effluent from the SBR reactor was below 200 mgO_2/dm^3 and that of total nitrogen – below 10 mg/dm^3. No biomass was found to have been washed out of the system, and most fats in the raw wastewater were biodegraded (47 % reduction in the AF reactor and over 99 % in the entire system). Also Spanish researchers conducted experiments to examine purification of dairy wastewater in SBR reactors [7].

In order to improve the quality of the wastewater after treatment, Korean researchers [8] installed membrane separators inside the SBR reactor. Such a mixed system is called a *membrane sequential bioreactor* (MSBR). The system was used to purify real dairy

wastewater from Haitai Diary Co. (Suwon, Korea) without pretreatment. The MSBR reactor worked in the following cycle: one hour of filling, 10 hours of reaction and two hours of decantation. Aerobic and anaerobic phases were introduced during the reaction phase in order to increase biogenic substance removal. Application of the membrane module (MF10, SK Chemicals Co., Korea) at the final phase of the wastewater treatment resulted in suspension-free effluent. The membrane was washed only once during the 110-day work cycle (after the pressure of 44 kPa was achieved, at the flow of 52 $dm^3 \cdot m^2/h$) without switching off the system. A polysulphone membrane was used with a pore size of 0.1 μm. The effective membrane area was 1.0 m^2. The efficiency of BOD removal was very high, about 97–98 %, regardless of the process phase system. Nitrogen removal was equal to 96 % in the most favourable system of process phases, which is a good result, considering the BOD/TKN (*Total Kjeldahl Nitrogen*) ratio equal to 30, which did not favour nitrification bacteria growth. A high phosphorus concentration in raw wastewater (66.9 mg/dm^3) reduced the effectiveness of its removal (80 %) limited by the processes of biological removal.

Experimental procedures

The experiment was conducted using the set-up as shown in Fig. 1. The system consisted of two SBR reactors working in a parallel arrangement. The effect of the selected factors of wastewater treatment was examined in one; the other was used as a reference. The active capacity of an individual reactor was equal to 12 dm^3. Synthetic

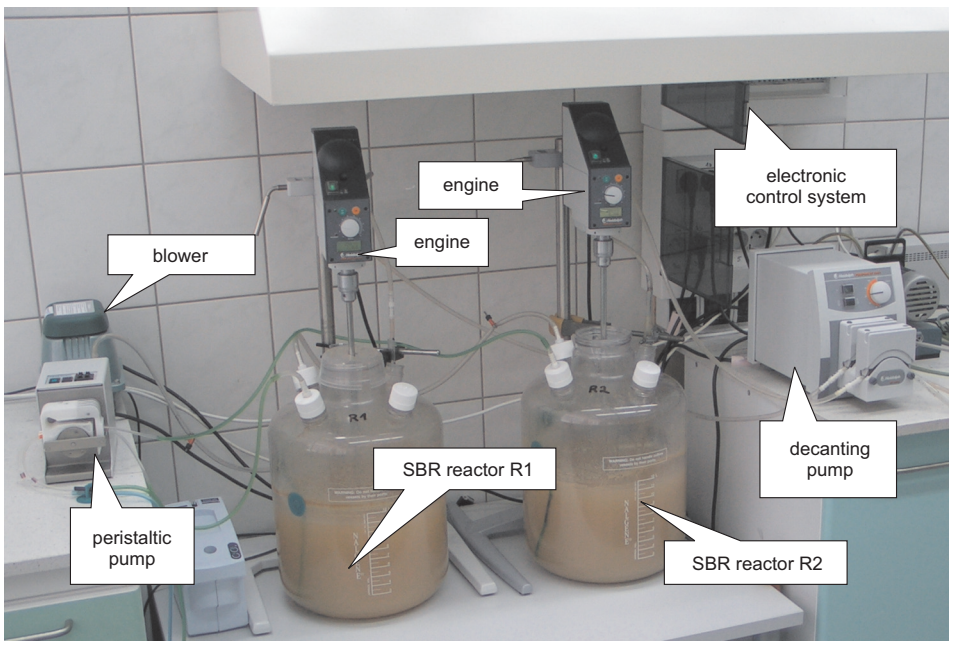

Fig. 1. The experimental set-up

wastewater was fed to the system with an ISM 828 peristaltic pump, manufactured by Ismatec Reglo; purified wastewater was decanted through a U-shaped decanter with a PumpDrive 5001 pump manufactured by Heidolph. Using such a decanter shape meant that wastewater could be channelled off without any improperly settling sludge being carried away with it. The depth of the decanter fixing ensured the amount of decantation of c. 33 % of the reactor's active volume. Wastewater was stirred with HEIDOLPH RZR 2051 agitators at a speed that ensured its proper mixing. Stirring was effected using VISCO JET stirring tips, whose shape ensured the flow without destroying the flocculate structure of the activated sludge. Air was fed into the reactors with HIBLOW AIR PUMP H810, type SPP-15GA blowers through aerating curtains of small air bubbles, the curtains being 90 cm long and situated at the bottom around the container's perimeter. The duration of individual process phases was controlled by a PCm.08 3k digital programmer, manufactured by METRON. Only excessive sludge was removed manually. The values of the basic system indicators were monitored with the computer connected to the experimental set-up; the indicators included oxygen concentration, pH, redox potential and monitoring was effected using MultiLab® pilot communication software and the appropriate electrodes. The basic cycle of the system operation lasted 12 hours and consisted of the following process phases:

I. Static filling – 1 hour;

II. Dynamic filling (combined with stirring) – 2 hours;

III. Aeration combined with stirring – 7 hours;

IV. Sedimentation – 1 hour;

V. Decantation – 0.5 hours;

VI. Idle phase (technical break) – 0.5 hours.

The mean BOD concentration in the synthetic raw wastewater at that stage of research was equal to 1350 mgO_2/dm^3, mean TOC (*Total Organic Carbon*) value was 610 mgC/dm^3, ammonium nitrogen 15.93 mgN_{NH4}/dm^3, nitrate nitrogen(V) 0.57 mgN_{NO_3}/dm^3, Kjeldahl nitrogen 71.33 mgN_{KJ}/dm^3, total phosphorus – 16.97 mgP_{tot}/dm^3.

The overall experiment was aimed at determining the wastewater treatment kinetics in the SBR system, the effect of changes of organic impurities' concentration in raw wastewater on the treatment process, the effect of raw wastewater reaction on their biological treatment, the effect of the sludge age on the wastewater treatment process, the effect of modification of the basic process phase system on the wastewater treatment effectiveness and supporting dairy wastewater treatment with natural zeolites. During the purification process of dairy wastewater, as well as other types of wastewater, problems are faced with the removal of biogenic substances and their reduction to the normative amounts. A lot of experiments are being carried out aimed at reducing the amount of biogenic substances without using chemical substances. Using zeolites is one of the solutions. Zeolites are aluminosilicates made of aluminate and silicate tetrahedrons, which impart them with electronegative character and high affinity to cations, especially to ammonium ions. Vast research into using natural zeolites in water and wastewater treatment has been conducted for several years by Prof. A.M. Anielak at the Department of Water and Wastewater Technology of the Koszalin University of

Technology. As a result of the research, natural zeolite in a powdery form was successfully used in community wastewater treatment at the Dygowo wastewater treatment plant [9]. A reactor with zeolite produces a 50 % increase in ammonium nitrogen removal effectiveness and an over 30 % increase in total nitrogen removal. Phosphorus removal increased by several percent too. Moreover, zeolite increased and stabilised the extent to which organic compounds were removed from the wastewater and provided an effective bed for microorganism growth.

The following were the independent factors in the experiment: sludge age (15 d), load of organic impurities on the sludge, raw wastewater reaction, type and amount of reagent. The resulting factors for purified wastewater included: ammonium nitrogen, nitrate nitrogen(V), total phosphorus, phosphates, redox potential, pH, dissolved oxygen concentration, BOD, TOC. The weight of activated sludge and its volume index were determined. The paper presents a fragment of a study on the effect of modification of the process phase system, which involves introduction of preaeration of raw wastewater and additional anoxic phases and using powdered natural zeolite to remove selected impurities from the wastewater.

Discussion of results

Figure 2 shows the effectiveness of ammonium nitrogen removal in the study. The lowest ammonium nitrogen removal index was achieved after additional anoxic phases were introduced; it was equal to 64 % for a 1-hour anoxic phase and 72 % for a 2-hour phase. The removal index reduction resulted from the essence of the nitrification process. A higher effectiveness of removal of that form of nitrogen was achieved by introducing powdered natural zeolite to the system – 89.5 % and by the application of preaeration of raw wastewater – 94 %. The highest removal effectiveness – 97.5 % – was achieved after preaeration of the raw wastewater and after introducing natural zeolite to the system. Mean N_{NH_4} removal in the system with no modifications was equal to 80.5 %.

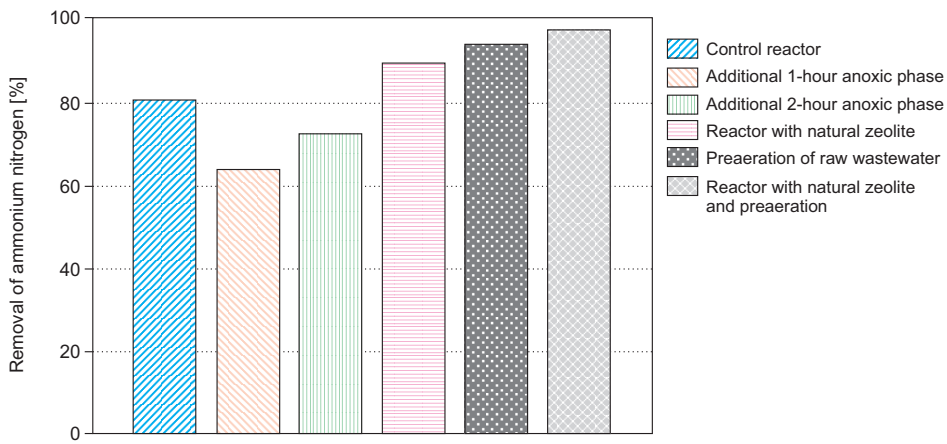

Fig. 2. Effectiveness of ammonium nitrogen removal

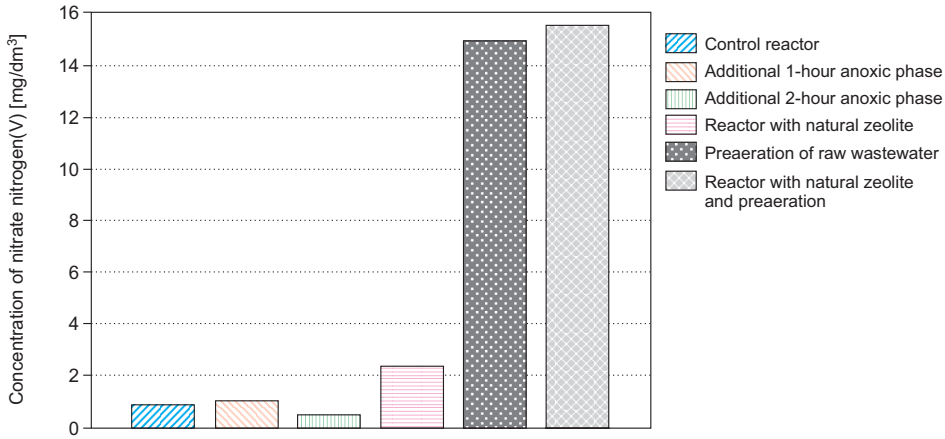

Fig. 3. Concentration of nitrate nitrogen(V) in the study

Concentration of nitrate nitrogen(V) in purified wastewater is shown in Fig. 3. At each stage of the study, the concentration of nitrate nitrogen(V) in purified wastewater was equal to or lower than that in the raw wastewater (0.57 mg/dm^3). A higher concentration of nitrate nitrogen(V), *ca* 2 mg/dm^3, was recorded after natural zeolite was introduced to the system; it provided a perfect substrate for microorganism growth, thereby making nitrification more efficient. A high concentration of that form of nitrogen during the raw wastewater aeration phase (ca. 15 mg/dm^3) may be proof of very intensive nitrification. The air pumped into raw wastewater provided better conditions for nitrification bacteria growth. The highest concentration of nitrate nitrogen(V) was observed in the reactor where natural zeolite was added, into which preaerated wastewater was pumped.

Removal of total phosphorus is shown in Fig. 4. The average effectiveness of its removal in the control reactor was equal to 75 %; a slightly higher value – 80 % – was

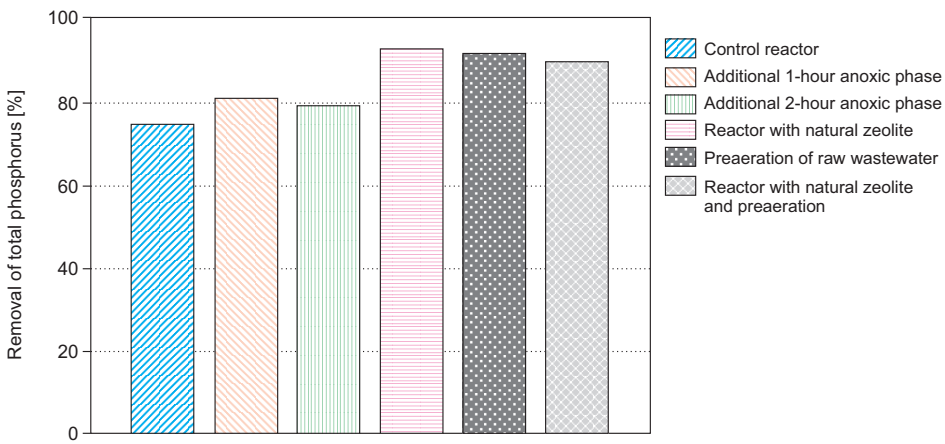

Fig. 4. Effectiveness of total phosphorus removal in the study

achieved by introducing an additional anoxic phase, regardless of its duration. The effectiveness of total phosphorus removal was highest in the SBR reactor after addition of natural zeolite and with preaeration of raw wastewater. The effectiveness of its removal under such conditions was equal to 90–93 %.

The effectiveness of total organic carbon (TOC) removal in the system was very high. It was no lower than 96 % throughout the experiment (Fig. 5). The average effectiveness increased to 97.6 % after additional anoxic phases were introduced and the effectiveness of total organic carbon removal was equal to 98 % after preaeration of raw wastewater was applied; it increased to nearly 99 % after natural zeolite was introduced, which is a perfect substrate for microorganism growth and can adsorb simple biodegradation products; under normal conditions, as a result of dynamic purification, they could get to wastewater as secondary impurities [9].

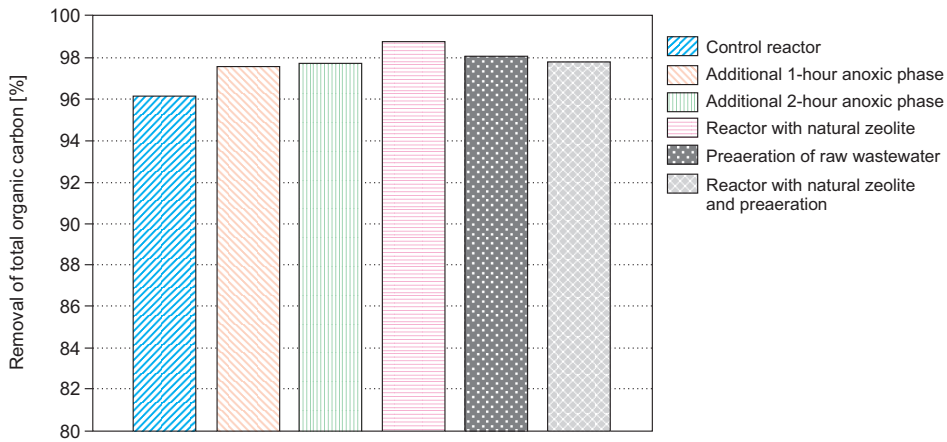

Fig. 5. Effectiveness of total organic carbon removal in the study

Conclusions

The experiment conducted over the 12-hour work cycle of an SBR reactor has ensured the effective removal of contaminants from modelled wastewater. Modification of the process phase system which involved preaeration of raw wastewater resulted in improvement of the purified wastewater quality as compared with the system without preaeration. Nitrogen ammonium removal increased by 14 % on average and total phosphorus removal by 17 %. A high concentration of nitrate nitrogen(V) was also recorded, which could indicate intensive nitrification. The system modification of introducing an additional anoxic phase increased the total phosphorus removal index by several percent; the ammonium nitrogen removal decreased; however, this was expected and resulted from the essence of the nitrification process. Introducing natural zeolite to the system also increased the effectiveness of impurities' removal as compared with the reference reactor. The TOC removal index was equal to 99 %, that of total phosphorus 93 %.

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OCZYSZCZANIE ŚCIEKÓW PRZEMYSŁU SPOZYWCZEGO W SYSTEMIE SEKWENCYJNYCH REAKTORÓW PORCJOWYCH

Katedra Technologii Wody i Ścieków
Politechnika Koszalińska

Abstrakt: W pracy zaprezentowano doświadczenia różnych badaczy w oczyszczaniu ścieków przemysłu spożywczego w sekwencyjnych reaktorach porcjowych (SBR). Przegląd literatury obejmuje takie zagadnienia, jak: 1) biologiczne oczyszczanie w reaktorach SBR ścieków zawierających melasę o stężeniu ChZT rzędu 100 000 mgO_2/dm^3, 2) oczyszczanie ścieków powstających w procesie mrożenia ryb w układzie hybrydowym, 3) reaktor SBR z osadem granulowanym oraz 4) filtracja membranowa czy oczyszczanie osadem granulowanym ścieków pochodzących z rzeźni. Sporym zainteresowaniem cieszy się również oczyszczanie ścieków przemysłu mleczarskiego w systemie SBR, dla tego w przeglądzie literatury zawarto różne metody oczyszczania tych ścieków m.in. oczyszczanie w membranowym systemie SBR, a także w układzie beztlenowo-tlenowym (AF-SBR), zastosowanym w skali ćwierć technicznej. Przedstawiono także wyniki badań własnych w oczyszczaniu modelowych ścieków przemysłu mleczarskiego w systemie SBR z zastosowaniem czynników wspomagających, takich jak wstępne napowietrzanie ścieków surowych, wprowadzenie dodatkowej fazy anoksycznej, czy zastosowanie zeolitów naturalnych. W przeprowadzonych badaniach uzyskano wysoki stopień usunięcia azotu amonowego wynoszący 97,5 %, fosforu ogólnego wynoszący maksymalnie 93 % i ogólnego węgla organicznego wynoszący w najbardziej sprzyjających warunkach prawie 99 %.

Keywords: SBR, ścieki przemysłu spożywczego, ścieki mleczarskie

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Elwira TOMCZAK[1]

QUALITY OF WELL WATER IN STARA WIES IN PIOTRKOW DISTRICT

JAKOŚĆ WÓD STUDZIENNYCH W STAREJ WSI W POWIECIE PIOTRKOWSKIM

Abstract: The aim of the research was to study the quality of drinking water in the agricultural region. Determination of the quality of water which is for immediate consumption by farmers who have not very deep wells is a very important problem. Farmers usually estimate the quality of water in their wells on the basis of subjective sensations – most frequently taste and flavour, without any professional control. Therefore, our tests had control and educational aspects.

The control tests were made for different 10 wells from Stara Wies in February, April and June 2010. The following parameters were taken into account: nitrates, nitrites, chlorides, chromium, copper, iron, manganese, hardness, pH, conductance, etc. Analysis were carried out by means of Spectrophotomer DR/2010 (Hach). The results were compared with the actually Polish Standards and EU Directive.

It was concluded that water quality was affected by climatic conditions and season of the year. Unfortunately in this region appearance local floods generated by intensive rainfalls.

Keywords: wells, water quality, standards

Introduction

Water is an indispensable element in human life and economy. It is the subject of consumption, a prerequisite for hygiene and health and, above all, it is the basis for the development of industry, agriculture and other sectors of the economy. The main factor that determines the usefulness of natural water for a particular purpose is its quality. Particular attention should be paid directly to water intended for human consumption. Drinking water consumed for many years and sometimes for life from a single source must be subject to special control. Potential pollutants occurring in the excessive amounts are potentially harmful to health, especially those that accumulate in the body and can lead to pathological changes [1].

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One of the important indicators of water quality is the presence of metals, especially heavy metals. Both deficiency and excess are detrimental to human health. Heavy metals can penetrate into the body in different ways (through the respiratory, digestive system, skin etc), but accepting them with water can lead to accumulation in the body.

The quality of groundwater is a function of many parameters, whose share in terms of the volume is variable in time. The composition of water is dependent on the characteristics of the area (geological structure, sorption properties of the medium, the processes of weathering and dissolution) processes in the water, deep wells and their location in relation to pollution sources. In the well water one can expect agricultural pollution, which mainly consist of plant protection products, fertilizers derived from surface runoff from fields and meadows, compounds derived from dunghill and animal farming areas [2].

Wells capture water from the first and the most shallow aquifer. The presence of contaminants can result in noticeable organoleptic changes and unpleasant taste, odor or color of the water and then more easily react to changing water quality and take appropriate preventive steps. It is worse when changes are physicochemical, undetectable by the consumer which directly can cause qualitative composition of drinking water being more significant in human health effects.

It should be noted that the safest solution for households is to connect the home to the local water supply as this water must be checked for quality by specialized units in order to minimize the risk of contamination.

Scope of experiments

The aim of this study was to determine the quality of water consumption, exploited from brick-lined wells in rural households. To determine the quality of water from the above-mentioned sources there was selected an agricultural area located in the south-eastern province of Lodz. In the spring season 2010 (February, April, June) there has been periodically collected water from selected ten brick-lined wells (water injection) with an average depth of 5 m to 10 m at different points in Stara Wies (Rozprza community, Piotrkow District). Wells were spaced apart from a few to several hundred meters. Weather conditions occurring on a day of water sample collection were as follows:

1. February: –10 ^{o}C, cloudy, snow cover thickness of 30 cm;
2. April: 3 °C, cloudy, without rain, the week after thaw;
3. June: 12 °C, overcast, drizzle, four weeks after the local flooding.

Wells 1–10 were located on farms in the Stara Wies in succession under the numbers “42”, “44”, “166”, “180”, “181”, “182”, “185”, “189”, “192”, “194”.

Using a spectrophotometer RD/2010 there was performed a qualitative analysis of water. There were identified 13 indicators adequately characterizing the water quality. Final results were expressed as mean of three determinations. In accordance with the procedures attached to the camera recommended by the U.S. Environmental Protection Agency there were determined:

- Hardness of water – (0 to 4.0 mg/dm^3 Ca and Mg as $CaCO_3$),
- Iron – (0 to 3.0 mg/dm^3 Fe),

- Sulphate – (0 to 70 mg/dm^3 SO_4^{2-}),
- Chlorine – (0 to 2.0 mg/dm^3 Cl_2),
- Chloride – (0 to 20 mg/dm^3 Cl^-),
- Chromium – (0 to 6 mg/dm^3 Cr^{3-}),
- Copper – (0 to 5 mg/dm^3 Cu),
- Manganese – (0 to 0.7 mg/dm^3 Mn),
- Nitrate – (0 to 4.5 mg/dm^3 $N\text{-}NO_3^-$),
- Nitrites – (0 to 3.0 mg/dm^3 $N\text{-}NO_2^-$),
- Turbidity – (0 to 450 FAU),
- Conductivity – (μS/cm at 20 °C),
- pH – (1 to 14).

Water supply network in the community of Rozprza

The primary source of income of Rozprza community is agriculture. It is mainly dominated by the cultivation of cereals and the breeding of pigs and cattle. Agricultural land has an area of 10704 ha, or 65.8 % of total area of the commune. They are dominated by private farms 7–15 ha. The community has 12115 inhabitants, who live in the area of 163 km^3.

Within the borders of Rozprza community, underground waters are mainly connected with the Quaternary and upper-cretaceous forms.

Basic utility Upper Cretaceous aquifer is a water-bearing floor and is formed by marls, limestone and tufa. This level is mainly at a depth of several to 60 m below the ground level. The quaternary aquifer is associated with fluvio-glacial sands and it occurs at a depth of several meters to 40 m below ground level. For municipal water intakes this level is of subordinate meaning.

Water intakes are located in towns: Bialocin, Milejow and Mierzyn (showing the operation of Upper Cretaceous aquifer) and in Lubien (quaternary aquifer). In the area of municipality there are 41 villages and a number of buildings connected to the network is 3200.

Acceptability of water supplied by the public does not relieve companies of water supply and sanitary inspection authorities from the obligation to monitor water quality. Depending on the type of water supply and the determined index there exists variation in the frequency of controls and water outlets. In case of the microbial assays (*Escherichia coli* and *fecal streptococci – enterococci*) sampling should take place on the length of the whole water supply system. Consumers should continue to have access to information and to obtain advice on the potential for improving water directly consumed. The guarantee of health security is the implementation of Water Safety Plans, which is the proper water quality management.

Control of water quality for drinking water supply system in the municipality is conducted on an ongoing basis by the District Sanitary and Epidemiological Station in Piotrkow. The frequency of sampling and testing of water are carried out 5 times a year with an average frequency of once every 3 months.

The work is acquainted with the reports of studies conducted in 2009 for drinking water intake located in Bialocin and Milejow. The water was characterized by good quality except for excess of levels of bacteriological indicators for inclusion in Milejow (19.01.2009). The results are shown in Table 1.

Table 1

The report of investigations of water intake – Milejów

Test	Unit	Result	Limit value
Colour	$mgPt/dm^3$	5	15
Turbidity	NTU	0.35	1
Reaction	pH	7.1	6.5–9.5
Conductivity	S/cm at 25 °C	830	2500
Smell	organoleptically	none	accept.
Taste	organoleptically	nb	accept.
Ammonia ion	$mgNH_4^+/dm^3$	< 0.10	0.50
Nitrates	$mgNO_3^-/dm^3$	1.6	50
Nitrites	$mgNO_2^-/dm^3$	0.007	0.50
Manganese	$mgMn/dm^3$	< 0.020	0.050
Iron	$mgFe/dm^3$	< 0.020	0.200
Fluorides	mg/dm^3	0.2	1.5
Chromium	$mgCr/dm^3$	< 0.005	0.050
Cadmium	$mgCd/dm^3$	< 0.001	0.005
Lead	$mgPb/dm^3$	< 0.010	0.025
Chlorides	$mgCl^-/dm^3$	33.0	250
Sulphates	$mgSO_4^-/dm^3$	9.6	250
The number of microorganism in 1 cm^3 on an agar at temperature of 22 °C after 72 h	cfu	*ca* 400	100
The number of microorganisms in 1 cm^3 on an agar at the temperature of 36 °C after 48 h	cfu	2	50
Bacteria *coli*	cfu	0	0
Escherichia coli in 100 cm^3	cfu	0	0
Enterococci in 100 cm^3	cfu	0	0

Results and discussion

Requirements for the quality of water intended for human consumption until recently were defined by the *Ministry of Health of 29 March 2007 on the quality of water intended for human consumption*. On 20 April of 2010 the Minister of Health Ewa Kopacz, has signed a regulation amending the aforementioned Regulation (*Decree of the Minister of Health of 20.04.2010 amending Regulation on the quality of water intended for human consumption – O.J. no. 72, item. 466*). The amendment was carried

to full and proper implementation of *Directive 98/83/EC* on the observations of the European Commission [3]. This authority previously noted that Polish legislation does not transpose the Directive requirements in relation to the number of such micro-organisms at 22 °C, turbidity, total organic carbon and color, taste and smell. These parameters are mainly used to determine the correctness of the process of water treatment and distribution without having a direct impact on consumer health, but they play an important role in the perception of water quality. Other observations/comments on amendments to existing legislation, de facto leading towards the security of consumers' health can be found in Wichrowski et al [4].

Figures 1–4 show examples of changes in water quality indicators by comparing them with the current regulatory framework.

Analyzing the results obtained it can be concluded that in most wells, there was a suspension of fine organic and inorganic particles, hence water was thus characterized by a different color, from transparent to yellow.

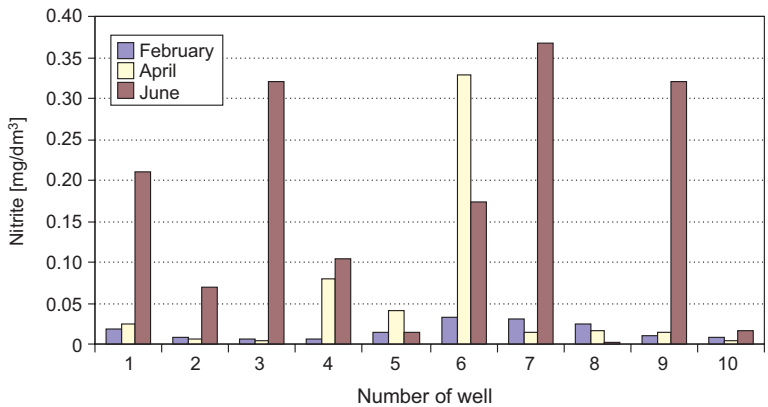

Fig. 1. Nitrite content in well water (standard 0.5 mg/dm^3)

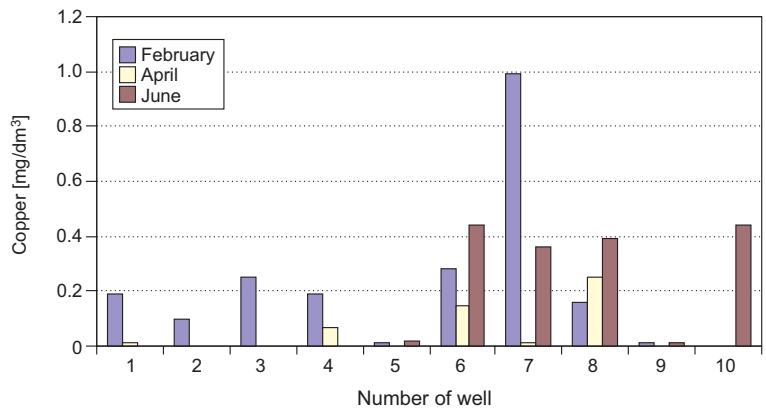

Fig. 2. Copper content in well water (standard 2.0 mg/dm^3)

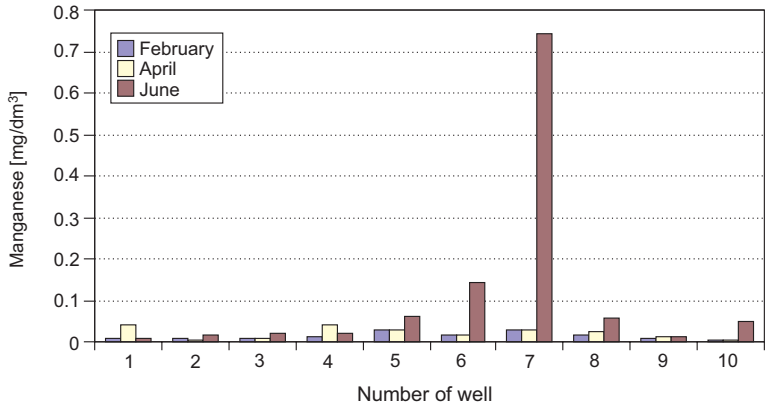

Fig. 3. Manganese content in well water (standard 0.05 mg/dm^3)

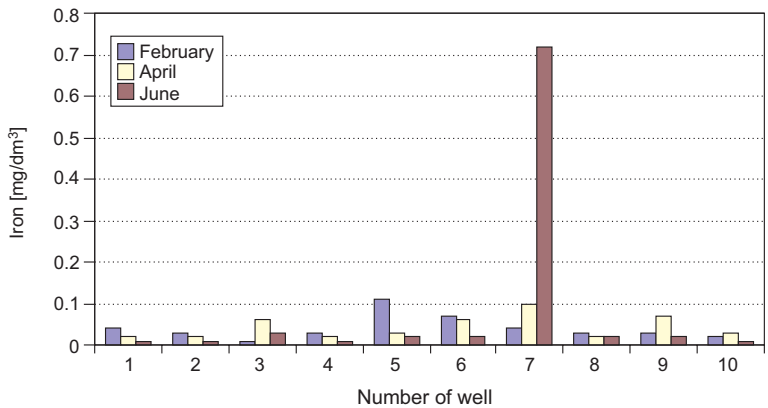

Fig. 4. Iron content in well water (standard 0.2 mg/dm^3)

All water samples were characterized by high turbidity above 1 NTU value. The pH of water was maintained at a constant level, within pH 7–8.

In two cases, for wells No. 6 and 8, the value of conductivity has been slightly exceeded.

In June for wells 5–8, there have been exceeded acceptable levels of manganese, in addition to the well no. 7 which was found to exceed the value of iron, but in other waters, these indices remained constant at a low level, not exceeding the standards. There was no excess of indicators such as nitrites, nitrates, copper, chlorides, sulfates, chlorine. In the case of nitrates and nitrites they have increased after the spring fertilization.

The final conclusion is that the worst water quality was characterized by well no. 7, while the best water came from well no. 10 and about the results of the studies there were informed the property owners in the area where water samples for quality assessment were collected.

Conclusions

Until recently, on agricultural land the well was the only source of drinking water. At present, despite the significant water service coverage of rural areas, there are still places where people use well water. This is due to economic reasons, from the lack of access to water supply system or unprofitability of its supply, and therefore, the problem of water quality derived from these shots is still very important. However, please note that access to clean drinking water is a fundamental right and need of every man in the world. According to the EU Water Framework Directive, water should not only be a commercial commodity like any other good natural resources, but the legacy of generations must be protected and appreciated. 22 March 2010 – World Water Day was celebrated under the theme *"Clean water for the health of the world"*. The ideas promoted in the framework of the celebrations aim to raise awareness that water quality has a direct impact on human health.

In rural areas, the greatest threat to groundwater quality is agriculture and the associated use of pesticides and fertilizers. Another source of water pollution may be inappropriate location of wells in relation to the collection tanks of liquid and solid.

There are also unforeseen natural phenomena with a random character, which may locally result in a significant deterioration in water quality. This group includes the floods and inundation. They may also be the result of human activity through the disruption of normal phenomena, or the result of technical failure of equipment. But the main cause of flooding is more rainfall in relation to the possible infiltration of the soil per unit time.

In the course of research in water in the area of water sample collection there was local flooding. The land was flooded to a height of 50–100 mm. This situation was related to the occurrence of floods throughout the country, causing a significant change in the organoleptic characteristics of water. In some wells there was observed the increase in turbidity, color from yellow to brown and smell, as well as the presence of numerous microflora and fauna.

Fortunately, the owners of wells intuitively abandon using that water for any purpose.

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JAKOŚĆ WÓD STUDZIENNYCH W STAREJ WSI W POWIECIE PIOTRKOWSKIM

Wydział Inżynierii Procesowej i Ochrony Środowiska
Politechnika Łódzka

Abstrakt: Badania obejmowały jakość wody gruntowej ze studni przydomowych znajdujących się na terenach rolniczych w miejscowości Stara Wieś. Określenie jakości wody bezpośrednio przeznaczonej do konsumpcji przez rolników, którzy eksploatują płytkie studnie, jest ważnym problemem. Konsumenci zwykle sami określają jakość wody do picia na podstawie subiektywnych odczuć, najczęściej smakowych i zapachowych, bez profesjonalnej kontroli. Dlatego też nasze badania miały kontrolny i edukacyjny aspekt. Analizą objęte były wody pochodzące z dziesięciu studni kręgowych w lutym, kwietniu i czerwcu 2010 r. Oznaczano następujące wskaźniki fizykochemiczne jakości wody: azotany, azotyny, chlorki, chrom, miedź, żelazo, mangan, twardość, pH oraz przewodność etc. Analizy prowadzono z użyciem Spektrofotomeru DR/2010 firmy Hach. Wyniki odniesiono do aktualnie obowiązujących unormowań polskich i europejskich.

Stwierdzono, że jakość wody zależy od warunków klimatycznych i pory roku. Niestety na tym terenie w okresie badań wystąpiły lokalne podtopienia wywołane znacznymi opadami, co skutkowało pogorszeniem jakości wody.

Słowa kluczowe: studnie, jakość wody, normy

Varia

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