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Barbara SYMANOWICZ and Stanisław KALEMBASA[1]

EFFECT OF IRON, MOLYBDENUM AND COBALT ON THE AMOUNT OF NITROGEN BIOLOGICALLY REDUCED BY *Rhizobium galegae*

WPŁYW ŻELAZA, MOLIBDENU I KOBALTU NA ILOŚĆ AZOTU BIOLOGICZNIE ZREDUKOWANEGO PRZEZ *Rhizobium galegae*

Abstract: Iron and molybdenum are microelements which have a decisive effect on the amount of nitrogen fixed by legume plants. They are mainly necessary in the synthesis of nitrogenase. Cobalt also takes part in the process of biological reduction of N_2.

In order to determine the effect of Fe, Mo and Co on the amount of nitrogen fixed by fodder galega (*Galega orientalis* Lam.), a field experiment was conducted in the years 2005–2007 at the experimental station of the University of Natural Sciences and Humanities in Siedlce. Nitrogen ^{15}N enriched at 10.3 at % was applied in early spring as $(^{15}NH_4)_2SO_4$ at 1.66g per $1m^2$. Simultaneously with fodder galega (*Galega orientalis* Lam.), a plant unable to reduce nitrogen N_2 was cultivated (spring barley – *Hordeum sativum*), which was also fertilised with ^{15}N as $(^{15}NH_4)_2SO_4$ with 10.3 at % enrichment. Upon harvesting the test plant in the budding phase (three cuts), samples were taken, dried and ground. Soil samples were also taken before each cut. Fe, Mo and Co content in soil and the plant was determined by the ICP-AES, following its dry mineralisation. Statistical calculations revealed significant differences in the content of iron, molybdenum and cobalt in the soil and in the plant. The soil contained the largest amount of iron, less cobalt and the smallest amount of molybdenum. The iron amount found in biomass of fodder galega lay within the optimum limits specifying the acceptable content of trace elements in fodder, whereas the molybdenum content was too high and that of cobalt was too low. The correlation coefficients indicate a significant relationship between the content of iron, molybdenum and cobalt in soil and the amount of nitrogen reduced biologically by *Rhizobium galegae* bacteria which live in symbiosis with fodder galega.

Keywords: fodder galega, nitrogen, iron, molybdenum, cobalt, soil, plant

Iron, molybdenum and cobalt are among the elements which are essential to plants and animals [1, 2]. The optimum content of the microelements in plants is an important quality feature in assessment of their usability as fodder. Restricting organic fertilisation

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and using exclusively mineral fertilisers leads very frequently to a negative balance of microelements in soil, which may affect a plant's ability to take them up [3]. Moreover, insufficient amounts of the elements available to plants may lead to disturb generative development of plants [4, 5]. Iron and molybdenum also have a decisive effect on the amount of nitrogen fixed by legume plants. Iron and molybdenum are essential in the synthesis of nitrogenase, whereas cobalt is incorporated in the molecules of porphyrins which are parts of the cobalamine, which is a coenzyme directly participating in the process of fixing N_2.

The process of biological reduction of nitrogen involves incorporation of molecular nitrogen in the biological system [6–10]. This ability is used by *Rhizobium* bacteria which live in symbiosis with legume plants, as well by free-living bacteria (*Azotobacter, Clostridium*) as well as fungi (*Rhizopus*) and actinobacteria (*Streptomyces*) [11, 12]. Their common feature is the presence of nitrogenase – the main enzyme which is responsible for fixing elemental nitrogen. Nitrogenase consists of two protein complexes: Protein comprising Mo-Fe is an enzyme which reduces N_2, whereas the protein that contains only Fe provides electrons necessary for reduction.

Recently, much interest has been attracted by the comprehensive research into fodder galega, conducted by Andrzejewska and Ignaczak [13], Ignaczak [14], Sienkiewicz et al [15], Symanowicz and Kalembasa [16]. It is well-known that the presence of Fe, Mo and Co in soil is associated with the process of biological reduction of N_2. However, there are no data on the effect of those elements on the process of fixing nitrogen by symbiotic and free-living bacteria.

The aim of the study was to determine the content of iron, molybdenum and cobalt in soil and plants as well as to determine the effect of those elements on the amount of nitrogen fixed by fodder galega (*Galega orientalis* Lam.) in three consecutive cuts and vegetation periods.

Materials and methods

A field experiment was conducted in the years 2005–2007 in a multi-year plantation of fodder galega (9th, 10th and 11th year of cultivation). Field experiments were conducted at the experimental station of the University of Natural Sciences and Humanities in Siedlce, on soil formed from loamy sand, with pH measured in 1mol $KCl \cdot dm^{-3}$ – 6.9. The soil on which the experiment was conducted contained 31.2 $g \cdot kg^{-1}$ of total carbon and 3.6 $g \cdot kg^{-1}$ of total nitrogen. The content of available phosphorus and potassium was determined to be of a medium level and that of magnesium to be low. The total content of iron in the soil on which galega was cultivated was equal to 4738.1 $mg \cdot kg^{-1}$, that of molybdenum was 0.31 $mg \cdot kg^{-1}$ and cobalt was 1.98 $mg \cdot kg^{-1}$. Nitrogen ^{15}N with 10.3 % enrichment was applied in early spring as $(^{15}NH_4)_2SO_4$ at 1.66 g per $1m^2$. Simultaneously with fodder galega (*Galega orientalis* Lam.), a plant unable to reduce nitrogen N_2 was cultivated (spring barley – *Hordeum sativum*) which was also fertilised with ^{15}N as $(^{15}NH_4)_2SO_4$ at 10.3 % enrichment.

Three cuts of the test plant during the budding phase were harvested in each year of the study. Samples of soil from the humus horizon were taken three times (before the first cut, before the second cut and before the third cut) and were subsequently dried and sifted through a 1 mm mesh sieve. During consecutive harvests of fodder galega (*Galega orientalis* Lam.), samples of entire plants were taken, which were subsequently dried and ground. The content of iron, molybdenum and cobalt in soil and in the plant was determined following dry mineralisation, by the ICP-AES method [17].

The results were worked out statistically and the analysis of variance was applied. Significant differences were calculated with Tukey's test at the level of significance of $\alpha = 0.05$. The effect of the content of iron, molybdenum and cobalt on the amount of nitrogen biologically reduced by fodder galega (*Galega orientalis* Lam.) was determined with correlation coefficients.

Results and discussion

Rainfall and temperature data for the 2005–2007 vegetation seasons are shown in Table 1.

Table 1

Air temperatures and rainfall in the vegetation in the years 2005–2007.
Reported by the measurement centre in Siedlce

Means air temperature [^{o}C]								
Months	Years	IV	V	VI	VII	VIII	IX	Mean
Mean monthly	2005	8.6	13.0	15.9	20.2	17.5	15.0	15.0
	2006	8.4	13.6	17.2	22.3	18.0	15.4	15.8
	2007	8.3	14.5	18.2	18.5	18.6	13.1	15.2
Multiyear mean		7.7	10.0	16.1	19.3	18.0	13.0	11.4
Total monthly rainfall [mm]								Sum
Sum monthly	2005	12.3	64.7	44.1	86.5	45.4	15.8	268.8
	2006	29.8	39.6	24.0	16.2	227.6	22.0	359.2
	2007	21.2	59.1	59.9	70.2	31.1	67.6	309.1
Multiyear sum		52.3	50.0	68.2	45.7	66.8	60.7	343.7

The average monthly temperature in consecutive vegetation periods was similar (15.0 ^{o}C to 15.8 ^{o}C) and it was much higher than the multi-year average. The temperatures recorded during the vegetation period favoured the process of biological reduction of N_2 [18]. The average rainfall during the vegetation period was lower than the multi-year total. Only in 2006 it was slightly higher (by 15.5 mm). This was a result of high rainfall in August, which was three times higher than the multi-year average.

The average iron content in the humus level of soil was equal to 4065.3 mg $\cdot$ kg^{-1} (Table 2) and it was significantly varied by the analysed factors and their combinations. The significantly highest iron content was determined in the soil sample taken before the first cut of fodder galega (4254.4 mg $\cdot$ kg^{-1}). The content of iron in 2005 and 2007 was similar (4285.8 and 4282.9 mg · kg^{-1}). Significant differences were found between iron content in soil in 2005 and 2006 and between 2006 and 2007. The results were confirmed by a study conducted by Kalembasa and Symanowicz [19].

Table 2

The content of total iron in the soil [mg · kg^{-1}]

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	4581.8	3732.1	4449.3	4254.4
II	4328.7	3670.1	4292.7	4097.2
III	3947.0	3479.1	4106.8	3844.3
Mean	4285.8	3627.1	4282.9	4065.3

LSD$_{0.05}$ for: cuts (A) – 31.8; research years (B) – 31.8; interaction (AxB) – 55.0.

The correlation coefficients indicate a significant positive relationship (r = 0.96*) between iron and molybdenum content in soil. A significant negative correlation was also shown to exist between iron content in soil and: cobalt content in soil (r = –0.99*); iron content in the plant (r = –0.95*); molybdenum content in the plant (r = –0.98*); cobalt content in the plant (r = –0.97*).

The average molybdenum content, determined at the humus horizon, was equal to 0.28 mg · kg^{-1} and was regarded as low (Table 3). The content was significantly differentiated by the analysed factors and their combinations. A statistical analysis showed that the largest amounts of the element were determined before harvesting the first cut of fodder galega (0.35 mg · kg^{-1}) and in the first year of the study (2005 – 0.29 mg · kg^{-1}). An analysis of variance showed a steady decrease in molybdenum content in soil in the consecutive years of the study. The correlation coefficients between the content of molybdenum in soil and the content of cobalt in soil, iron in the plant, molybdenum in the plant and cobalt in the plant all revealed a significant negative relationship.

Table 3

The content of total molybdenum in the soil [mg · kg^{-1}]

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	0.28	0.25	0.32	0.28
II	0.39	0.34	0.27	0.33
III	0.21	0.27	0.22	0.23
Mean	0.29	0.28	0.27	0.28

LSD$_{0.05}$ for: cuts (A) – 0.01; research years (B) – 0.01; interaction (A×B) – 0.02.

The cobalt content in soil was significantly varied for different cuts and years of the study and for combinations of the analysed factors (Table 4). The average cobalt content in soil was equal to 1.89 mg · kg^{-1}. Analyses of the soil from the humus horizon performed before re-growth of the consecutive cuts of fodder galega showed a steady increase in cobalt content in soil; the value decreased significantly in the third year of the study compared with the first year. The cobalt content in soil was significantly positively correlated with the content of iron, molybdenum and cobalt in the plant.

Table 4

The content of total cobalt in the soil [mg · kg^{-1}]

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	1.72	1.72	1.60	1.68
II	1.92	2.06	1.73	1.90
III	2.05	2.11	2.12	2.09
Mean	1.90	1.96	1.82	1.89

$LSD_{0.05}$ for: cuts (A) – 0.08; research years (B) – 0.08; interaction (A×B) – 0.14.

The average iron content in dry matter of fodder galega harvested during the budding phase was equal to 163.74 mg · kg^{-1} (Table 5) and it was significantly varied between the first and third cut and between the second and the third cut. A statistical analysis revealed significant differences for combinations of the analysed factors. The iron content in consecutive years of the study was similar (162.17–165.39 mg · kg^{-1} of d.m.). The results were confirmed by a study conducted by Kalembasa and Symanowicz [19], which examined changes in the content of iron in biomass of fodder galega depending on its development phase and years of cultivation. In addition, Kabata-Pendias and Pendias [2] report that iron content changes during the vegetation period and such changes are different for different plant organs. The content for legume plants may range from 75 to 400 mg · kg^{-1} of d.m. The iron content in biomass of fodder galega harvested in consecutive cuts lay within the limit values of the acceptable trace elements content in fodder [20]; according to Gorlach [21] and Jamroz et al [22], the content was optimal.

Table 5

The content of iron [mg · kg^{-1} d.m.] in the dry mass
of goat's rue (*Galega orientalis* Lam.) of fertilization ^{15}N

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	136.76	114.36	126.82	125.98
II	112.68	154.16	107.20	124.68
III	241.54	227.65	252.51	240.56
Mean	163.66	165.39	162.17	163.74

$LSD_{0.05}$ for: cuts (A) – 14.62; research years (B) – n.s.; interaction (A×B) – 25.3.

The average molybdenum content in the test plant was equal to 4.13 mg · kg^{-1} of d.m. (Table 6) and it was significantly varied by the analysed factors and their combinations. The highest significant molybdenum content was determined in the biomass of the test plant harvested in the third cut (4.78 mg · kg^{-1} of d.m.), as well as in 2007 (4.46 mg · kg^{-1} of d.m.). A statistical analysis found significant differences in molybdenum content between cuts of fodder galega. The results exceed the optimum and acceptable levels of molybdenum content in fodder [20–25]. It is supposed that the elevated content of molybdenum in the test plant biomass was caused by the neutral pH value of the soil. This causes the element to be more soluble and phyto-absorbable [2, 23, 26]. Such reasoning has been corroborated by the findings of a study conducted by Symanowicz and Kalembasa [27], in which molybdenum content in corn ranged from 0.89 to 0.96 mg · kg^{-1} of d.m. at a soil pH equal to 5.3. Moreover, Wysokinski et al [28] conducted some research on soil at pH 4.0 and determined the molybdenum content in dry matter of corn to be 0.57–0.68 mg · kg^{-1} and in that of silage sunflower to range from 0.71 to 1.12 mg · kg^{-1}.

Table 6

The content of molybdenum [mg · kg^{-1} d.m.] in the dry mass
of goat's rue (*Galega orientalis* Lam.) of fertilization ^{15}N

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	3.78	3.47	5.56	4.27
II	4.56	4.01	4.74	4.44
III	3.71	4.22	3.09	3.67
Mean	4.02	3.90	4.46	4.13

$LSD_{0.05}$ for: cuts (A) – 0.42; research years (B) – 0.42; interaction (A×B) – 0.72.

The analysed factors significantly differentiated the total content of cobalt in biomass of fodder galega (*Galega orientalis* Lam.) (Table 7).

Table 7

The content of cobalt [mg · kg^{-1} d.m.] in the dry mass
of goat's rue (*Galega orientalis* Lam.) of fertilization ^{15}N

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	0.21	0.19	0.10	0.17
II	0.23	0.13	0.21	0.19
III	0.15	0.48	0.49	0.37
Mean	0.20	0.27	0.27	0.24

$LSD_{0.05}$ for: cuts (A) – 0.06; research years (B) – 0.06; interaction (A×B) – 0.11.

The average cobalt content was low and equal to 0.24 mg · kg^{-1} of d.m. These findings confirm the results of earlier studies conducted in similar soil conditions by

Kalembasa and Symanowicz [29]. The highest significant content of the element was determined in dry matter of the test plant harvested in the third cut in 2006 and 2007 (0.48 and 0.49 mg · kg^{-1}) and the values lay within the limits of the optimum range [20, 21]. According to Kabata-Pendias and Pendias [23], cobalt content in dry matter of plants to be used as fodder, especially for ruminants, should not be lower than 0.08–0.1 mg · kg^{-1}, whereas Gorlach [21] determined animals' demand for trace elements and found the value of 0.3 mg Co · kg^{-1} of d.m. as a deficit. Cobalt is used by ruminants in the synthesis of vitamin B_{12}.

Fodder galega absorbed an average of 288.63 kgN · ha^{-1} by bio-reduction of N^2 from the air during a vegetation season (Table 8). The analysis of variance has shown that the amount of nitrogen from the air was significantly differentiated by the analysed factors and their combinations. The largest amounts of bio-reduced nitrogen were found in the biomass of the first cut of fodder galega harvested during the budding phase. The largest total amounts of nitrogen in three cuts were found in the first year of the study (2005) – 376.03 kgN · ha^{-1}. The results were similar to the findings of a study conducted by Symanowicz et al [30], in which the amount of nitrogen bio-reduced by fodder galega harvested during the blossoming phase was equal to 379.7 kgN · ha^{-1}. The correlation coefficient (r = 0.99*) indicates a significant relationship between the amount of bioreduced nitrogen in consecutive cuts and years of the study. The calculations were based on a paper by Kalembasa and Symanowicz [31].

Table 8

Amount of nitrogen biologically reduced for *Rhizobium galegae* cultures living together by goat's rue (*Galega orientalis* Lam.) [kgN · ha^{-1}]

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	280.34	183.75	91.65	185.25
II	81.33	37.07	81.18	66.53
III	14.36	39.98	56.23	36.86
Sum	376.03	260.80	229.06	288.63

$LSD_{0.05}$ for: cuts (A) – 0.72; research years (B) – 0.72; interaction (A×B) – 1.25.

The coefficients of simple correlation were calculated in order to determine the effect of the content of iron, molybdenum and cobalt in soil on the amount of nitrogen bio-reduced by *Rhizobium galegae* bacteria which live in symbiosis with fodder galega. The findings of the study have shown that there is a significant relationship between the content of iron (r = 0.91*), molybdenum (r = 0.99*) and cobalt (–0.96*) in soil and the amount of nitrogen bioreduced by *Rhizobium galegae* bacteria which live in symbiosis with fodder galega (Table 9). The correlation coefficients have also shown a significant negative correlation (–0.97*) between the content of molybdenum in the test plant and the amount of nitrogen bio-reduced by *Rhizobium galegae*.

Table 9

Values of the correlation coefficients between the average content of Fe, Mo, Co in the soil and content of Fe, Mo, Co in the dry mass goat's rye and N_2 biologically reduced from air

	N_2	Fe_s	Mo_s	Co_s	Fe_p	Mo_p	Co_p
N_2	1.00						
Fe_s	0.91*	1.00					
Mo_s	0.99*	0.96*	1.00				
Co_s	–0.96*	–0.99*	–0.99*	1.00			
Fe_p	–0.72	–0.95*	–0.81*	0.88*	1.00		
Mo_p	–0.97*	–0.98*	–0.99*	0.99*	0.87	1.00	
Co_p	–0.78	–0.97*	–0.86	0.92*	0.99*	0.91*	1.00

* – significant at $\alpha = 0.05$; N_2 – N_2 biologically reduced from air; Fe_s – iron in the soil; Mo_s – molybdenum in the soil; Co_s – cobalt in the soil; Fe_p – iron in the plant; Mo_p – molybdenum in the plant; Co_p – cobalt in the plant.

Conclusions

1. The soil contained the largest amount of iron, less cobalt and the smallest amount of molybdenum.
2. The iron amount found in biomass of fodder galega lay within the optimum limits specifying the acceptable content of trace elements in fodder, whereas the molybdenum content was too high and that of cobalt was too low.
3. The average amount of nitrogen bio-reduced during the vegetation period by fodder galega (*Galega orientalis* Lam.) was equal to 288.63 kg N · ha^{-1}, and it decreased in consecutive cuts.
4. The simple correlation coefficients indicate a significant relationship between the amount of N_2 bioreduced by *Rhizobium galegae* and the content of Fe, Mo and Co in the soil and the plant.

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WPŁYW ŻELAZA, MOLIBDENU I KOBALTU NA ILOŚĆ AZOTU BIOLOGICZNIE ZREDUKOWANEGO PRZEZ *Rhizobium galegae*

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Abstrakt: Żelazo i molibden to mikroelementy, które mają decydujący wpływ na ilość azotu biologicznie związanego przez rośliny bobowate. Są one niezbędne do budowy nitrogenazy i innych enzymów. Również kobalt pośrednio uczestniczy w procesie biologicznej redukcji N_2. W celu określenia wpływu Fe, Mo i Co na ilość azotu związanego przez rutwicę wschodnią (*Galega orientalis* Lam.) przeprowadzono doświadczenie polowe w latach 2005–2007 na terenie należącym do stacji doświadczalnej UP-H w Siedlcach. Azot ^{15}N o wzbogaceniu 10,3 at % stosowano w formie $(^{15}NH_4)_2SO_4$ w ilości 1,66 g na 1 m^2 wczesną wiosną. Równolegle z uprawą rutwicy wschodniej (*Galega orientalis* Lam.) uprawiano roślinę nie mającą zdolności biologicznej redukcji N_2 (jęczmień jary – *Hordeum sativum*), którą również nawożono ^{15}N w formie $(^{15}NH_4)_2SO_4$ o wzbogaceniu 10,3 at %. Podczas zbioru rośliny testowej w fazie pąkowania (trzy pokosy) pobierano próbki, które następnie wysuszono i rozdrobniono. Przed każdym pokosem pobierano także próbki gleby. Zawartość Fe, Mo i Co w glebie i roślinie oznaczono metodą ICP-AES po mineralizacji „na sucho". Obliczenia statystyczne wykazały istotne zróżnicowanie w zawartości żelaza, molibdenu i kobaltu w glebie i roślinie. W glebie najwięcej oznaczono żelaza, mniej kobaltu, a najmniej molibdenu. Oznaczona zawartość żelaza w biomasie rutwicy wschodniej mieściła się w optymalnym zakresie liczb granicznych określających dopuszczalne ilości pierwiastków śladowych w paszy, natomiast molibden występował w nadmiarze, a kobalt w niedoborze. Obliczone współczynniki korelacji wskazują na istotną zależność między zawartością żelaza, molibdenu i kobaltu w glebie a ilością biologicznie zredukowanego azotu przez bakterie *Rhizobium galegae* żyjące w symbiozie z rutwicą wschodnią.

Słowa kluczowe: rutwica wschodnia, azot, żelazo, molibden, kobalt, gleba, roślina

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FRACTIONS OF LEAD AND CADMIUM IN SEWAGE SLUDGE COMPOSTED WITH ADDITION OF CALCIUM OXIDE AND POWER STATION ASHES

FRAKCJE OŁOWIU I KADMU W OSADACH ŚCIEKOWYCH KOMPOSTOWANYCH Z DODATKIEM TLENKU WAPNIA I POPIOŁÓW Z ELEKTROWNI

Abstract: This study determined the effect of mixing sludge with calcium oxide, brown coal and hard coal ash and composting the obtained mixtures on the total content and fractions of lead and cadmium isolated by the sequential method. Mixtures of sludge with those components usually contained less lead and cadmium than the sludge itself. Composting usually resulted in a slight increase in the content of heavy metals in sludge and its mixtures with all the components. The content of lead was the highest in the organic (average 54.1 %) and residual (average 33.0 %) fraction in both fresh and composted sludge, with and without all the additions. The content of cadmium was the highest in sludge and their mixtures in the residual (average 59.3 %) and the organic (average 13.2 %) fraction. The content of the most mobile fractions of lead and cadmium fractions (soluble and exchangeable) in the sludge alone as well as in its mixtures with all the components, was rather small and it did not exceed several percent of the whole. The content of lead in the exchangeable fraction which was adsorbed on oxides in the organic and residual fraction was found to increase during the composting process and it was found to decrease in the water-soluble and carbonate fractions. Composting the waste material under study reduced the content of cadmium in the water-soluble and carbonate fraction (adsorbed on oxides) and in the organic fraction, whereas the amounts of the exchangeable and residual fractions increased.

Keywords: sewage sludge, calcium oxide, ash, composting process, lead, cadmium

Introduction

It is common in Poland to use sludge in agriculture as fertiliser. This is economically justifiable because the large amounts of organic matter and biogenous elements which enrich the soil can be put to good use [1]. Agricultural use of sludge has some limitations because of the above-normal content of undesired components, such as

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heavy metals or sanitary contaminations [2]. When introduced to soil, excessive amounts of heavy metals can reduce the yield and lower its quality and, consequently, pose a hazard to consumers. The amount of heavy metals taken up by plants fertilised by sludge depends not only on its amount added to soil, but also on the measures used to relieve the effects of increased content of the elements. Solubility, mobility and the availability to plants of most heavy metals can be reduced by soil liming [3–5]. Sanitation of sludge by mixing it with calcium oxide or substances with high calcium content can be an interesting solution in regard to the phytotoxicity of the elements. Such procedures lead not only to quantitative changes in the heavy metal content, but they can also affect their speciation [6]. Monitoring the hazard that is posed to ecosystems by heavy metals should include determination of their total content, but it should be supplemented by assessment of their mobility based on identification of their species [7, 8].

According to the Regulation of the Minister of Environment on the catalogue of waste [9], by-products of combustion (such as ashes produced by the power industry) are not hazardous products and, as such, they can be used in agriculture. Along with a high content of alkaline compounds, ashes contain large amounts of microelements and heavy metals. The content of heavy metals in ash is not hazardous to plants which are fertilised with them [10].

The aim of the study was to determine the total content of lead and cadmium and their fractions in fresh and composted sludge with the addition of CaO, brown coal ash and hard coal ash, used in the hygienisation of sludge.

Materials and methods

Sludge from mechanical and biological wastewater treatment plants in Siedlce and Lukow, produced as a result of purification of communal waste with a small proportion of industrial sewage, was used in the study. In the technological process in the wastewater treatment plant in Siedlce, sludge is subjected to methane fermentation at the final stage of separation and compaction. Stabilisation of sludge from Lukow was performed under aerobic conditions and the excess water was removed by centrifuging. Organic waste material used in the study contained 18.7 % and 13.8 % of dry matter, respectively. Fresh sludge was mixed separately with CaO, ash from brown coal and ash from hard coal at a 2 : 1 dry matter ratio (w/w). The prepared mixtures were then composted in 200 dm^3 plastic containers for 3 months at a temperature of about 20 ^oC. The compost was stirred every 30 days and samples for examination were taken. Brown coal ash from the third flue gas de-dusting filter in the Patnow CHP plant (burning brown coal from the Belchatow coal mine) contained 17.57 mg Pb and 2.77 mgCd in 1 kg of d.m. Hard coal ash from the PEC power company in Siedlce, contained 74.66 mgPb and 1.30 mgCd in 1 kg of d.m.

The total content of lead and cadmium was determined by the ICP-AES method in stock solutions obtained by dry mineralisation of the materials at the temperature of 450 ^oC. After mineralisation, the ash was poured over with 6 mol $\cdot$ dm^{-3} HCl to decompose carbonates and evaporated to dryness on a sand bath. Subsequently, the chlorides obtained in the process were transferred to volumetric flasks in 10 % hydrochloric acid.

Lead and cadmium fractions were separated by the sequential method (according to Tessier), using the following solutions for extraction in this sequence: H_2O, 1 mol · dm^{-3} $CaCl_2$, 1 mol · dm^3 CH_3COONa, pH ca 5.0, 0.75 mol · dm^{-3} $(NH_4)_2C_2O_4$, pH 3.25, 0.1 mol · dm^{-3} $Na_4P_2O_7$ [11, 12]. Extraction was carried out for 3 hours in centrifuge tubes and the deposit was then centrifuged for 20 minutes at a rotational speed of 5000 rpm. After decantation, before the next extraction solvent was used, the solid phase was washed twice with H_2O, centrifuged (as above) and the relevant stock solution was prepared. Subsequently, all the solutions were mineralised in H_2O_2 and the stock solutions were evaporated to *ca* 5 cm^3. The residue after evaporation was transferred to volumetric flasks in 10 % hydrochloric acid and the content of the heavy metals was determined by the ICP-AES method.

Results and discussion

The lead content in sludge from Lukow (111.35 mg · kg^{-1}) was more than twice higher than in the sludge from Siedlce (51.18 mg · kg^{-1}, Table 1), whereas the content of cadmium in sludge from Siedlce (4.13 mg · kg^{-1}) was higher by nearly 2/3 than in sludge from Lukow (2.65 mg · kg^{-1}, Table 2). The content of the heavy metals in sludge used in the study did not exceed the maximum acceptable content for the substances to be used in agriculture [2].

The addition of CaO to sludge from Siedlce and Lukow produced a mixture with a lower content of lead – 36.6 and 32.9 %, respectively (Table 1). The cadmium content in the sludge with CaO was lower by 39.1 and 44.5 %, respectively, than in the sludge without the addition (Table 2). The addition of brown coal ash to sludge from Siedlce and Lukow produced a mixture with a lower content of lead (23.6 and 29.8 %, respectively) than the sludge without the addition. Mixtures of sludge from Siedlce with brown coal ash contained less cadmium by 8.2 % than the sludge alone, whereas the content of the metal in mixtures of sludge from Lukow with the ash was higher by 12.6 % than in the sludge alone. Mixtures of sludge from Lukow with hard coal ash contained less lead by 10.4 % than the sludge alone, whereas the content of the metal in mixtures of sludge from Siedlce with the ash was higher by 14.1 % than in the sludge alone. The addition of hard coal ash to sludge produced mixtures with a lower content of cadmium (by 22.3 and 16.2 %, respectively) than the sludge without the addition. Composting sludge after methane fermentation, which was later stabilised in aerobic conditions, increased the content of lead by 9.2 and 24.8 %, respectively. Composted mixtures of sludge with CaO, brown and hard coal ash contained more lead by 7.7; 6.1 and 3.8 %, respectively, as compared with its content before composting (average values for sludge from Siedlce and Lukow, Table 3). The total content of cadmium in sludge from Siedlce, after being composted for three months, increased slightly by 4.4 %. The metal content in sludge from Lukow increased as a result of composting by 13.3 % (Table 2). The average content of cadmium in the composted sludge and its mixtures with all the components was slightly higher than in the materials before

Table 1

Total content and fraction of lead in sewage sludge and their mixtures with CaO and ashes, [mgPb · kg^{-1} d.m.]

Kind of sludge	Kind of addition	Before or after composting process	Fraction						Total content
			water-soluble	exchangeable	carbonate	oxide	organic	residual	
Sewage sludge from Siedlce	without addition	fresh	0.575	0.085	1.948	2.590	29.978	16.002	51.177
		composted	0.285	0.000	2.190	3.216	31.914	18.296	55.901
	CaO	fresh	1.197	0.000	3.115	2.801	6.436	18.899	32.447
		composted	1.194	3.668	1.264	2.117	7.813	17.882	33.937
	ash 1^2	fresh	1.370	0.000	3.141	1.663	24.018	8.892	39.083
		composted	0.497	0.000	3.130	2.645	21.854	11.692	39.817
	ash 2^3	fresh	2.241	1.655	2.556	2.795	40.021	9.123	58.390
		composted	1.935	0.000	4.890	3.408	40.532	3.464	54.230
Sewage sludge from Lukow	without addition	fresh	1.121	0.000	4.574	5.246	65.678	34.732	111.350
		composted	1.530	0.000	2.423	5.952	82.702	46.395	139.002
	CaO	fresh	0.744	0.000	1.652	3.055	22.088	47.166	74.703
		composted	0.888	4.602	1.324	3.495	22.167	49.009	81.483
	ash 1^2	fresh	1.439	1.030	2.971	1.873	50.212	20.649	78.180
		composted	0.668	0.000	3.790	3.123	55.354	21.649	84.583
	ash 2^3	fresh	1.472	0.000	3.949	5.778	77.836	10.692	99.727
		composted	0.857	0.000	1.236	4.667	71.500	31.581	109.840
Averages for additives		without addition	0.878	0.021	2.784	4.251	52.568	28.856	89.358
		CaO	1.006	2.068	1.839	2.867	14.626	33.239	55.643
		ash 1^2	0.994	0.258	3.258	2.326	37.860	15.721	60.416
		ash 2^3	1.626	0.414	3.158	4.162	57.472	13.715	80.547
$LSD_{0.05}$			0.171	1.609	0.522	0.367	5.820	3.773	3.828

Ash 1^2 – ash from brown coal, ash 2^3 – ash from pit-coal.

Table 2

Total content and fraction of cadmium in sewage sludge and their mixtures with CaO and ashes, mg Cd · kg^{-1} d.m.

Kind of sludge	Kind of addition	Before or after composting process	Fraction						Total content
			water-soluble	exchangeable	carbonate	oxide	organic	residual	
Sewage sludge from Siedlce	without addition	fresh	0.076	0.419	0.265	0.225	0.534	2.609	4.128
		composted	0.077	0.253	0.152	0.309	0.421	3.096	4.308
	CaO	fresh	0.079	0.050	0.129	0.381	0.333	1.541	2.513
		composted	0.061	0.212	0.171	0.216	0.262	1.645	2.567
	ash 1^2	fresh	0.069	0.282	0.406	0.090	0.234	2.707	3.788
		composted	0.098	0.313	0.327	0.167	0.420	2.590	3.915
	ash 2^3	fresh	0.250	0.519	0.557	0.096	0.478	1.308	3.208
		composted	0.114	0.685	0.420	0.081	0.282	1.980	3.562
Sewage sludge from Lukow	without addition	fresh	0.122	0.120	0.078	0.240	0.562	1.531	2.653
		composted	0.063	0.036	0.058	0.264	0.611	1.975	3.007
	CaO	fresh	0.039	0.101	0.071	0.283	0.285	0.694	1.473
		composted	0.054	0.213	0.012	0.128	0.292	0.805	1.504
	ash 1^2	fresh	0.025	0.198	0.192	0.151	0.379	2.042	2.987
		composted	0.026	0.472	0.356	0.109	0.163	1.865	2.991
	ash 2^3	fresh	0.072	0.326	0.296	0.148	0.389	0.992	2.223
		composted	0.059	0.302	0.281	0.135	0.223	1.221	2.221
Averages for additives		without addition	0.085	0.207	0.138	0.260	0.532	2.303	3.524
		CaO	0.058	0.144	0.096	0.252	0.293	1.171	2.014
		ash 1^2	0.055	0.316	0.320	0.129	0.299	2.301	3.419
		ash 2^3	0.124	0.458	0.389	0.115	0.343	1.375	2.803
$LSD_{0.05}$			0.011	0.039	0.035	0.021	0.041	0.208	0.307

Ash 1^2 – ash from brown coal, ash 2^3 – ash from pit-coal.

Table 3

The average of total content and fraction of lead [mg · kg^{-1} d.m.] in sewage sludge for kind of sludge as well as for composting process

Studied factors		Fraction						Total content
		water-soluble	exchangeable	carbonate	oxide	organic	residual	
Kind of sewage sludge	from Siedlce	1.162	0.676	2.779	2.654	25.321	13.031	45.623
	from Lukow	1.090	0.704	2.740	4.149	55.942	32.734	97.359
	LSD$_{0.05}$	n.i.	n.i.	n.i.	0.195	3.094	2.006	2.035
Composting process of sludge	fresh sludge	1.270	0.346	2.988	3.225	39.533	20.769	68.132
	composted sludge	0.982	1.034	2.531	3.578	41.730	24.996	74.849
	LSD$_{0.05}$	0.091	0.655	0.173	0.195	n.i.	2.006	2.035

n.i. – differences not important.

Table 4

The average of total content and fraction of cadmium [mg · kg^{-1} d.m.] in sewage sludge for kind of sludge as well as for composting process

Studied factors		Fraction						Total content
		water-soluble	exchangeable	carbonate	oxide	organic	residual	
Kind of sewage sludge	from Siedlce	0.103	0.342	0.303	0.196	0.371	2.185	3.499
	from Lukow	0.058	0.221	0.168	0.182	0.363	1.391	2.382
	LSD$_{0.05}$	0.006	0.021	0.018	0.011	n.i.	0.110	0.163
Compos-ting process of sludge	fresh sludge	0.092	0.252	0.249	0.202	0.399	1.678	2.871
	composted sludge	0.069	0.311	0.222	0.176	0.334	1.897	3.009
	LSD$_{0.05}$	0.006	0.021	0.018	0.011	0.023	0.110	n.i.

composting, but the difference was not statistically significant (Table 4). The effect of a slight increase in lead and cadmium content in the organic and mineral-organic materials under study was caused by mineralisation of the organic matter contained in them.

Organic fraction accounted for the highest part of lead content in fresh sludge: 58.6 % in sludge from Siedlce and 59.0 % in sludge from Lukow (Table 1, Fig. 1). About 1/3 (31.2 %) of the total content of lead in the sludge was in the fraction which was unextractable by the reagents used in the experiment, *ie* in the extraction residue. The proportion of lead in the water soluble, exchangeable, carbonate and oxide fraction was small and equal to: 1.0, 0.04, 4.0 and 4.8 %. Residual fraction (63.2 % in sludge from Siedlce and 57.7 % in sludge from Lukow [Table 2, Fig. 2]), contained the highest proportion of cadmium in fresh sludge. It was followed by the organic fraction, which accounted for 12.9 and 21.2 % of sludge from Siedlce and Lukow, respectively. The proportion of the most mobile fractions, *ie* the water-soluble and exchangeable fractions, in the total cadmium content, was equal to 1.8 % and 10.2 % in sludge from Siedlce and 4.6 and 4.5 % in sludge from Lukow. The carbonate and oxide fractions accounted for 11.9 % each of the total cadmium content, both in sludge from Siedlce and from Lukow. The results confirm the thesis that a small part of the total content of heavy metals is present in mobile fractions, but they usually form combinations with the organic and aluminosilicate fractions [13]. However, the mobility of cadmium contained in sludge is higher compared with other heavy metals [14, 15].

The addition of CaO to sludge from Siedlce and from Lukow increased to the largest extent the portion of the lead fraction which could be extracted with the reagents used, *ie* from 31.2 to 61.7 % of its total content. The portion of the exchangeable, carbonate fraction and that adsorbed on oxides increased to 1.8, 4.5 and 5.5 %, respectively. After CaO was added to sludge, exchangeable lead was not determined in it, and the portion

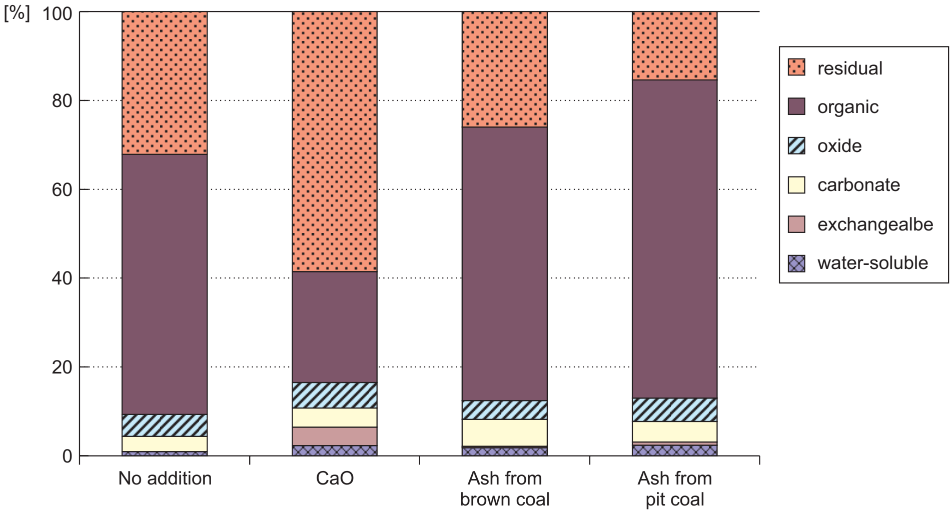

Fig. 1. The proportional part [%] of lead fractions in sewage sludge depending on additives to them

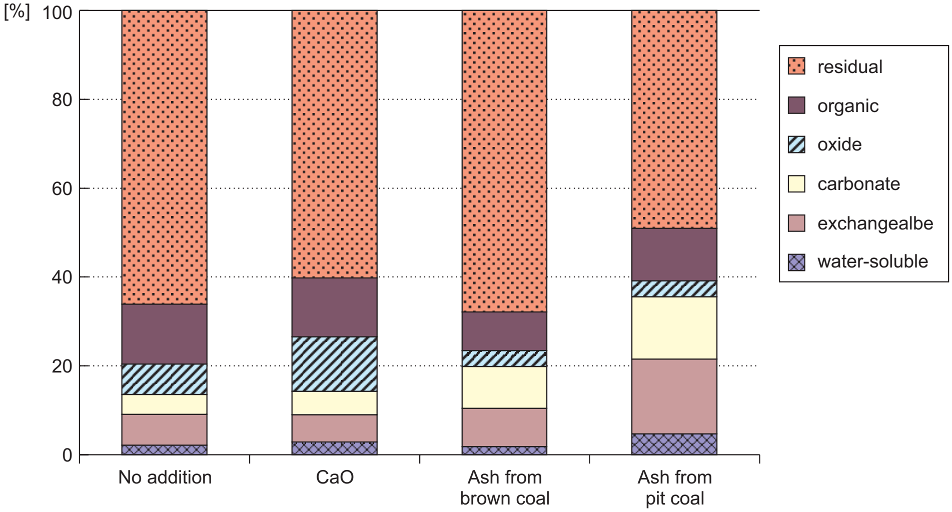

Fig. 2. The proportional part [%] of cadmium fractions in sewage sludge depending on additives to them

of the organic fraction decreased by more than a half, *ie* to 26.6 % of the total content. No considerable changes in the portion of each lead fraction in its total content were observed after brown and hard coal ash were added to sludge. The portion of lead in water-soluble, exchangeable and carbonate fraction increased slightly and that in the residual fraction decreased. After CaO was added to both types of sludge, the portion of oxide fraction of cadmium increased from 7.2 to 17.2 %. At the same time, the portion of residual fraction decreased from 60.5 to 54.2 %. After CaO was added to sludge from Siedlce, the portion of exchangeable fraction decreased considerably, from 10.2 to 2.0 %. Different results were presented by Szymanski and Janowska [16], who stabilised sludge with lime and observed an increase in heavy metal content in the ion-exchange fraction and increase in the organic matter mineralisation rate, which resulted in formation of their mobile species. The portion of water-soluble, carbonate and organic fraction of cadmium did not change considerably after CaO was added to the sludge under study. After brown coal ash was added to sludge from Siedlce, the portion of the exchangeable, oxide and organic fractions decreased, whereas the portion of carbonate and residual fractions increased. After the same ash was added to sludge from Lukow, the portion of the water-soluble, oxide and organic fractions decreased, whereas the portion of exchangeable, carbonate and residual fractions increased. After hard coal ash was added to sludge from Siedlce, the portion of water-soluble, exchangeable and carbonate fractions increased, whereas the portion of oxide and residual fractions decreased. Mixtures of sludge from Lukow with hard coal ash contained smaller portions of water-soluble, oxide, organic and residual fractions and larger portions of exchangeable and carbonate fractions than in sludge alone. Research conducted by Rosik-Dulewska [6] found a small portion of mobile fractions of lead and cadmium in sludge with an addition of mineral waste. The elements are bound to poorly-soluble fractions, with limited availability to plants.

The content of lead in the exchangeable fraction (one which was adsorbed on oxides, in the organic and residual fraction) was found to increase as a result of composting sludge and its mixtures with brown and hard coal ash and it was found to decrease in the water-soluble and carbonate fractions (Table 3). The portion of cadmium in the exchangeable and residual fraction increased, whereas its amount decreased in the fractions: water-soluble, carbonate, adsorbed on oxides and organics (Table 4). The results of this experiment and literature data on forms in which heavy metals occur in fresh and composted sludge and its mixtures with CaO and with mineral waste with high calcium content, *eg* with power plant ash, suggest a low hazard to the environment posed by the presence of lead and cadmium as the elements are present as species of low mobility [6, 15, 17, 18].

Conclusions

1. The addition of power plant ash to sludge in most cases resulted in a decrease in lead and cadmium content in the mixtures.
2. The largest portions of lead and cadmium in sludge with no additions and in mixtures with CaO and brown and hard coal ash were present in organic and residual (unextractable) fractions. The content of mobile species of cadmium was small.
3. The addition of power plant ash to sludge in most cases resulted in an increase in lead and cadmium content in the exchangeable and carbonate fractions and in a decrease in their content in the oxide, organic and residual fractions. The addition of CaO usually increased the portion of the oxide fraction and decrease the organic fraction of the elements.
4. Composting increased the portions of lead and cadmium in sludge with no additions and in mixtures with CaO and brown and hard coal ash. However, the portions of water-soluble and carbonate fractions decreased, whereas the portion of exchangeable and residual fractions of the heavy metals under study increased.

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FRAKCJE OŁOWIU I KADMU W OSADACH ŚCIEKOWYCH KOMPOSTOWANYCH Z DODATKIEM TLENKU WAPNIA I POPIOŁÓW Z ELEKTROWNI

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Abstrakt: Określono wpływ mieszania osadów ściekowych z tlenkiem wapnia, popiołem z węgla brunatnego i kamiennego oraz kompostowania otrzymanych mieszanin na zawartość całkowitą oraz frakcje ołowiu i kadmu wydzielone sekwencyjnie. Mieszaniny osadów ściekowych z wymienionymi komponentami zawierały najczęściej mniej ołowiu i kadmu niż same osady. W trakcie kompostowania stwierdzono na ogół niewielkie zwiększenie się zawartości badanych metali ciężkich w osadach ściekowych i ich mieszaninach ze wszystkimi komponentami. Zarówno w świeżych, jak i w kompostowanych osadach ściekowych bez dodatku i ze wszystkimi dodatkami, największy udział stanowił ołów we frakcji organicznej (średnio 54.1 %) i rezydualnej (średnio 33.0 %). Kadm w największej ilości występował w osadach ściekowych i ich mieszaninach we frakcji rezydualnej (średnio 59.3 %) oraz frakcji organicznej (średnio 13.2 %). W samych osadach ściekowych, a także w ich mieszaninach ze wszystkimi komponentami udział frakcji ołowiu i kadmu o największej mobilności (rozpuszczalnej w wodzie i wymiennej) był niewielki i nie przekraczał kilkunastu procent całkowitej zawartości. W trakcie kompostowania osadów ściekowych i ich mieszanin stwierdzono zwiększenie ilości ołowiu we frakcji wymiennej, zaadsorbowanej na tlenkach, organicznej i rezydualnej, natomiast zmniejszyła się ilość frakcji rozpuszczalnej w wodzie i węglanowej. Kompostowanie badanych materiałów odpadowych zmniejszyło ilość kadmu występującej we frakcji rozpuszczalnej w wodzie, węglanowej, zaadsorbowanej na tlenkach i organicznej, natomiast zwiększeniu uległa ilość frakcji wymiennej i rezydualnej.

Słowa kluczowe: osady ściekowe, tlenek wapnia, popiół, kompostowanie, ołów, kadm

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EFFECT OF DIFFERENT FACTORS ON THE QUALITY OF FEED COMPONENTS

WPŁYW RÓŻNYCH CZYNNIKÓW NA JAKOŚĆ MIESZANEK PASZOWYCH

Abstract: The aim of this study was to determine the effect of different doses of nitrogen and soil's fertilizers on the feed value of *Festulolium braunii* mixture with red clover and alfalfa. The experience with cultivation of *Festulolium braunii* (Felopa variety) in mixture with red clover (Tenia variety) and alfalfa (Tula variety), was established in April 2007. Full period, three – cuts of experimental objects using, were in 2008–2010. The first study factor was the following levels of nitrogen fertilization: N0 – control^{o} (without nitrogen fertilization), N1 – 60 kgN · ha^{-1}, N2 – 120 kgN · ha^{-1}. The second factor was the tested soil's fertilizer. The studies included total protein content, crude fiber and organic or dry matter digestibility [%].The best nutritional value (concentration of protein, fiber and digestibility of dry matter) were characterized the crops on the objects supplied with a lower dose of nitrogen (60 kgN · ha^{-1}). The study showed lack of efficacy for increasing nitrogen doses to improve the feed quality of *Festulolium braunii* in a mixture with red clover and alfalfa. Application of soil's fertilizer to nutrition of tested mixtures not caused significant differences in protein and fiber content in the feed. Its significant impact was noted only on the objects supplied with 60 kgN · ha^{-1}, where it caused a decrease in dry matter digestibility (an average about 2 %) of *Festulolium braunii* mixture with red clover and alfalfa.

Keywords: soil's fertilizer, nitrogen dose, mixture, *Festulolium braunii*, red clover, alfalfa

The basic of grassland functioning in the rural economy, is the production of full value forage for ruminants. For this purpose, especially useful are convertible grasslands [1]. The fodder from grass – legumes mixtures belongs to one of the most valuable and cheapest feed used in animal nutrition [2]. The main advantages of this crop is a higher yield level and more favorable nutritional value compared with monocultures, the ability to extend the utilization period, better health of the individual components of the mixture, reducing the sward infestation, easier feed conservation (hay, silage) and reduction of losses during the storage [3, 4]. Some authors [5] underlined the importance of soil conditions in the yield formation of cultivated mixtures. In the time of simplified rotations, more often we meet with a progressive

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degradation of soils, and as a result, with the reduction of crop yields and with the increase of expenditure on plant production. In addition, soil without the structure is difficult to tillering, and it clumping and acidified. Are also disrupted air-water relations, and hence, there is inhibition of decomposition processes of organic matter and humus formation [6]. According to many authors [7, 8], to improve of this state can be use a soil's fertilizer, which increases the biological activity of soil and restoration of humus cavities, thereby improving soil fertility.

In addition, some studies [9], have shown that the use of microbial preparations to feed the grass – legumes mixtures, caused a significant increase in dry matter yield of plants and preferably effected on the floristic composition of the sward. Therefore, the aim of this study was to determine the effect of different doses of nitrogen and soil's fertilizers on the feed value of *Festulolium braunii* mixture with red clover and alfalfa.

Materials and method

The experience with cultivation of *Festulolium braunii* (Felopa variety) in mixture with red clover (Tenia variety) and alfalfa (Tula variety), was established in April 2007, in randomized blocks in 3 replicants on the experimental object of Grassland Department and Green Areas Creation in Siedlce (geographic coordinates: 52.169^o N, 22.280^o E). Share of individual components of mixtures and standard of seeds sowing were as follows:

- – *Festulolium braunii* 50 % (40 kg $\cdot$ ha^{-1}),
- – *Trifolium pratense* 25 % (21 kg $\cdot$ ha^{-1}),
- – *Medicago sativa* ssp. *media*, 25 % (26 kg $\cdot$ ha^{-1}).

Surface plot was 6 m^2. In the year of sowing only infestation cuts was carried out. Full period, when three – cuts of experimental objects were used, were in 2008–2010. The first experience factor was the following levels of nitrogen fertilization: N0 – control^o (without nitrogen fertilization), N1 – 60 kgN $\cdot$ ha^{-1}, N2 – 120 kgN $\cdot$ ha^{-1}.

Nitrogen (34 % ammonium nitrate) was used in three divided doses, subsequently sown into each regrowth.

The substrate of experience belongs to soil with hortisole type formed from medium sand. Granulometric composition of the soil shows Table 1.

Table 1

Granulometric composition of soil material existing as subsoil in experiment

Percentage share of soil fractions [diameter in mm]								
1–0.1	0.1–0.05	0.05–0.02	0.02–0.06	0.06–0.002	< 0.002	Sum of fraction 0.1–0.02	Sum of fraction < 0.02	Grain group
76	9	5	4	4	2	14	10	psg

On the basis of soil chemical analysis performed at the Station Chemical Agriculture in Wesola was found that soil under cultivation was characterized by neutral reaction and medium high humus content (Table 2). In addition, it was characterized by a very high content of phosphorus, high magnesium and an average of available forms of potassium, total nitrogen, nitrate and ammonium. Soil's fertilizer was used once for watering plants in a phase of *Festulolium* shooting in the first regrowth, as a solution at a dose 0.9 $dm^3 \cdot ha^{-1}$ diluted in 350 dm^3 of water. Combinations of soil's fertilizer labeled as UG, and without fertilizer as BUG. According to Trawczynski [7], in soil's fertilizer are yeast, lactic acid bacteria, photosynthetic bacteria and *Azotobacter*, *Pseudomonas* and *Actinomycetes*. In addition, it contains the following macro- and micronutrients: potassium (3500 $mg \cdot dm^{-3}$), nitrogen (1200 $mg \cdot dm^{-3}$), sulfur (1000 $mg \cdot dm^{-3}$), phosphorus (500 $mg \cdot dm^{-3}$), sodium (200 $mg \cdot dm^{-3}$), magnesium (100 $mg \cdot dm^{-3}$), zinc (20 $mg \cdot dm^{-3}$) and manganese (0.3 $mg \cdot dm^{-3}$).

Table 2

Chemical composition of soil as a subsoil in experiment

pH in KCl	Content of assimilated components [$mg \cdot 100\ g^{-1}$ of soil]			Content [%]		Content [$mg \cdot kg^{-1}$ d.m.]	
	P_2O_5	K_2O	Mg	N_{tot}	Humus	N-NO_3	N-NH_4
6.99	90.0	19.0	8.4	0.18	3.78	10.10	7.47
Results uncertainty*							
± 3 %	± 20 %	± 20 %	± 20 %	± 20 %	± 17 %	± 22 %	± 25 %

* Widened uncertainty calculated with using of widen index 2, what gives the level 95 %.

In addition, on all the plots potassium fertilization (60 % potassium salt) were used in an amount of 120 $kgK_2O \cdot ha^{-1}$. But phosphorus (46 % superphosphate) at a dose 80 $kgP_2O_5 \cdot ha^{-1}$, was sown once in early spring.

The chemical analysis of plant material included total protein content, crude fiber and dry matter digestibility [%]. The plant material in terms of those characteristics, were collected from all the cuts in the last two years of the experiment. It was done at the Institute of Technology and Life Sciences in Falenty, PL.

The study results were evaluated statistically by using analysis of variance for multivariate experiments. Differentiation of means was verified by Tukey's test at significance level $p \le 0.05$.

The climatic conditions of research area were typical for IX – eastern of agriculture and climatic district of Poland. Average annual air temperature varied within 6.7–6.9 ^{o}C, and in the summer, the average daily temperature was 15 ^{o}C. Annual precipitation levels amounted to 550–650 mm, while they are not belong to frequent, but to heavy. Growing season starts in the first decade of April and ends in the third of October, and so takes from 200 to 220 days. Meteorological data from the research years were obtained from the Meteorology and Hydrology Station in Siedlce. However, in order to determine the temporal and spatial variability of meteorological elements and their effects on plants vegetation, was calculated the hydrometrical ratio of Sielianinow.

From the data presented in Table 3 resulted, that the most preferred and size distribution of precipitation, optimal air temperatures per growing season of plants, was in 2009. This year were not the months with drought but in the other years were the months with strong and week drought.

Table 3

Value of hydrometrical index of Sielianinow (K) in individual months of vegetation period

Study years	Month						
	IV	V	VI	VII	VIII	IX	X
R1 – 2008	0.82	1.34	1.08	1.23	0.54	0.69	1.72
R2 – 2009	1.03	2.24	1.03	1.26	1.36	1.01	1.73
R3 – 2010	0.40	2.21	1.19	1.18	1.79	2.81	0.53

$K < 0.5$ – high drought; 0.51–0.69 – drought; 0.70–0.99 – week drought, $K > 1.0$ – not drought.

Results and discussion

In the first year of full using of the experiment, nitrogen fertilization did not differentiate the protein content within the tested combinations (Table 4).

Table 4

Protein content [% d.m.] of the *Festulolium braunii* mixture
with red clover and alfalfa (average from cuts)

Combination of fertilization		Study years		Mean
		2009	2010	
N0	UG	18.90	18.64	18.77
	BUG	17.24	18.08	17.66
N1	UG	18.54	19.26	18.90
	BUG	18.21	20.62	19.41
N2	UG	18.39	19.34	18.86
	BUG	18.26	18.07	18.16
Mean for soil's fertilizer				
UG		18.61	19.08	18.84
BUG		17.90	18.80	18.35
Mean for nitrogen dose				
N0		18.07	18.36	18.21
N1		18.37	19.94	19.16
N2		18.32	18.70	18.51
Mean		18.25	19.00	18.62
LSD$_{0.05}$ for: study year (A) – n.s. soil's fertilizer (B) – n.s. nitrogen dose (C) – 0.90		interaction: (A×B) – 0.88 (A×C) – 1.52 (B×C) – n.s. (A×B×C) – n.s.		

Nitrogen dose: N0 – no nitrogen, N1 – 60 kgN · ha^{-1}, N2 – 120 kgN · ha^{-1}. UG – soil's fertilizer, BUG – no soil's fertilizer.

In the second year of the study, the highest protein content occurred in the plants from the plots fertilized with 60 kgN · ha^{-1} (19.94 % d.m.). It should also be noted that regardless of study years and applied soil's fertilizer, the statistical analysis showed significantly higher protein content in dry matter of the mixture cultivated on the objects supplied with a lower dose of nitrogen (60 kgN · ha^{-1}).

A similar relationship was noted in other study [10]. This author analyzed the average yield of *Festulolium* mixtures with red clover for objects fertilized with different doses of nitrogen, generally showed, that increasing amounts of this factor not caused a significant increase in the yield of protein. The other authors [11] showed, that in the first two years of cultivation, significantly higher yield of protein for mixtures of meadow fescue with red clover were obtained after fertilization with dose of 60 kgN · ha^{-1}, compared with control dose. But increasing the amount of nitrogen applied to 120 kg · ha^{-1} did not result a significant increase in the yield of protein. Microbiological preparation used in the experiment did not differentiate significantly these characteristics, however, other studies [9], have shown, that it caused the increase in dry matter yield of the tested mixture, what in consequences caused an increase in the yield of protein in feed.

Table 5

The crude fiber content [% d.m.] of the *Festulolium braunii* mixture
with red clover and alfalfa (average from cuts)

Combination of fertilization		Study years		Mean
		2009	2010	
N0	UG	25.52	23.45	24.48
	BUG	24.18	23.65	23.91
N1	UG	22.08	24.91	23.49
	BUG	22.81	23.85	23.33
N2	UG	25.53	26.90	26.21
	BUG	26.11	24.95	25.53
Mean for soil's fertilizer				
UG		24.27	25.08	24.67
BUG		24.36	24.15	24.25
Mean for nitrogen dose				
N0		24.85	23.55	24.20
N1		22.44	24.38	23.41
N2		25.82	24.40	25.10
Mean		24.37	24.11	24.23
LSD$_{0.05}$ for: study year (A) – n.s. soil's fertilizer (B) – n.s. nitrogen dose (C) – 0.78		interaction: (A×B) – n.s. (A×C) – 1.82 (B×C) – n.s. (A×B×C) – 1.27		

Nitrogen dose: N0 – no nitrogen, N1 – 60 kgN · ha^{-1}, N2 – 120 kgN · ha^{-1}. UG – soil's fertilizer, BUG – no soil's fertilizer.

Conversely to the protein content in dry matter yield of plants, has developed the crude fiber content. It was confirmed by other studies [12, 13]. The largest amount of fiber (Table 5) occurred at the objects supplied with the highest dose of nitrogen (N2 – 120 kg N · ha^{-1}) and amounted an average 25 %. The using of soil's fertilizer, similar as in the case of proteins, not differenced significantly of received values.

The tested mixture regardless of the fertilizer combination characterized by a low dry matter digestibility, on the level an average 57 % (Table 6).

Table 6

Digestibility of dry matter [%] of *Festulolium braunii* mixture
with red clover and alfalfa (average from cuts)

Combination of fertilization		Study years		Mean
		2009	2010	
N0	UG	56.10	56.04	56.07
	BUG	56.14	57.05	56.59
N1	UG	58.17	57.94	58.05
	BUG	59.84	60.22	60.03
N2	UG	55.19	57.34	56.26
	BUG	55.08	56.22	55.65
Mean for soil's fertilizer				
UG		56.48	57.10	56.79
BUG		57.02	57.03	57.02
Mean for nitrogen dose				
N0		56.12	56.54	56.33
N1		59.00	59.08	59.04
N2		55.13	56.78	55.95
Mean		56.75	57.46	57.10

$LSD_{0,05}$ for:
study year (A) – n.s.
soil's fertilizer (B) – n.s.
nitrogen dose (C) – 2.27

interaction:
(A×B) – n.s.
(A×C) – n.s.
(B×C) – 1.89
(A×B×C) – n.s.

Nitrogen dose: N0 – no nitrogen, N1 – 60 kgN · ha^{-1}, N2 – 120 kgN · ha^{-1}. UG – soil's fertilizer, BUG – no soil's fertilizer.

This fact is a consequence of three – cuts using of plots [14–17]. However it should be noted, that the highest digestibility were characterized the cultivations reached with 60 kgN · ha^{-1} (average 59 %). Significant influence of soil's fertilizer on this feature, regardless of study years, it has been noted only on the objects supplied with a lower dose of nitrogen (N1). In this case, the supply with soil's fertilizer to *Festulolium braunii* cultivation with red clover and alfalfa caused a decrease in digestibility on average about 2 %. Analyzing the means for soil's fertilizer in the whole study cycle, it should be noted that it had no significant effect on the digestibility of dry matter of investigated plants. Similar tendency was also for organic matter digestibility (Table 7).

Table 7

Digestibility of organic matter [%] for *Festulolium braunii* mixture with red clover and alfalfa (average from cuts)

Combination of fertilization		Study years		Mean
		2009	2010	
N0	UG	57.78	57.72	57.75
	BUG	57.82	58.76	58.30
N1	UG	59.91	59.67	59.79
	BUG	61.64	62.02	61.83
N2	UG	56.84	59.06	57.95
	BUG	56.73	57.90	57.32
Mean for soil's fertilizer				
UG		58.17	58.81	58.49
BUG		58.73	58.74	58.73
Mean for nitrogen dose				
N0		57.80	58.23	58.02
N1		60.77	60.85	60.81
N2		56.78	58.48	57.63
Mean		58.45	59.18	58.81
$LSD_{0.05}$ for: study year (A) – n.s. soil's fertilizer (B) – n.s. nitrogen dose (C) – 2.17		interaction: (A×B) – n.s. (A×C) – n.s. (B×C) – 1.95 (A×B×C) – n.s.		

Nitrogen dose: N0 – no nitrogen, N1 – 60 kgN · ha^{-1}, N2 – 120 kgN · ha^{-1}. UG – soil's fertilizer, BUG – no soil's fertilizer.

According to other author [18], a mixture of *Festulolium* with red clover and alfalfa gets very good scores in a scale of Fliege-Zimmer, and as a maize silage can be a primary feed in the feeding of cattle, especially cows with a capacity about 8000 liters of milk. Higher fiber content and thus lower the digestibility of dry matter, indicates the need of earlier harvest of the first cut and greater frequency of mowing. However, it should be noted, that the tested mixture, covered the basic feed criteria for ruminants [19, 20].

Conclusion

The best nutritional value (concentration of protein, fiber and digestibility of dry matter) were characterized the crops on the objects supplied with a lower dose of nitrogen (60 kgN · ha^{-1}).

The study showed lack of efficacy for increasing nitrogen doses to improve the feed quality of *Festulolium braunii* in a mixture with red clover and alfalfa.

Application of soil's fertilizer to nutrition of tested mixtures not caused significant differences in protein and fiber content in the feed. Its significant impact was noted only on the objects supplied with 60 kgN · ha^{-1}, where it caused a decrease in dry matter digestibility (an average about 2 %) of *Festulolium braunii* mixture with red clover and alfalfa.

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WPŁYW RÓŻNYCH CZYNNIKÓW NA JAKOŚĆ MIESZANEK PASZOWYCH

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Abstrakt: Doświadczenie z uprawą *Festulolium braunii* (odmiana Felopa) w mieszance z koniczyną łąkowa (odmiana Tenia) i lucerna mieszańcową (odmiana Tula) założono w kwietniu 2007 r. Udział poszczególnych komponentów mieszanki były następujące: *Festulolium braunii* 50 %, koniczyna łąkowa 25 %, lucerna mieszańcowa 25 %. Okres pełnego, trzykośnego użytkowania obiektów doświadczalnych przypadał na lata 2008–2010. Pierwszym czynnikiem doświadczalnym były następujące poziomy nawożenia azotem: N0 – obiekt kontrolny (bez nawożenia azotem), N1 – 60 kgN $\cdot$ ha^{-1}, N2 – 120 kgN $\cdot$ ha^{-1}. Azot (34 % saletra amonowa) zastosowano w trzech dzielonych dawkach, wysiewanych kolejno na każdy odrost. Drugi czynnik to użyźniacz glebowy: BUG – bez użyźniacza, UG – użyźniacz glebowy. Ponadto na wszystkich poletkach zastosowano nawożenie potasem (60 % sól potasowa) w ilości 120 kgK_2O $\cdot$ ha^{-1} – użyto na odrosty. Natomiast fosfor (46 % superfosfat) w dawce 80 kgP_2O_5 $\cdot$ ha^{-1}, wysiano jednorazowo wczesną wiosną. Szczegółowymi badaniami objęto zawartość białka ogólnego, włókna surowego oraz strawność organicznej i suchej masy (%). Przeprowadzone badania wykazały, że najlepszą wartością pokarmową (koncentracją białka, włókna i strawnością suchej masy) odznaczały się uprawy prowadzone na obiektach zasilanych niższą dawka azotu (60 kgN $\cdot$ ha^{-1}). Zastosowanie użyźniacza glebowego do zasilania badanych mieszanek nie przyczyniło się do istotnego zróżnicowania omawianych parametrów jakościowych surowca paszowego.

Słowa kluczowe: użyźniacz glebowy, dawka azotu, mieszanka, *Festulolium braunii*, koniczyna łąkowa, lucerna

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DYNAMICS OF CHANGES IN SELENIUM CONTENT IN SOIL AND WINTER WHEAT AFFECTED BY LONG-TERM ORGANIC FERTILIZATION

DYNAMIKA ZMIAN ZAWARTOŚCI SELENU W GLEBIE I ROŚLINACH PSZENICY OZIMEJ POD WPŁYWEM WIELOLETNIEGO NAWOŻENIA NAWOZEM NATURALNYM

Abstract: The objective of this study was to determine the selenium content in soil and its accumulation and distribution in winter wheat plants affected by organic fertilization. There was found a significant effect of FYM application on the total selenium in soil. A supplement of manure at the dose of 80 $\mathrm{Mg \cdot ha^{-1}}$ resulted in the significantly highest increase of the total selenium content almost 50 % in soil, as compared with the soil from control plots. The total selenium content in soil was significantly correlated with organic carbon content. The highest selenium concentrations were observed in aboveground biomass of winter wheat gathered from plots treated with FYM with the doses of 20 and 40 $\mathrm{Mg \cdot ha^{-1}}$, which was about 70 % higher in comparison with the control plants. The application of manure in the dose of 80 $\mathrm{Mg \cdot ha^{-1}}$ resulted in a decrease in the selenium content in aboveground wheat biomass. The bioaccumulation and translocation coefficients of selenium demonstrated that aboveground winter wheat biomass absorbed and transported selenium more easily from soil treated with FYM at the doses of 20 or 40 $\mathrm{Mg \cdot ha^{-1}}$. Both coefficients decreased considerably due to the highest doses of FYM.

Keywords: selenium, soil, winter wheat, organic fertilization

Selenium (Se) is considered as an essential trace element for humans, animals and plants. The concentration of this microelement in plants depends on the chemical form of it, its concentration and bioavailability in soils and on the accumulation capacity of the plant [1, 2]. Selenium is one of the trace elements strongly affected by microbiologically mediated redox processes affecting its solubility and, consequently, its mobility, bioavailability and uptake in the soil-plant system [3]. Although all the plants

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are able to take up and to metabolize selenium, the assumption about its necessity for plants has not been fully confirmed yet. Numerous studies have shown that at low concentrations Se exerts a beneficial effect promoting growth and increasing stress tolerance of plants by enhancing their antioxidative capacity, reducing lipid peroxidation and enhancing the accumulation of starch and sugars [4,5,6,7,8]. Higher plants vary in their capacity to accumulate and tolerate selenium and they are classified into non-accumulators, indicators and accumulators [9,10]. According to Whanger [11], the currently observed interest in selenium focuses on the health benefits of high-Se plants as a source of cancer-preventative Se compounds, for its unique role in recycling and delivering selenium from the soil to the food chain. In soils the natural total selenium content varies, ranging from 0.1 to 2 mg · kg^{-1}, however, in most agricultural areas, soils contain so little available selenium that crops usually do not absorb more than traces of this element. In many low-selenium countries the possibility of applying selenium fertilizer or foliar application of selenium to plants has been studied [5], however according to Stadlober et al [12], enhancing the selenium levels in crops is also possible by adding organic supplements of high level of selenium (sewage sludge, manure of selenium supplemented farm animals).

The objective of this study was to determine the selenium content in soil and its accumulation and distribution in winter wheat plants affected by organic fertilization.

Material and methods

Soil and plant samples were taken from a long-term static field experiment carried out since 1980 by the Department of Plant Nutrition of the Institute of Soil Science and Cultivation in Pulawy in the area of the Agricultural Experimental Station at Grabow on the Vistula River. Soil samples were collected in the 22nd year of the experiment, in May 2002, from the 0–20 cm layer in the winter wheat interrows (cv. Korweta). The experiment was designed in a split-plot with four replications on the 8 × 5 m (40 m^2) plots. The width of paths between the plots was 1 m. Crop rotation included: potato – winter wheat + intercrop – spring barley + undersown and red clover + grasses. The soil was treated with cattle *farmyard manure* (FYM) under potato in the doses of 0, 20, 40, 60 and 80 Mg · ha^{-1}. Soil samples were air-dried and sieved through a 2 mm screen. Plant material was sampled at the begging of the shooting stage into blade, rinsed in deionised water to remove soil particles, separated into aboveground biomass and roots, and dried. The total selenium content in soils and plants was determined using the method of Watkinson [13] with a Hitachi F-2000 spectrofluorometer. Samples were microwave digested with concentrated nitric (HNO_3) and perchloric ($HClO_4$) acids. Different forms of selenium in the samples were reduced by boiling with 10 % HCl. The selenium was complexed with 2,3-diaminonaphtalene (DAN) to give the fluorescent compound, which was extracted with cyclohexane and read on the spectrofluorometer at the excitation and emission wavelengths of λ = 376 and 519 nm, respectively. The analytical procedures provided satisfactory values for the standard reference material CRM024-050 Resource Technology Corporation (RTC), soil from the western part of the US of the loamy sand texture; Se 0.558 mg · kg^{-1} (certified value 0.540 mg · kg^{-1}).

The certified reference material was included in each batch of samples for quality control. The soil samples were analysed for granulometric composition according to Bouyoucos-Casagrande method, organic carbon – using wet oxidation with potassium dichromate, and pH in distilled water and 0.1M KCl – potentiometrically. All the analyses were performed in triplicate samples. The multireplication data from the analyses of soils and plants samples were evaluated using the statistical procedure. The analysis of variance for one-factore experiment in the split-plot design was made. The data were verified with the analysis of variance (ANOVA) and the Tukey test at $p < 0.05$. The analysis was facilitated with the use of Statistica for Windows software.

Results and discussion

The general properties 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. The soil pH values were found in the slightly acidic range 5.7–6.2. The application of manure resulted in the highest amounts of organic carbon in soil, especially in soil from the plots fertilized with FYM with the doses of 60 and 80 Mg · ha^{-1}.

Table 1

General properties and total selenium content in soil under study

Dose of manure [Mg · ha^{-1}]	Soil particle size fraction [%]		pH in		C_{org} [g · kg^{-1}]	Se_{tot} [mg · kg^{-1}]
	< 0.02 [mm]	< 0.002 [mm]	H_2O	KCl		
0	18	7	6.2	5.8	8.6	0.099
20	16	7	6.2	5.8	9.9	0.171
40	17	6	6.0	5.7	10.3	0.179
60	18	6	6.2	5.8	10.6	0.184
80	15	5	6.2	5.8	11.1	0.191
						$LSD_{0.05}$ 0.010

The selenium content from the control plots ranged from 0.086 to 0.117 mg · kg^{-1} (average 0.101 mg · kg^{-1}) (Table 1). Statistical analyses confirmed that the FYM application resulted in the highest amounts of total selenium content in soil (Table 1), which increased with increasing doses of manure. The soil fertilized with the highest dose of manure showed a two-fold higher rate of total selenium than the soil from the control plots, which could have been due to the amount of this microelement in farmyard manure since, as reported in literature, in various FYM the selenium content varies from 0.32 to 2.4 mg · kg^{-1} [14, 15]. According to Kabata-Pendias [7], 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 to 1.5 mg · kg^{-1} being the lowest in Podzols and the highest in Histosols. Aro and Alfthang [16] and Hartikainen

[6] claim that soils containing less than 0.5 mgSe · kg^{-1} are likely to lead to crops and pastures with inadequate selenium concentrations (< 0.05 mg · kg^{-1} d.m.). The total selenium content in soil under study was significantly correlated with the organic carbon content, which coincides with our earlier findings [17] and those reported by other authors [7,14,18]. Navarro-Alarcon and Cabrera-Vique [19] report on selenium levels in soil generally being reflected in food and the Se levels in human populations. Se food content is influenced by the geographical location, seasonal changes, protein content and food processing. In the present study the average selenium content in upper parts of winter wheat from control plots reached 0.133 mg · kg^{-1} d.m. (Fig. 1). There were observed the highest selenium concentrations in aboveground parts of winter wheat from the plots treated with the doses of 20 and 40 Mg · ha^{-1}, the Se content increased on average above 70 % against the control. The application of manure in the doses of 60 or 80 Mg · ha^{-1} resulted in the decrease in selenium content in aboveground biomass of winter wheat. The selenium content in winter wheat roots from the control plots was on average above 30 % higher than from the FYM plots (Fig. 1).

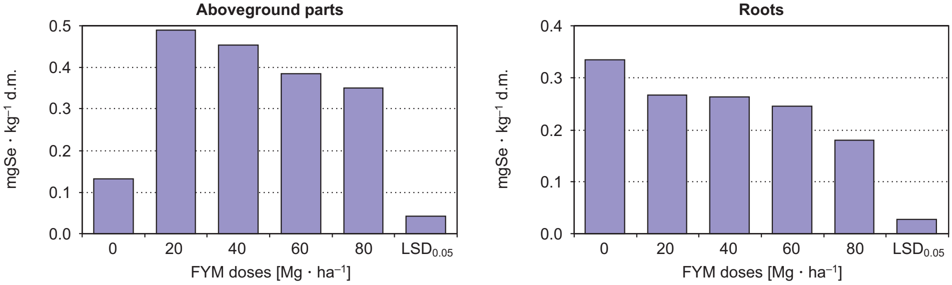

Fig. 1. Selenium content in winter wheat aboveground parts and roots

The value of bioaccumulation coefficient (BC) reflects plant capacity for the nutrients uptake from soil and informs about the amount and the rate of the nutrient translocation from soil solution to aboveground plant parts [20, 21]. The parameter is a ratio of the element concentration in plant aboveground parts or roots to its amount in soil. The bioaccumulation coefficients (BC) of selenium demonstrated that aboveground parts of winter wheat absorbed selenium more easily from soil of the control plots or from soil treated with FYM in the doses of 20 and 40 Mg · ha^{-1} (Fig. 2). The bioaccumulation coefficient (BC) decreased considerably as a result of the highest doses of manure. The highest selenium absorption in winter wheat roots was observed in the plants from plots without FYM (Fig. 2). The FYM application resulted in a 5-fold decrease in selenium bioaccumulation by winter wheat roots, as compared with the no-manure treatment. The distribution of selenium in various parts of the plant differs according to the species, its phase of development and its physiological conditions [9]. In Se-accumulators, Se is accumulated in young leaves during the early vegetative stage of growth, however at the reproductive stage, high levels of selenium were found in seeds, while the Se content in leaves is reduced [22]. The selenium concentration in grain and roots of cereal plants is often the same level, with lower amounts in the stems

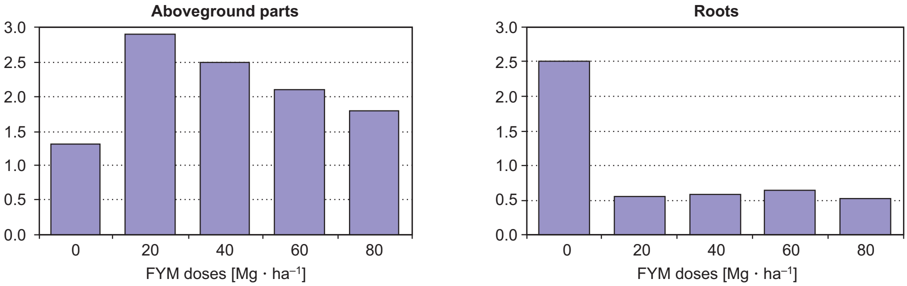

Fig. 2. Bioaccumulation of selenium in aboveground parts and roots of winter wheat in relation to its content in soil

and leaves. Zayed et al [23] report on the distribution of selenium in plants also depending on the form and the concentration of selenium supplies to the roots and on the nature and concentration of other ions, especially sulphates and on the degree of Se fixation in soils. Plants absorb Se easily from alkaline soils, where it often exists in water-soluble forms. Although acid soils may contain high selenium concentrations, plants assimilate only small amounts since Se is bound by insoluble iron compounds or by organic matter of soil [18]. According to Terry et al [20], since selenium and sulphur share similar properties, the Se compounds are absorbed and metabolized by mechanisms similar to those for S analogues. Soluble Se forms are likely to be taken up by plants – selenates and organic selenium are driven metabolically, however the uptake of selenites can be a result of passive mechanism. In general, organic Se is more readily absorbed by plants than inorganic forms. On the other hand, selenates are likely to be easily transported to aboveground parts than selenite or organic species (*eg* SeMet) [7].

The value of *translocation coefficient* (TC) informs about the amount and the rate of element translocation from roots to plant aboveground parts. The parameter is a ratio of the element concentration in aboveground plant parts to its amount in roots. Translocation coefficients (BC) demonstrated that selenium is more easily transported from roots to aboveground parts of plants on the plots with the rate of FYM application of 20 and 40 Mg · ha^{-1} (Fig. 3). The translocation of Se from root to shoot depends on the form of Se supplied, selenate being transported much more easily than selenite [9]. Munier-Lamy et al [21] observed the differences in transporting Se from roots to shoots between plant species, which could be related either to the root system, *ie* to soil exploration by roots and root exudation, or to the chemical conditions resulting of microbial activity in the rhizosphere. Indeed, some microorganisms may excrete organic compounds that increase bioavailability, and facilitate root absorption of essential metals, such as Fe as well as non-essential metals, such as Cd. Soil microorganisms can also affect metal solubility directly by changing their chemical forms. When Se is supplied as selenate to plants, Zayed et al [23] report on most of it being transported to the shoots unchanged with very little Se remaining in roots, which seems to suggest a reduction in selenate to selenium organic forms, which are less available in soil.

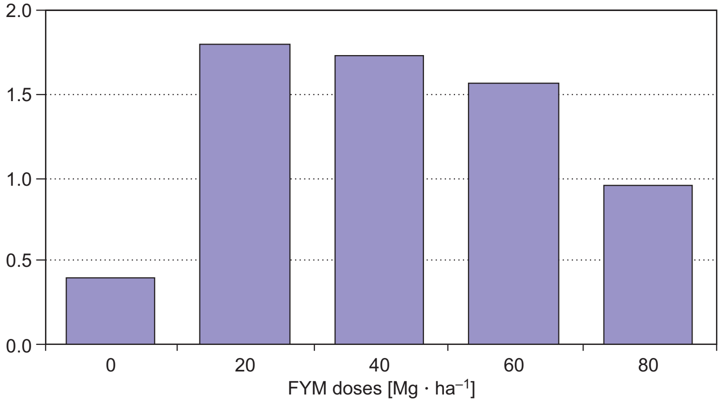

Fig. 3. Translocation of selenium from roots to aboveground parts of winter wheat

Conclusions

1. There was found a significant effect of FYM application on the total selenium content in the soil investigated. A supplement of manure at the dose of 80 Mg · ha^{-1} resulted in the significantly highest increase in total selenium content (almost 50 %) in soil, as compared with the control soil. The total selenium content in soil was significantly correlated with the organic carbon content.

2. There were observed the highest selenium concentrations in the upper parts of winter wheat from the plots treated with FYM with the doses of 20 and 40 Mg · ha^{-1}, namely about 70 % higher in comparison with the control plants. The FYM application in the dose of 80 Mg · ha^{-1} resulted in a decrease in the selenium content in aboveground parts of wheat.

3. The bioaccumulation and translocation coefficients of selenium demonstrated that aboveground biomass of winter wheat absorbed and transported selenium more easily from soil treated with FYM at the doses of 20 or 40 Mg · ha^{-1}. Both coefficients decreased considerably due to the highest doses of FYM.

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DYNAMIKA ZMIAN ZAWARTOŚCI SELENU W GLEBIE I ROŚLINACH PSZENICY OZIMEJ POD WPŁYWEM WIELOLETNIEGO NAWOŻENIA NAWOZEM NATURALNYM

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Abstrakt: Celem badań było określenie zmian zawartości selenu w glebie i roślinach pszenicy ozimej po wieloletniej aplikacji nawozu naturalnego. Wykazano istotny wpływ nawożenia obornikiem na zawartość selenu w glebie. Aplikacja obornika na poziomie 80 Mg · ha^{-1} istotnie zwiększyła zawartość tego mikroelementu o prawie 50 % w odniesieniu do jego zawartości w glebie z obiektu kontrolnego. Zawartość selenu ogółem w glebie była istotnie dodatnio skorelowana z zawartością węgla organicznego. Najwyższą zawartość selenu w nadziemnych częściach pszenicy ozimej wykazano na obiektach, na których obornik stosowano w dawce 20 i 40 Mg · ha^{-1}, w porównaniu z roślinami kontrolnymi. Po zastosowaniu obornika w tych dawkach zawartość selenu wzrosła o ponad 70 %. Aplikacja obornika w dawce 80 Mg · ha^{-1} spowodowała natomiast istotne obniżenie zawartości tego pierwiastka w częściach nadziemnych pszenicy.

Słowa kluczowe: selen, gleba, pszenica ozima, obornik

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ACTIVITY OF ALKALINE AND ACID PHOSPHATASES AND THE CONTENT OF PHOSPHORUS IN SOIL UNDER SELECTED CROPS FERTILIZED WITH SLURRY

AKTYWNOŚĆ ALKALICZNEJ I KWAŚNEJ FOSFATAZY I ZAWARTOŚĆ FOSFORU W GLEBIE SPOD WYBRANYCH ROŚLIN UPRAWNYCH NAWOŻONYCH GNOJOWICĄ

Abstract: The aim of the present paper was to determine the effect of fertilization with pig slurry and with nitrogen in a form of ammonium nitrate on the changes in the activity of alkaline and acid phosphatase determined with the Tabatabai and Bremner method, the contents of available phosphorus – with the Egnera–Riehma method (DL) in the soil under red clover, winter wheat and corn. The experiment was made in 2008 as a single-factor experiment by IUNG in Pulawy. There was found a significant effect of fertilization both with slurry and with nitrogen on the changes in the enzymes and in the content of available phosphorus in the soil. The content of available phosphorus as well as the activity of acid phosphatase were greater in the soil fertilized with slurry, as compared with that fertilized with nitrogen. The activity of phosphomonoesterases varied depending on the crop species.

Keywords: phosphorus, alkaline and acid phosphatase, soil, nitrogen, slurry

Slurry is a natural fertilizer providing high amounts of indispensable nutrients in the forms easily available to plants. Its application affects the physicochemical soil properties and thus intensifies its biological processes. However, a long-term and unreasonable use of that fertilizer can trigger negative effects in the natural environment [1]. Depending on the slurry doses and the application frequency, the effect of slurry on the soil environment can vary both in the topsoil and in deeper soil profile horizons [2]. Upon Poland's joining the European Union, including the applicable laws, following the Nitrates Directive of 1991 [Directive 91/676/EEC] [3], into the domestic law, Regulation of Minister of the Environment [Dz. U. of 2003 No. 4, item 44] [Regulation

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2002] [4], limits the annual burden of agricultural land with nitrogen derived from slurry to 170 kgN · ha^{-1}, which corresponds to 45 m^3 of slurry per hectare.

The aim of the paper was to evaluate the content of available phosphorus and the activity of alkaline and acid phosphatase in soil as affected by the fertilization of red clover, winter wheat and red clover with slurry and nitrogen.

Material and methods

Soil was sampled from a many-year plot experiment, performed in 2008 by the Department of Plant Nutrition and Fertilization, the Institute of Soil Science and Plant Cultivation in Pulawy, under red clover, winter wheat and corn. The experiment was performed on 1 m^2 plots filled 1.2 m deep with the soil material sampled from the arable field. The plots were formed while maintaining the natural soil profile which, in its granulometric composition, in the 0–25 cm layer, contained heavy loamy sand. The experiment was set up following the randomized block design. The soil formation, further referred to as ‘soil’, was fertilized with mineral nitrogen in a form of ammonium nitrate at the amount of 100 kgN · ha^{-1} under red clover, winter wheat and corn, while nitrogen fertilization in a form of slurry varied: 80 kgN · ha^{-1} under red clover, 100 kgN · ha^{-1} under winter wheat and 160 kgN · ha^{-1} under corn. Pig slurry showed the following chemical composition: dry matter – 4.45 %, N_{tot} – 0.774 %, N-NH_4 – 0.680 %, P_2O_5 – 0.400 %, K_2O – 0.350 %, MgO – 0.060 %, CaO – 0.080 %. The soil was sampled three times (April, June, October) during the vegetation period of red clover, winter wheat and corn.

In the adequately prepared material, the following were determined: the content of available phosphorus ($P_{E\text{-}R}$) with the Egner–Riehm method – DL [5], the activity of alkaline (AlP) and acid (AcP) phosphatase with the Tabatabai, Bremner method [6], pH in H_2O [5].

The results were exposed to the analysis of variance and the significance of differences between means was verified with the Tukey test at the confidence level of $p = 0.05$. The calculations involved the use of FR-ANALWAR software based on Microsoft Excel. To identify the potential correlations between soil parameters, the statistical analysis of the results was made applying the Statistica software.

Results and discussion

The mineral and natural fertilization in a form of slurry, applied in the experiment, slightly changed the soil reaction. The pH_{H_2O} values ranged from 5.9 to 6.3, while pH_{KCl} – from 5.3 to 5.9 in soil, depending on fertilization and the plant species (Table 1). Drawing from the pH values recorded, the soil can be qualified as acid and slightly acid. A release of phosphorus to the soil solution is the highest at neutral reaction.

The content of available phosphorus in soil ranged from 13.08 to 23.41 mgP · kg^{-1} depending on the fertilization applied and the plant species, which according to PN-R-04023 1996 [7] classifies it as a soil of a very low phosphorus content (class V).

Table 1

Changes in soil reaction (H_2O and in 1 M · dm^{-3} KCl)

Fertilization	Red clover		Winter wheat		Corn	
	H_2O	KCl	H_2O	KCl	H_2O	KCl
Control	6.2	5.8	6.1	5.6	6.1	5.5
Mineral nitrogen	6.2	5.7	5.9	5.3	6.2	5.7
Slurry	6.3	5.9	6.3	5.8	6.3	5.8

Bearing in mind the environmental aspect, the optimal phosphorus condition in soil should fall within the average richness class (45–66 mgP · kg^{-1}). The highest content of available phosphorus was recorded in the soil under clover (20.35 mgP · kg^{-1}), winter wheat (20.00 mgP · kg^{-1}), corn (20.18 mgP · kg^{-1}) fertilized with slurry (Fig. 1).

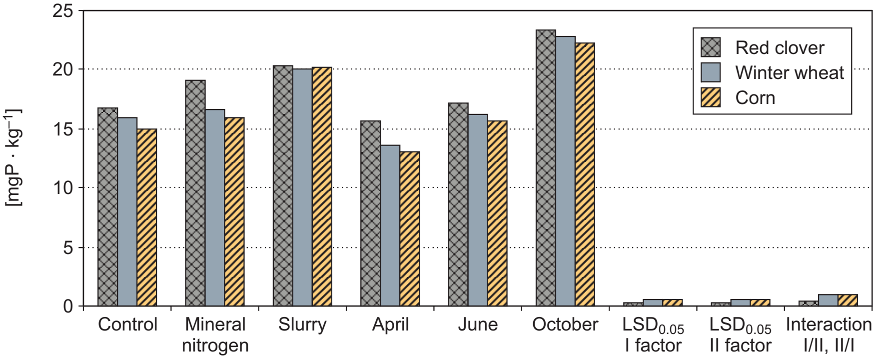

Fig. 1. Content of available phosphorus [mgP · kg^{-1}] in the soil depending on fertilization and sampling date

As reported by Potarzycki [8], as a result of a many-year slurry application there was noted an increase in the humus content which, as a result, can increase the amount of phosphorus available to plants. As for the application of mineral fertilization only, there was observed a decrease in the content of the available form of phosphorus in soil, as compared with the treatments with slurry application (Fig. 1). A negative effect of nitrogen can be due to a strong yield-forming effect as a result of which the uptake of phosphorus from soil increases [9]. According to Mackowiak [2], in the soils with slurry fertilization in many-year period or as combined with mineral fertilization, there is observed a high increase in the content of the available form of phosphorus in soil as well as the mobility of that macroelement deep down the soil profile, which due to its poor mobility, is a desired effect. In integrated agriculture there operates a principle of mineral and organic fertilization. According to Mazur and Mazur [10], in mineral fertilizers the nutrient introduced into soil as a result of transformations becomes available to plants. In natural and organic fertilizers, on the other hand, nutrients which occur in organic bonds get released as a result of mineralization processes microbiologically or biochemically.

The lowest content of available phosphorus was noted in the soil sampled under corn (17.03 mgP $\cdot$ kg^{-1}), as compared with the content of $P_{E\text{-}R}$ in the soil under clover (18.73 mgP $\cdot$ kg^{-1}), and wheat (17.55 mgP $\cdot$ kg^{-1}) (Fig. 1), which is connected with the fact that corn is a plant with high nutrition requirements [11].

The highest accumulation of available phosphorus was recorded in the soil sampled in autumn (October), while the lowest – in spring (April) under all the three crop species (Fig. 1). A low content of available phosphorus in the soil sampled in April could have been due to high requirements of plants in the initial development period when phosphorus determines the root system growth rate. At the same time it defines the capacity of the plant for water and nutrients uptake from soil. Steineck et al [12], on the other hand, add that some of the phosphorus available in soil is uptaken with the crop yield, at the same time, some amount of that macroelement returns to soil with post-harvest residue.

The activity of alkaline phosphatase ranged from 0.682 to 0.910 mM pNP $\cdot$ kg^{-1} $\cdot$ h^{-1}, while the activity of acid phosphatase was higher 1.032–1.737 mM pNP $\cdot$ kg^{-1} $\cdot$ h^{-1} depending on the fertilization applied, soil sampling date and the crop species (Table 2).

Table 2

Activity of alkaline (AlP) and acid (AcP) phosphatase (AcP) [mM pNP $\cdot$ kg^{-1} $\cdot$ h^{-1}] in the soil depending on fertilization and sampling date

Fertilization		Red clover			Winter wheat			Corn		
		AlP	AcP	AlP/ AcP	AlP	AcP	AlP/ AcP	AlP	AcP	AlP/ AcP
I factor	Control	0.682	1.078	0.63	0.723	1.330	0.54	0.788	1.378	0.57
	Mineral nitrogen	0.793	1.202	0.66	0.773	1.437	0.51	0.837	1.477	0.51
	Slurry	0.843	1.430	0.59	0.847	1.503	0.59	0.898	1.643	0.61
Date II factor	April	0.860	1.470	0.59	0.863	1.713	0.50	0.910	1.737	0.52
	June	0.765	1.208	0.63	0.777	1.327	0.58	0.853	1.505	0.57
	October	0.693	1.032	0.67	0.703	1.215	0.58	0.760	1.257	0.60
	Mean	0.773	1.237	0.62	0.781	1.423	0.55	0.841	1.499	0.56
$LSD_{0.05}$	I factor	0.022	0.033		0.017	0.025		0.022	0.020	
	II factor	0.022	0.033		0.017	0.025		0.022	0.020	
Interaction	I/II	n.s.*	0.056		0.029	0.044		n.s.*	0.035	
	II/I	n.s.*	0.056		0.029	0.044		n.s.*	0.035	

n.s.* – non-significant

In the soil fertilized with slurry the activity of alkaline phosphatase increased, as compared with the soil sampled from the control by 23 % under red clover, by 17 % under winter wheat and by 13 % under corn. Similarly the activity of acid phosphatase was higher in the soil fertilized with slurry, as compared with the control (Table 2). Martens et al [13] also noted in earlier research that the activity of enzymes participating in the transformations of such elements as P, S, N, C increases a few-fold due to the application of natural fertilizers (barley straw, FYM, bird litter and green manure). The results reported by Jezierska-Tys and Frac [14], on the other hand, showed that the slurry dose used resulted in a decrease in the activity of the enzymes (protease, urease and dehydrogenase) below the value in the control soil. The application of nitrogen in a mineral form under the crops resulted in a significant increase in the activity of acid phosphatase in soil, however, the increase was lower than after the use of natural fertilizer (Table 2). Mineral fertilization results in the proliferation of soil microorganisms less considerably than natural or organic fertilization, and so the effect of mineral fertilization on the enzymatic activity is lower than that of natural fertilization [15]. According to Skowronska and Filipek [16], introducing fertilizers physiologically acid, including ammonium nitrate, into soil results in inducing the soil acidification and, as such, affects the bioavailability of nutrients and the activity of soil enzymes.

The highest enzymatic activity was recorded in the soil sampled in April under all the crops (Table 2). Furczak [17] also found a high activity of soil phosphatases in spring and summer, and a decrease in the soil sampled in autumn. The intensity of secretion of phosphatases by plants and microorganisms remains closely connected with the phosphorus requirements of the plants. Nutrition requirements of young plants are high, and so the mineralization rate of organic phosphorus bonds in soil by enzymatic activity is, over that period, more intensive. Natywa et al [18], on the other hand, claim that an increased activity of phosphomonoesterases in soil in autumn can be stimulated by the supply of fresh organic weight in a form of post-harvest residue which stimulates the development of microorganisms and, at the same time, the secretion by microorganisms. The activity of the phosphatases was undergoing changes depending on the plant species (Table 2). The highest activity of alkaline (0.841 mM pNP $\cdot$ kg^{-1} $\cdot$ h^{-1}) and acid (1.499 mM pNP $\cdot$ kg^{-1} $\cdot$ h^{-1}) phosphatase was noted in the soil under corn, while the lowest – under red clover (AlP –0.773 mM pNP $\cdot$ kg^{-1} $\cdot$ h^{-1}, AcP – 1.237 mM pNP $\cdot$ kg^{-1} $\cdot$ h^{-1}). Earlier reports by Balot et al [19], Gilewskiej and Płociniczak [20] show that the enzymatic activity of soil remains closely connected with the species, and even the crop cultivar. It is common knowledge that the quantitative and qualitative composition of root secretions and the successive effect of plant residue entering the soil are considerably affected by that biochemical parameter.

Based on the values of activity of alkaline and acid phosphatase recorded, there was calculated the ratio of AlP : AcP, referred to as the enzymatic index of the pH level [21]. The values of the AlP : AcP ratio throughout the research ranged from 0.51 to 0.67 (Table 2). The soil pH value to be considered adequate for the plant growth and development can be the one under the conditions of which there occurs the right ratio of the activity of AlP : AcP [21]. According to Dick et al [21], the value of the AlP : AcP ratio lower than 0.50 points to the acid reaction of soil and limiting is recommended.

There was demonstrated a significantly negative relation between the content of available phosphorus in soil and the activity of acid phosphatase ($r = -0.54$, $p < 0.05$) (Table 3). In general, the activity of soil phosphatases is inversely proportional to its content of mineral phosphorus since an elevated level of inorganic phosphorus in soil decreases the activity of phosphatases [22]. However, as reported by Bielinska and Ligeza [23], there was recorded a strict interaction between the activity of phosphatases and the content of available phosphorus: a high activity of the phosphatases was connected with a many-times higher, than in the control soils, content of $P_{E\text{-}R}$. There was recorded a significant negative value of the coefficient of correlation between pH_{H2O} of soil and the activity of acid phosphatase ($r = -0.19$, $p < 0.05$) (Table 3). Similarly Acosta-Martinez and Tabatabai [24] recorded a significant, yet negative, coefficient of correlation ($r = -0.69$, $p < 0.05$) for the activity of acid phosphatase and soil pH.

Table 3

Correlation coefficients between enzymatic activity of soil
and available phosphorus contents and pH

	Available phosphorus	pH_{H_2O}	pH_{KCl}
Alkaline phosphatase	n.s.	n.s.	n.s.
Acid phosphatase	–0.54	–0.19	n.s.

n.s. – non-significant.

Conclusions

1. Considering the criteria provided for in PN-R-04023, the soil investigated showed a very low content of phosphorus available to plants, which classifies it as representing phosphorus richness class V.
2. A single-side mineral fertilization of plants resulted in a decrease in the content of available phosphorus, as compared with the soil fertilized with slurry, and so the measurable effects are brought by a combination of mineral fertilization with natural or organic fertilization, which can enhance the supply of crops with phosphorus considerably.
3. There was found a varied activity of alkaline and acid phosphatase depending on the fertilization applied. The slurry application resulted in an increase in the enzymatic activity.
4. The activity of the phosphomonoesterases investigated was greater in the soil sampled in spring (April), as compared with the activity of those enzymes in the soil sampled in summer and autumn.

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AKTYWNOŚĆ ALKALICZNEJ I KWAŚNEJ FOSFATAZY I ZAWARTOŚĆ FOSFORU W GLEBIE SPOD WYBRANYCH ROŚLIN UPRAWNYCH NAWOŻONYCH GNOJOWICĄ

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Abstrakt: Celem pracy było określenie wpływu nawożenia gnojowicą trzody chlewnej oraz azotem w postaci saletry amonowej na zmiany aktywności fosfatazy alkalicznej i kwaśnej oznaczonych metodą Tabatabai i Bremnera, zawartości fosforu przyswajalnego metodą Egnera–Riehma (DL), fosforu ogółem oraz fosforu związków organicznych w glebie spod uprawy koniczyny łąkowej, pszenicy ozimej i kukurydzy zwyczajnej. Doświadczenie prowadzone było w roku 2008 jako jednoczynnikowe przez IUNG w Puławach. Stwierdzono istotny wpływ nawożenia zarówno gnojowicą, jak i azotem na zmiany badanych enzymów, jak i zawartości fosforu przyswajalnego w badanej glebie. Zawartość fosforu przyswajalnego jak również aktywność kwaśnej fosfatazy była większa w glebie nawożonej gnojowicą w porównaniu do nawożenia azotem. Aktywność fosfomonoesteraz była zróżnicowana w zależności od gatunku uprawianej rośliny.

Słowa kluczowe: fosfor, alkaliczna i kwaśna fosfataza, gleba, azot, gnojowica

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EFFECT OF FOLIAR SULPHUR FERTILIZATION ON THE PRODUCTIVITY OF THE PERMANENT AND ALTERNATE MEADOW
PART I. THE DRY MATTER YIELD AND THE CONTENT OF MACROELEMENTS

WPŁYW DOLISTNEJ APLIKACJI SIARKI NA PRODUKCYJNOŚĆ ŁĄKI TRWAŁEJ I PRZEMIENNEJ
CZ. I. PLONY SUCHEJ MASY ORAZ ZAWARTOŚĆ MAKROELEMENTÓW

Abstract: In the three-year field experiment the dry matter yields and the content of macroelements were compared with the plants derived from permanent and alternate meadows. Foliar fertilization with sulphur was applied in both types of meadows. In the green forage samples dry matter content was determined at 105 °C. On the basis of dry matter content in hay the yield of dry matter was calculated. After the mineralization the hay samples were subjected to the analyses of total nitrogen content – by the Kiejdahl method, phosphorus and magnesium content – by colorimetric method, potassium, sodium and calcium contents – using flame photometry and sulphur content – by nephelometric method.

Foliar fertilization with sulphur positively affected the dry matter yield in the case of both permanent and alternate meadows. The application of sulphur in the form of Super S-450 preparation influenced respectively 10.5 and 22.2 % higher dry matter yields in comparison with the fields not supplemented with this fertilizer.

The weighted mean content of macroelements in plants derived from both meadows fluctuated in the range of: 1.8–4.5 gP; 15.9–27.0 gK; 0.7–3.3 gCa; 1.2–3.1 gMg; 0.15–0.95 gNa; 1.3–3.5 $gS \cdot kg^{-1}$ d.m.

Fertilization with sulphur resulted in higher content of all macroelements and had the positive effect on the N : S proportion narrowing it to the level of 9.03 and 9.28 for the permanent and alternate meadow, respectively. The lower values of the N:S ratio in the forage are beneficial from the nutritional point of view.

Keywords: meadow sward, sulphur fertilization, content of macroelements, dry matter yield

Among all nutrients sulphur is the basic one which is important for the proper growth and development of the plants, together with nitrogen, phosphorus and magnesium

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[1–3]. It is a component of many organic and inorganic compounds which play an important role in plant metabolism. Sulphur can be found in amino acids like: methionine, cystine, cysteine, which are essential for the protein synthesis [4]. Moreover, sulphur is a component of many enzymes whose function is to regulate the plant metabolism and nitrate nitrogen to amide nitrogen transformation. Sulphur deficiency symptoms are especially visible in plants under the conditions of intensive nitrogen fertilization. For the proper plant growth and development the suitable quantitative N : S proportions, 10–15 : 1 being the optimal, are very important [5–7].

The requirements of the reduction of SO_2 release into the atmosphere as well as limited use of mineral and organic fertilizers resulted in significantly lower sulphur concentration in soil [8, 9]. The problem of sulphur deficiency in plant production exists in 73 countries all over the world, involving 18 European countries [10, 11]. In Poland 53 % of soils are characterized with a low sulphur level [12] and according to the predictions of the World Sulphur Institute the deficiency of this element in 2010 will amount to 11.1 mln Mg/year [10].

The aim of the present study was an estimation of the effect of sulphur foliar application on the productivity of the permanent and alternate meadows as well as yields and the content of macroelements.

Materials and methods

The field experiment was conducted in the years 2006–2008 in an individual farm in Pilica administrative district, in Zawiercie county, in the Silesia province, at the altitude of 320 m. The experiment was established by means of random block sampling, in four replicants, on the acid, brown soil ($pH_{KCl} = 5.2$) which belonged to the V soil quality class. The investigated objects were located on the permanent and on the newly-established, alternate meadow in the second year of full utilization. The soil contained medium levels of potassium, manganese and zinc and it was poor in assimilable forms of phosphorus and copper. During the vegetation period (IV–IX) total rainfall and average air temperatures for each year of the study were as follows: 2006 – 338 mm and 15.2 ^oC; 2007 – 375 mm and 14.3 ^oC; 2008 – 320 mm and 14.9 ^oC.

Foliar fertilization with sulphur was the determining factor in the study. Foliar fertilizer in the form of Super S-450 suspension in a dose of 2 $dm^3 \cdot ha^{-1}$ (equal to 900 $gS \cdot ha^{-1}$) was applied under each regrowth.

In each year of the experiment the following mineral fertilization was used for both meadow types: under the first regrowth – 80 $kgN \cdot ha^{-1}$, under the second and third regrowths – 60 $kgN \cdot ha^{-1}$ (for each regrowth) in the form of ammonium saltpetre, phosphorus – once in the spring, in the amount of 120 $kgP_2O_5 \cdot ha^{-1}$ in the form of triple superphosphate and potassium – under the first and third regrowths – 60 $kgK_2O \cdot ha^{-1}$ (for each regrowth) in the form of 57 % potassium salt. The experimental fields were characterized with the area of 10 m^2. Collected plant samples were subjected to the analysis of the fodder chemical composition. The dry matter content was determined by drying at 105 ^oC. Nitrogen content was determined by the Kiejdahl method; phosphorus and magnesium content – by the vanadium-molybdenic method; potassium, sodium and calcium contents – using flame photometry and sulphur content – by the nephelometric method [13].

The obtained results were subjected to the statistical analysis of variance. The mean results were compared on the basis of the Duncan test on the significance level of 0.05.

Results and discussion

The average yield collected from the fields located on the permanent meadow not fertilized with sulphur amounted to 6.8 Mg · ha^{-1} (Table 1). The application of sulphur in the form of Super S-450 resulted in 10.5 % higher yield. The effect was more significant in the case of alternate meadow, where the obtained yields were higher by 22.2 %. The plants derived from all fields gave the highest yields in the year 2007 and the lowest – in 2008. The difference was equal to 19 % on average and was statistically significant.

Table 1

Dry matter yields of the meadow sward [Mg · ha^{-1}]

Variant	Years			Mean of years (2006–2008)
	2006	2007	2008	
	[Mg · ha^{-1}]			
Permanent meadow (–S)	6.89a*	7.58a	5.88a	6.8a
Permanent meadow (+S)	7.26a	8.98ab	6.62b	7.6ab
Alternate meadow (–S)	9.65b	10.26b	9.32c	9.7b
Alternate meadow (+S)	12.38c	12.74c	10.41d	11.8c

* Mean values followed by the same letters are not significantly different according to the results of the Duncan test at P = 0.05; (–S) – series without sulphur; (+S) – series with sulphur.

The concentration of mineral components in the investigated objects was diversified. The application of sulphur fertilizer affected higher contents of all macroelements. The weighted mean content of macroelements fluctuated in the range of: 17.2–28.0 gN; 1.8–4.5 gP; 15.9–27.0 gK; 0.7–3.3 gCa; 1.2–3.1 gMg; 0.15–0.95 gNa; 1.3–3.5 gS · kg^{-1} d.m. (Table 2). According to the feeding requirements the fodder of good quality should contain at least: 15–30 gN; 3.0 gP; 17–20 gK; 7.0 gCa; 2.0 gMg; 1.5–2.5 gNa and 2.0–3.0 g S · kg^{-1} d.m. [6, 7].

In the conducted study the nitrogen content reached the satisfactory level in all objects. Phosphorus, calcium, magnesium and sodium levels in the examined grasses were low and below the optimal values. Only legumes as well as herbs and weeds were characterized with optimal contents of these elements. Sulphur content was below the optimal value in the case of two objects not fertilized with this element, other objects contained desirable sulphur amounts.

The value of N : S ratio is often reported as the indicator of sulphur content. The N : S = 15 is assumed as the proper value for cereals. The N : S proportion in the meadow sward of the examined fields fluctuated in the range of 8.0–15.7 and lower

Table 2

Weighted mean content of macroelements [g · kg^{-1} d.m.] in analysed plant groups as affected by the sulphur fertilization (mean values for three years of the investigations)

Species	Permanent meadow							Alternate meadow						
	N	P	K	Ca	Mg	Na	S	N	P	K	Ca	Mg	Na	S
Series without sulphur														
Grasses	22.4a	1.8a	17.6a	0.7a	1.2a	0.36ab	2.24a	20.4ab	2.0a	17.4a	0.9a	1.4a	0.35a	2.00b
Legumes	26.8b	3.0b	16.1a	1.2ab	1.7b	0.15a	2.65b	22.6b	3.8cd	15.9a	1.5a	2.0ab	0.16a	1.44a
Herbs and weeds	23.8ab	2.3a	24.0c	2.4c	2.4c	0.95c	2.08a	17.2a	2.8b	26.7c	3.3c	3.1c	0.89c	1.30a
Meadow sward	19.2a	2.4a	19.1b	2.0bc	1.9b	0.52b	1.90a	21.0ab	2.6ab	19.7b	2.5b	2.3b	0.59b	2.00b
Series with sulphur														
Grasses	24.3ab	2.0a	17.7ab	0.8a	1.3a	0.36ab	2.58b	22.1b	2.5ab	17.5a	1.4a	1.5a	0.36a	3.40c
Legumes	28.0b	3.4b	16.3a	1.6b	1.7b	0.16a	3.50c	23.2c	4.5d	16.1a	1.7ab	2.1ab	0.17a	1.84ab
Herbs and weeds	24.2ab	2.5ab	24.3c	2.4c	2.5c	0.93c	2.33ab	18.8a	3.4c	27.0c	3.3c	3.1c	0.89c	1.77ab
Meadow sward	19.9a	2.7b	19.3b	2.1c	1.9b	0.52b	2.40ab	22.2b	2.9b	19.9b	2.8b	2.4ab	0.60b	3.00bc
V %	12.9	20.7	16.6	41.5	25.3	62.4	19.8	9.8	26.1	22.3	42.3	28.5	57.9	35.1

values were stated for the sward fertilized with sulphur (Table 3). Lower values of N : S ratio in fodders are more advantageous from the nutritional point of view. According to Alloway and Thompson the optimal N : S proportion in the fodder destined for ruminants ranges from 10 : 1 to 15 : 1, so it is lower than the value assumed for the optimal plant growth [14]. In the opinion of Falkowski et al [7] critical sulphur concentration, below which the inhibition of plant development may occur, amounts to 1.0 for grasses. In that light, all examined samples were characterized with higher than critical values.

Table 3

Nitrogen to sulphur ratio (means for three years)

Species	Permanent meadow	Alternate meadow
Series without sulphur		
Grasses	10.00	10.20
Legumes	10.10	15.70
Herbs and weeds	11.41	13.20
Meadow sward	10.10	10.50
Mean value	10.40	12.40
Series with sulphur		
Grasses	9.40	6.50
Legumes	8.00	12.60
Herbs and weeds	10.40	10.60
Meadow sward	8.30	7.40
Mean value	9.03	9.28

The highest N and P contents were noticed in legumes, also herbs and weeds were characterized with high K, Ca, Na and Mg levels, which is consistent with the results by Trzaskos [15] as well as Czyz and Dzida [16]. Grasses were the poorest in macroelements, except the content of K and Na, which was higher than legumes.

Conclusions

1. The 10.5 % and 22.2 % higher dry matter yields were observed for both permanent and alternate (respectively) meadows as a result of sulphur application.
2. Treatment with sulphur fertilizer resulted in increased levels of all macroelements in all plant groups.
3. Fertilization with sulphur in the form of Super S-450 had a positive effect on the narrowing of the N : S ratio, which is beneficial from the nutritional point of view.

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WPŁYW DOLISTNEJ APLIKACJI SIARKI NA PRODUKCYJNOŚĆ ŁĄKI TRWAŁEJ I PRZEMIENNEJ CZ. I. PLONY SUCHEJ MASY ORAZ ZAWARTOŚĆ MAKROELEMENTÓW

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Abstrakt: W trzyletnim doświadczeniu polowym porównywano plony suchej masy i zawartość makroelementów w roślinności łąki trwałej i przemiennej nawożonej dolistnie siarką. W próbkach zielonki oznaczono zawartość suchej masy metodą suszarkową w temperaturze 105 °C. Na podstawie zawartości suchej masy w sianie obliczono plony suchej masy. Po mineralizacji próbek siana oznaczono zawartość N – metodą Kiejdahla; zawartość fosforu i magnezu – kolorymetrycznie; potasu, sodu i wapnia – metodą fotometrii płomieniowej oraz siarkę – metodą nefelometryczną.

Dolistne stosowanie siarki korzystnie oddziaływało na zwyżkę plonu suchej masy zarówno na łące trwałej, jak i przemiennej. Aplikacja siarki w formie nawozu Super S-450 spowodowała wzrost plonów suchej masy odpowiednio o 10.5 i 22.2 % w porównaniu z obiektami, na których nie stosowano tego składnika.

Średnia ważona zawartości makroelementów w roślinach obu łąk wahała się w zakresie: 1.8–4.5 gP; 15.9–27.0 gK; 0.7–3.3 gCa; 1.2–3.1 gMg; 0.15–0.95 gNa; 1.3–3.5 g S · kg^{-1} s.m.

Zastosowane nawożenie siarką spowodowało zwiększenie zawartości wszystkich makroelementów oraz korzystnie wpłynęło na zawężenie stosunku N : S do wartości średnio 9.03 i 9.28 dla łąki trwałej i przemiennej. Niższe wartości stosunku N : S w paszy są korzystniejsze ze względów żywieniowych.

Słowa kluczowe: ruń łąkowa, nawożenie siarką, zawartość makroelementów, plony suchej masy

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DIVERSIFICATION OF YIELDING AND QUALITY TRAITS OF MEADOW GRASS ON AN EXAMPLE OF SELECTED CULTIVARS AND STRAINS

ZRÓŻNICOWANIE PLONOWANIA I CECH JAKOŚCIOWYCH WIECHLINY ŁĄKOWEJ NA PRZYKŁADZIE WYBRANYCH ODMIAN I RODÓW

Abstract: The paper presents an assessment of varieties and strains of meadow grass regarding dry mass yields, total protein and crude fibre content. Obtained results of strain value testing reveal that the collection resources contain forms which have a high breeding potential. The studied strains and varieties were characterized by big yields of dry matter with differences reaching even 39 %. Individual studied objects differed also with the content of total protein reaching from 11.60 to 19.10 %. On the other hand for crude fibre the values fluctuated from 22.50 to 29.20 %.

Keywords: variety of meadow grass, strain, total protein, crude fibre, yield

Meadow grass (*Poa pratensis* L.) is a species with high fodder value, revealing good digestibility and tastefulness, as well as advantageous mineral composition. The species is adapted to a wide spectrum of habitat conditions, but develops best on moist soils, tolerating pH from 4.5 to 8.5. It is resistant to big differences in temperatures ranging from 43 to –44 ^{o}C. Variability of environment in which meadow grass occurs led to its considerable morphological plasticity and formation of different genetic phenotypes [1, 2]. Scientific research confirmed a wide variability of functional features, which evidences a considerable genetic diversity of its varieties [3, 4, 5]. Moreover, a pheno-

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menon of apomixis, which occurs in meadow grass can make cross-breeding difficult, but is helpful in maintaining pure breeding lines, which are both morphologically and genetically homogenous [6].

Fodder varieties of meadow grass characterized by a considerable proportion of leaves ensure high productivity with maintained advantageous fodder value. Due to its resistance to trampling, nibbling and cutting it is particularly recommended for pastures where it constitutes the main component of sward, it is also appropriate for park lawns and golf courses.

Breeding works aim in two directions: selecting high yielding varieties for fodder purposes and varieties for lawns in which preferred are narrow type of leaf and slow regrowth.

Success in breeding new varieties of meadow grass is to a considerable degree determined by the initial material and particularly a wide variety of biological and functional (morphological) features determining the value of the variety [4].

A review of literature shows the variety dependent diversification of the rate of biomass accumulation and differences in chemical composition. Research of Lemaire et al [7] reveals a strict dependence between plant morphological composition and the quality of obtained fodder.

Therefore the aim of the presented research was an assessment of diversification of strains and varieties of meadow grass regarding dry mass yields, total protein and crude fibre content.

Materials and methods

The research was conducted in 2005–2007 at Plant Breeding Station in Skrzeszowice near Krakow (220 m a.s.l.), on degraded chernozem developed from loess. Chemical properties of the soil were as follows: pH_{KCl} – 7.2, bioavailable P – 54, K – 127.2 and Mg – 48.1 $g \cdot kg^{-1}$. Annual precipitation totals during the investigated period (years 2005–2007) fluctuated from 569.5 to 722.0 mm, whereas mean precipitation totals during the vegetation period (April–September) ranged from 325–465 mm. Average annual temperature in the years of the research ranged between 6.0 and 6.9 ^{o}C and during the April–September period from 12.3 to 13.9 ^{o}C. The experiment set up using randomized block method in three replications (plots 1 × 9.8 m^2) considered the standard Eska 46 variety, accompanying Skiz variety and three strains: SKW-15, SKW-17 and SKW-18. Component families of strains:

- SKW-15:
  1. Self-pollinated 46/2 with Eska 46,
  2. Self-pollinated 28/2 with Eska 46.
- SKW-17:
  1. Self-pollinated 10/2 with Eska 46,
  2. Self-pollinated 6/2 with Eska 46,
  3. Self-pollinated 12/3 with Eska 46.

- SKW-18:
  1. Self-pollinated 46/2 with Eska 46,
  2. Self-pollinated 46/2 with Eska 46.
- ESKA 46:
  43 component families.

All objects composing the strains originate from ESKA46 seeds treated with chemical mutagens: colchicine and phenylmercury acetate in 2000. Selected seedlings were planted on the field and following the observations, seeds of chosen self-pollinated plants made up constituents of strains.

The experiment was set out on 23rd May 2005. The standard sowing rate was 10 kg/ha. In autumn, phosphorus fertilizers were sown in the amount of 70 $kgP_2O_5 \cdot ha^{-1}$ as triple superphosphate and potassium fertilizers in the amount of 100 $kgK_2O \cdot ha^{-1}$ as potassium salt. Nitrogen fertilization was applied prior to sowing in the dose of 20 $kgN \cdot ha^{-1}$ of ammonium nitrate. The second dose of nitrogen fertilizer in the amount of 50 $kgN \cdot ha^{-1}$ was applied after the seeds sowing.

In the years of full utilisation the following fertilization was applied: 80 $kg\ N \cdot ha^{-1}$ under the first cut and 60 $kgN \cdot ha^{-1}$ of ammonium nitrate after the second and third cuts each, phosphorus – 120 $kgP_2O_5 \cdot ha^{-1}$ as triple superphosphate once, in the autumn preceding the harvest and potassium dosed 60 $kgK_2O \cdot ha^{-1}$ as 57 % potassium salt.

Total nitrogen was assessed by means of Kjeldahl's method in mean weighted average plant samples after their drying and grounding.

The obtained results were verified by the analysis of variance. Differences between means were assessed using Tukey multiple range test and then the least significant difference was calculated.

Results and discussion

Considering the analysed varieties and strains, Eska 46 standard variety yielded best over the total 2 years of utilisation, whereas the accompanying Skiz variety produced slightly poorer yields (Table 1). The lowest yields were registered for the plants of SKW-18 strain. The difference in dry mass yield between the standard variety and strains ranged from 20 to 27 %.

Table 2 shows a diversification of total protein contents between compared varieties and strains in the years of utilisation and in cuts. Regarding total protein content in the plant dry mass, the studied strains exceeded Eska 46 standard variety and Skiz variety as they proved to be definitely more abundant in protein. The situation may be explained by the fact that these varieties were characterised by poorer foliage which, as results from Buxton et al [8] and Julier et al [9] research on alfalfa, may have affected a decline in total protein content in dry mass. Diversified crude fibre content was presented in Table 3. In dry mass of the tested meadow grass strains crude fibre content was lower than in the varieties. According to some authors fibre content in plants to a considerable degree depends on the weather factors [10]. The lowest concentration of this factor was noted in the vegetation season in which the temperature and precipitations were favourable for the growth and development of plants. It was the second regrowth in the second year of full utilisation. On the other hand, the higher level of fibre occurred in the season with high air temperatures and low precipitations, which accelerated the process of cell walls saturation with lignins.

Table 1

Yield of dry matter [Mg · ha^{-1}]

Object	Year of utilisation							Total for two years
	2006			2007				
	Cut			Cut				
	I	II	I + II	I	II	III	I + II + III	
SKW-15	3.68	2.64	6.32	3.80	5.02	3.40	12.22	18.54
SKW-17	3.69	2.36	6.05	3.52	4.76	3.01	11.29	17.34
SKW-18	3.69	2.46	6.15	3.47	4.32	2.99	10.78	16.93
SKIZ	6.27	3.93	10.20	5.78	4.00	3.02	12.80	23.00
ESKA 46	5.93	3.99	9.92	5.87	3.92	3.37	13.16	23.08
Mean	4.65	3.08	7.73	4.49	4.40	3.16	12.05	19.78
V(%)	2.85	2.65	2.76	2.74	1.08	0.66	0.83	1.54
NIR$_{(0.05)}$	0.77	0.57	1.32	0.81	0.39	0.14	1.01	2.25

Table 2

Crude protein content in the dry matter [g · kg^{-1} d.m.]

Object	Year of utilisation					Mean
	2006		2007			
	Cut		Cut			
	I	II	I	II	III	
SKW-15	181.0	145.0	183.0	144.0	159.0	162.4
SKW-17	171.0	143.0	182.0	147.0	174.0	163.4
SKW-18	169.0	140.0	191.0	142.0	170.0	162.4
SKIZ	153.0	149.0	160.0	116.0	152.0	146.0
ESKA 46	161.0	146.0	158.0	132.0	144.0	148.2
Mean	167.0	144.6	174.8	136.2	159.8	156.5
V(%)	6.34	2.32	8.50	9.26	7.77	5.50
NIR$_{(0.05)}$	11.1	3.6	13.3	15.9	12.5	8.3

From the perspective of fodder value, the content of analysed components was adequate. The studied varieties proved slightly less abundant in total protein but no decline in the yield of this element was registered in result. For two years of utilisation the harvest for Eska 46 standard variety was 3.47 Mg · ha^{-1} and 3.39 Mg · ha^{-1} for Skiz. On the other hand, total protein yield for strains was on average lower by 1.74 Mg · ha^{-1}

in comparison with the standard (total yield of total protein for two years of full utilisation).

Table 3

Crude fiber content in the dry matter [g · kg^{-1} d.m.]

Object	Year of utilisation					Mean
	2006		2007			
	Cut		Cut			
	I	II	I	II	III	
SKW-15	250.0	248.0	240.0	225.0	260.0	244.6
SKW-17	249.0	251.0	239.0	231.0	255.0	245.0
SKW-18	260.0	252.0	236.0	239.0	261.0	249.6
SKIZ	292.0	247.0	249.0	247.0	253.0	257.6
ESKA 46	287.0	246.0	272.0	259.0	264.0	265.6
Mean	267.6	248.8	247.2	240.2	258.6	252.5
V(%)	7.67	1.04	5.94	5.57	1.74	3.57
NIR$_{(0.05)}$	14.7	2.2	8.8	12.0	4.4	6.2

Table 4

Yield of crude protein [Mg · ha^{-1}]

Object	Year of utilisation		Total for two years
	2006	2007	
SKW-15	1.05	1.96	3.01
SKW-17	0.97	1.86	2.83
SKW-18	0.97	1.78	2.75
SKIZ	1.54	1.85	3.39
ESKA 46	1.54	1.93	3.47
Mean	1.21	1.88	3.09
V(%)	2.48	0.37	1.05
NIR$_{(0.05)}$	0.19	0.07	0.27

The analysed populations of varieties and strains of meadow grass are diversified regarding many traits. Accumulated genotypes widen the diversity within the genus of meadow grass which may be used in breeding and research works.

Conclusions

1. Both varieties and strains differed slightly from one another regarding dry mass yields and the content of total protein and crude fibre.

2. The feature which significantly differed varieties and strains were meteorological conditions.
3. On the basis of many-year observations it was determined that further thorough observations should be conducted in order to identify valuable strains.

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ZRÓŻNICOWANIE PLONOWANIA I CECH JAKOŚCIOWYCH WIECHLINY ŁĄKOWEJ NA PRZYKŁADZIE WYBRANYCH ODMIAN I RODÓW

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Abstrakt: Praca prezentuje ocenę zróżnicowania odmian i rodów wiechliny łąkowej pod względem plonów suchej masy, zawartości białka ogólnego oraz włókna surowego. Uzyskane wyniki badania wartości rodów wskazują, że w zasobach kolekcyjnych są formy, które mają dużą wartość hodowlaną. Badane rośliny charakteryzowały się dużymi plonami suchej masy, gdzie różnice sięgały nawet 39 %. Poszczególne rośliny różniły się także zawartością białka ogólnego, które wynosiły od 11,60 do 19,10 %. Natomiast dla włókna surowego wartości te kształtowały się od 22,50 do 29,20 %.

Słowa kluczowe: odmiana wiechliny łąkowej, ród, białko ogólne, włókno surowe, plon

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SURVIVAL RATE OF REDWORMS AND WOODLICE IN SOIL CONTAMINED WITH PETROL, DIESEL OIL AND ENGINE OIL

PRZEŻYWALNOŚĆ DŻDŻOWNIC I PROSIONKÓW PRZY SKAŻENIU GLEBY BENZYNĄ, OLEJEM NAPĘDOWYM I OLEJEM SILNIKOWYM

Abstract: The investigations aimed at an assessment of representatives of Isopoda and Lumbricidae sensitivity to soil pollution with oil derivatives from the perspective of their use as bioindicators. The laboratory experiment, conducted in 3 replications, comprised the following objects: soil contaminated with unleaded petrol; soil contaminated with diesel oil; soil contaminated with used engine oil; control. Doses of 3000 mg, 6000 mg and 10 000 mg of oil derivative per 1 kg soil d.m. were applied. The test animal most sensitive to soil pollution with petrol, diesel and engine oil were redworms. They revealed a rapid and clear response in the objects contaminated with these pollutants in comparison with the control. Woodlice proved the most sensitive to soil contamination with petrol and diesel oil. Used engine oil revealed the weakest effect on the tested invertebrate species among all three applied pollutants, whereas petrol had the most toxic effect.

Keywords: oil derivatives, soil pollution, redworms, woodlice

Representatives of terrestrial fauna, such as Isopoda, Lumbricidae are regarded as good bioindicators of the environmental contamination [1]. However, there are not many reports about oil derivatives influence on these invertebrates. Among others, survival rate and reproduction of *Eisenia foetida* redworm has been investigated with progressing bioremediation process of soil contaminated with oil derivatives [2] and its influence on properties of soil contaminated with these substances [3]. This species was also used to reveal the optimal indicators of sublethal doses of polycyclic aromatic hydrocarbons [4]. Redworms may positively contribute to bioremediation of soil contaminated with oil derivatives, although it depends on their species [5]. A number of papers have also addressed the issue of the ability of among others redworms or *Isopoda* representatives to metabolize polycyclic aromatic hydrocarbons (PAHs) [6–8].

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Redworms were also used as indicators for an assessment of oil derivative contaminated soil quality [9].

The investigations aimed at an assessment of selected invertebrate groups (representatives of Isopoda, Lumbricidae) sensitivity to soil pollution with oil derivatives from the perspective of their use as bioindicators.

Material and methods

The laboratory experiment, conducted in 3 replications, comprised the following objects:

1. Soil contaminated with unleaded petrol;
2. Soil contaminated with diesel oil;
3. Soil contaminated with used engine oil;
4. Control.

The experiment was conducted on the representatives of redworms of *Lumbricus* genus and rough woodlouse (*Porcellio scaber*). Three contamination levels were used for redworms: 3000 mg of oil derivative per 1 kg soil d.m. (level I), 6000 mg of oil derivative per 1 kg of soil d.m. (level II) and 10 000 mg of oil derivative per 1 kg soil d.m. (level III). Doses of 3 000 mg and 10 000 mg of oil derivative per 1 kg soil d.m. were applied for rough woodlice (*Porcellio scaber*). The soil used for the experiment was degraded chernozem formed from loess, classified as the very good wheat complex and soil quality class. The soil was dried at 60 ^{o}C for 5 days in a dryer and then contaminated using a syringe with formerly calculated and precisely measured amount of oil derivatives.

Redworms used in the experiment were collected in Barwald Gorny village near Kalwaria Zebrzydowska. The experiment made use of redworm of similar length and well formed clitellum. The redworms were cultured in 500 cm^3 containers. 10 redworms were put in each container with 400 g of prepared soil. Containers were covered with gauze for good ventilation. The redworms were cultured at 20 ± 2 ^{o}C. Every week 4 g of dry and crushed horse excreta was supplied as feed and of the specimens viability was checked regularly. Each week redworm weight was measured. Dead specimens were removed. If the soil in the containers dried, it was sprinkled with 20 cm^3 of distilled water. Rough woodlice were collected on 19 and 20 June 2009 in Zawadka village near Wadowice. The woodlice were cultured in 100 cm^3 containers and 6 specimens were placed per container. The woodlice were fed with small pieces of apple and carrot. Otherwise the culturing conditions were the same as for redworms.

Statistical analysis comprised one-way ANOVA. Means were differentiated using LSD Fisher's test. All computations were conducted using "Statistica 8.0" programme.

Results and discussion

Among the tested oil derivatives petrol proved the most toxic for redworms (Fig. 1). Already a day after the experiment outset mortality rate of these invertebrates in soil contaminated with 6000 and 10 000 mg of petrol · kg^{-1} soil d.m. reached 100 %, whereas over 80 % died in soil contaminated with 3 000 mg · kg^{-1} soil d.m. On the following days of the experiment mortality rate was also increasing in the other objects containing soil contaminated with diesel and engine oil. On the fourth day of observa-

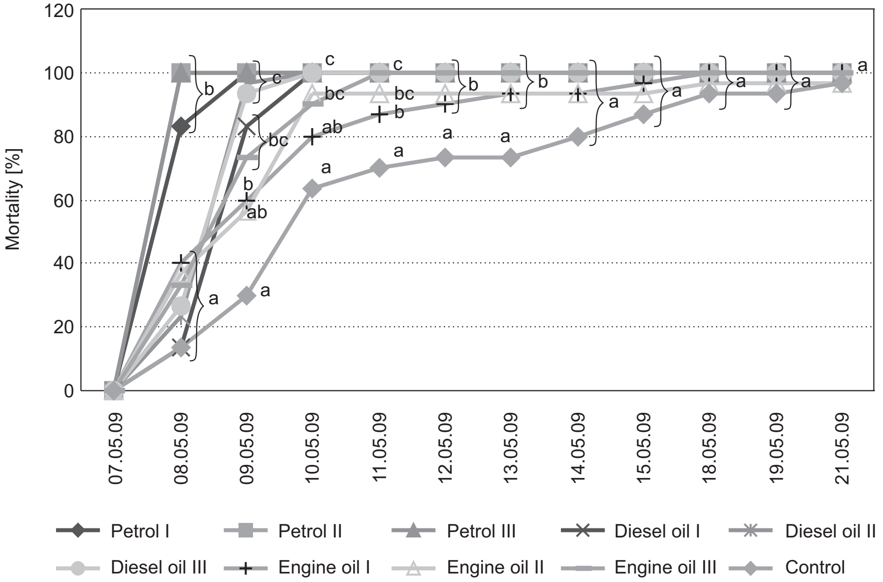

Fig. 1. Mortality rate of redworms [%] cultured in soil contaminated with oil derivatives. Means marked with different letters for individual dates of observation differ statistically at p = 0.05

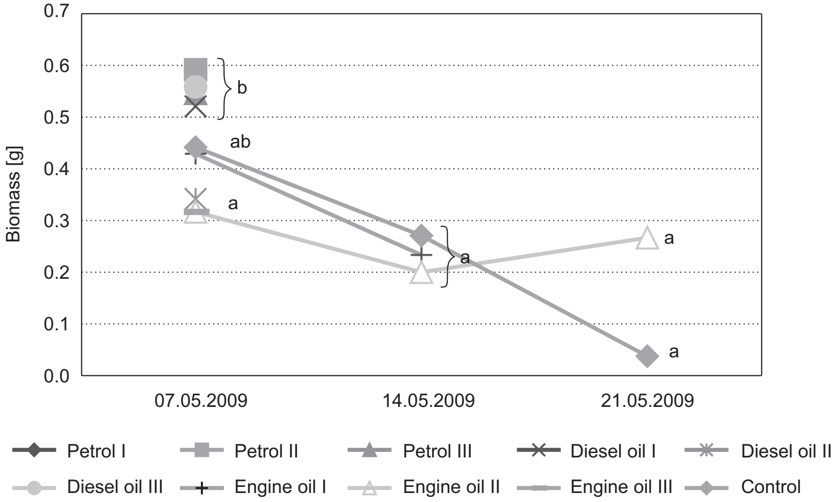

Fig. 2. Mean body weight of a single specimen of Lumbricidae cultured in soil contaminated with oil derivatives. Means marked with different letters for individual dates of observations differ statistically at p = 0.05

tion live specimens were still registered, beside the control, in the objects where soil was contaminated with doses of 3 000 and 6 000 mg of engine oil per 1 kg of soil d.m. Due to very high mortality rate of redworms in most objects with contaminated soil, it

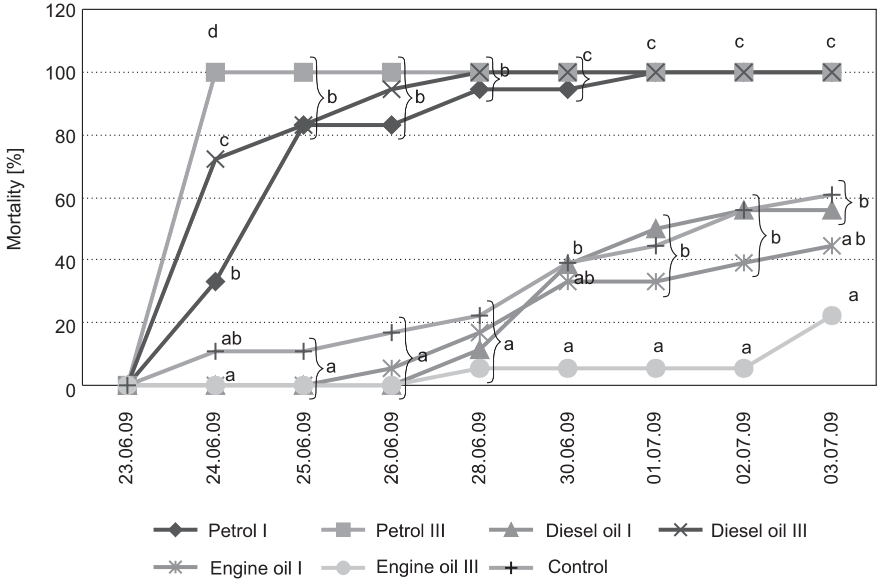

Fig. 3. Mortality rate of Isopoda [%] cultured in soil contaminated with oil derivatives. Means marked with different letters for individual dates of observation differ statistically at p = 0.05

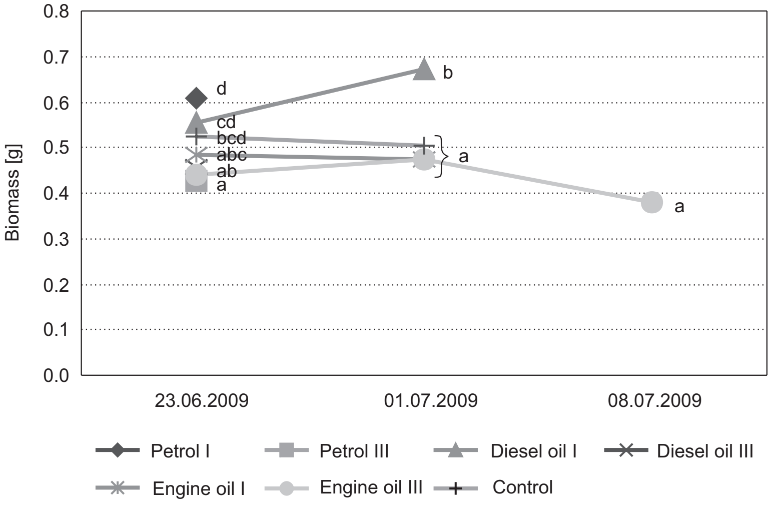

Fig. 4. Mean body weight of a single specimen of Isopoda cultured in soil contaminated with oil derivatives. Means marked with different letters for individual dates of observations differ statistically at p = 0.05

proved impossible to measure changes in body weight of live specimens after a week and two weeks from the experiment outset. In the objects where the measurement was possible an apparent decline in body weight was noted in all objects (also in the control) (Fig. 2). Investigations on the use of *Eisenia foetida* redworm for the quality assessment of soils contaminated with various kinds of crude oils (heavy, medium and light (API gravity 16–18.30 and 53) after remediation tests, demonstrated that the species was between 1.4 and 14 times more sensitive than mictrotox test and between 1.3 and > 77 times more sensitive than plant toxicity tests. Light oil in the silty low organic carbon soil proved the most toxic [9].

The tested species of Isopoda order, *ie* rough woodlouse showed weaker sensitivity to the applied substances (Fig. 3). Only the highest dose of petrol caused a 100 % mortality of these animals already after one day from the soil contamination. Relatively high mortality rate was observed among woodlice in the object where the soil was contaminated with the highest dose of engine oil. On the second day of observations mortality rate of woodlice increased also in the second object contaminated with petrol (a dose of 3000 mg $\cdot$ kg^{-1} d.m.), where it reached over 80 %. At the same time woodlice mortality rate in the objects with soil contaminated with both doses of engine oil and diesel oil in the lower dose did not exceed 20 %, similar as in the control. During the whole period of the experiment the lowest percentage of dead woodlice (significantly lower than in the control) was registered in the object where soil was contaminated with the higher dose of engine oil. No significant changes in the weight of live specimens were observed during culturing in most objects (Fig. 4). Only in the object with soil contaminated with diesel oil in a dose of 3000 mg $\cdot$ kg^{-1} soil d.m. an increase in *Porcelio* weight was noted a week after the experiment outset. Research conducted under field conditions by the Author on Isopoda occurrence in soil polluted with oil derivatives (petrol, diesel and engine oil dosed 2 dm^3 $\cdot$ m^{-2}) did not demonstrate any marked differences in these invertebrate number caught into pitfall traps, as dependant on the pollutant substance [10], however they were caught sporadically. There were no clear dependencies either between Isopoda presence and soil pollution with heavy metals (Cd, Zn and Pb) in case when weakly polluted soils were analyzed. On the other hand, no Isopoda were caught in strongly polluted soils [11].

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Conclusions

1. The test animal more sensitive to soil pollution with petrol, diesel and engine oil were redworms. They revealed a rapid and clear response in the objects contaminated with these pollutants in comparison with the control.
2. Woodlice proved the most sensitive to soil contamination with petrol and diesel oil.

3. Used engine oil revealed the weakest effect on the tested invertebrate species among all three applied pollutants, whereas petrol had the most toxic effect.

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PRZEŻYWALNOŚĆ DŻDŻOWNIC I PROSIONKÓW PRZY SKAŻENIU GLEBY BENZYNĄ, OLEJEM NAPĘDOWYM I OLEJEM SILNIKOWYM

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Abstrakt: Celem badań była ocena wrażliwości wybranych grup bezkręgowców (przedstawicieli *Isopoda, Lumbricidae*) na zanieczyszczenia gleby substancjami ropopochodnymi pod kątem możliwości ich wykorzystania jako biowskaźników. Doświadczenie laboratoryjne przeprowadzono w 3 powtórzeniach i obejmowało ono następujące obiekty: gleba skażona benzyną bezołowiową, gleba skażona olejem napędowym, gleba skażona przepracowanym olejem silnikowym, kontrola. Zastosowano dawki 3000, 6000 i 10 000 mg substancji ropopochodnej na kg s.m. gleby. Zwierzęciem testowym najbardziej wrażliwym na zanieczyszczenia gleby benzyną oraz olejami (napędowym i silnikowym) były dżdżownice. Cechowała je szybka i wyraźna odpowiedź w obiektach skażonych tymi polutantami w stosunku do obiektów kontrolnych. Stonogi okazały się najbardziej wrażliwe na skażenie gleby benzyną i olejem napędowym. Najsłabszym działaniem na testowane gatunki bezkręgowców spośród wszystkich trzech zastosowanych polutantów charakteryzował się zużyty olej silnikowy, natomiast najbardziej toksycznie oddziaływała benzyna.

Słowa kluczowe: ropopochodne, skażenie gleby, dżdżownice, równonogie

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THERAPEUTIC ROLE OF ANIMALS IN HUMAN LIFE – EXAMPLES OF DOG AND CAT ASSISTED THERAPY

TERAPEUTYCZNA ROLA ZWIERZĄT W ŻYCIU CZŁOWIEKA NA PRZYKŁADZIE DOGO- I FELINOTERAPII

Abstract: Pet assisted activities are addressed to healthy people and to those with various developmental, psychological, physical and emotional disorders. Most often pet assisted therapy is used to support treatment of children suffering from cerebral palsy, motricity problems, limb paralysis, muscle atrophy, autism, attention deficit hyperactivity disorders and attention deficit disorders (ADHD and ADD), emotional instability and mental retardation.

This paper is aimed at describing the role the animals played in history and still play in the life of contemporary people and at presenting therapeutic programmes that support treatment of various illnesses in children and adults.

Keywords: pet therapy, dog therapy, felinotherapy

Human being has a need of contact with other members of the group. This is the so-called need for the membership of a definite social group. Social meetings with close people fulfil also the need for security [1, 2]. When social ties started to loosen with time, people began to look for substitutes. Pets being the faithful creatures, able to love, capable of manifesting obedience and gratitude to their master started to play such a role. In many cases they are hold up as a model of discipline, self-control, politeness

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and nobleness for young generation teaching them the ability of living in a group and establishing friendly contacts with its members.

Care of animals helps also building a sense of self-esteem. It is often so, that our low self-appreciation discourages us from undertaking various actions, makes us feel useless and helpless and sometimes gives rise to aggression towards other persons whom we blame for our failures. That is why the faith in man's own abilities and relatively high self-esteem triggers positive emotions which in turn motivate to better functioning and undertaking new challenges. Care of animals may largely increase self-esteem in children and positively change self-perceivability in adults [3, 4]. Based on observations of children's relations with animals one may predict with a high probability what kind of people they will be in the future. Studies performed in American penitentiaries showed a close relationship between harassing animals in the childhood and "dissocial personality disorders". Prisoners who demonstrated not normal attitude to animals being young were more often arrogant, ruthless, aggressive and even cruel towards other people in the adulthood [5, 6]. It was also noticed that the ideal animal for a family with little child was a large, over 3-year-old gentle bitch with fluffy hair which had already lived with the family before [3].

Sum of these experiences changed the current role of pets which began to be used in supporting rehabilitation processes in people or allowed disabled persons to function better in the society, to activate them and to increase their sense of self-esteem. Such method of supporting rehabilitation of disabled persons which is adapted to specific needs of a given person we call zootherapy [7]. Depending on the group of animals involved one may distinguish canine therapy and feline therapy.

Animal therapy is a new "natural and medical science whose aim is to study the use of animals in solving human problems" [8] which was divided into the following categories:

1. AAA – Animal Assisted Activities which are a form of occupation with an animal oriented to mobilisation of patient's physical activity. Such meetings motivate, relax or help in education of patients with various disorders. AAA is carried out in various places – in nursing homes, medical institutions and private apartments – mainly by volunteers.

The method is specific in that volunteers and therapists do not keep notes, the programme must not closely follow the established plan. Time and proceeding of the visit is not precisely defined and depends on many factors like *eg* predispositions of the patient and the animal. Animal assisted activities may assume various forms. They often consist in a visit of volunteers group paid once a month to residents of a nursing home together with accompanying animals. Meetings take place in a large group under the supervision of the workers of such institution. Another form of such activity is the exercises performed by dog trainers in the young offenders' home [8–11].

2. AAT – Animal Assisted Therapy is the therapeutic programme more precise than the AAA. It has strictly defined goal and animal is an inherent element of treatment supporting process. Aims and occupations are strictly defined for each participant and progress is documented in this therapy. The therapy is led by properly trained specialists like medicine doctors, rehabilitants (physiotherapists) [7, 10, 11].

Advantages and goals of this therapy were divided into psycho-motoric, emotional, social and motivative. Psycho-motoric functions are associated with growth. The main focus is put on motoric and cognitive functions and on mobilisation of short- and long-term memory. Emotional and social behaviours affect the expression of needs and feelings, increase the self-esteem, decrease the sense of fear and loneliness and develop the capability of group communication. Motivative functions of the therapy should first of all increase the interest in group activities, teach cooperation with other group members, develop the interactions with carers and enhance motivation for exercises [8, 9, 12, 13].

There is one more form of animal therapy in the world. It is animal assisted education (AAE). In Poland it does not exist as a separate programme but some of its elements are being included into AAA or AAT. Exercises in animal therapy are directed to healthy people and to people with some developmental, psychic, physical or emotional disorders. Most often animal therapy is used to support treatment in children suffering from cerebral palsy, motricity problems, limb paresis, muscle atrophy, autism, attention deficit hyperactivity disorders and attention deficit disorders (ADHD and ADD), emotional instability and mental retardation[2, 12–14].

One of the most common methods of supporting human treatment and rehabilitation is the therapy with dog assistance named dog therapy. Pet therapy being English equivalent of dog therapy was first applied in 1792 in England in patients of psychiatric wards where traditional methods were replaced with a contact with animals. Taking care over animals the patients had to learn self-control. The experiment brought a great success which resulted in introducing this innovative method to other hospitals [1, 7, 8].

The term “dog therapy” first appeared in Poland in 1996 during the press conference in Warsaw Animal Fair organised on the occasion of “Animal Day”. The term was used by a writer and journalist Maria Czerwinska [15]. She was one of the first to notice the calming effect dogs exerted of blind children during works on a film set in the 1980s. Czerwinska soon became the president of Polish foundation CZE-NE-KA dealing with the natural method of supporting treatment and rehabilitation. In the year 2002 she organised a training course of dog therapy with the participation of 20 persons from all over the country. Three years later there were several similar organisations in Poland including *ia* ALTERI – Association for Supporting Therapy in Krakow, AMA CANEM – Foundation for Disabled Persons in Lodz, CZE-NE-KA – Foundation of the Human and Animal Friendship in Warszawa and DOGTOR – a foundation in Gdynia with branches in Warszawa and Kozminek [15,16].

Most important objective of the contact therapy is to provide patient with conditions, under which he/she will feel safe and will accept him/herself [17].

Four-legged therapist fully accepts clumsy motions of a disabled patient and patiently bears various treatments it is subjected to. It tolerates rapid reactions and noisy behaviour of a child, who may feel satisfied with performed exercise and is eager to undertake next challenges. Well done task and friendly and jolly four-legged therapist motivate little patients to make various works without help which favours acquiring self-reliance by them [17].

It is important that the dog therapy programme be adopted to developmental possibilities and the type of disorder in the patient. This way the improvement of disabled patient's functions proceeds faster.

Characteristics of dog therapy

The following dog races are now trained in Poland for the dog therapy: Labrador retriever, golden retriever, samoyed, Syberian husky, malamute, collie, Alsatian sheepdog and cross-breeds of these races of specific features [1, 8, 18]. The first stage of training the dog therapist is the course for the 1st degree Accompanying Dog (PT1). Decision on having a dog for therapy must be responsible since its training is a multi-stage and multi-aspect process that lasts since the puppy period till the animal's "retirement". One has always to remind the dog what it has learned before and to introduce new elements. Dogs have to learn basic commands like "sit" and "give a paw" (which is not included in PT1 training), to lay motionless and to work in the company of other dogs. To be able to cooperate, dogs should at least once a week meet for common play and learning [3, 4, 9, 17]. One has to remember that a dog may work up to 2 hours a day with a 20 minutes break during training. After work the dog should have a long walk [17]. When dogs work in a group, they need to have time (c. 15 minutes) to welcome each other. Carer should always have water and dogs' favourite titbits which would not make harm to children participating in training. All this contributes to better working conditions for an animal which motivated by good food and caresses eagerly returns to people and places with which it will have positive associations [17].

Dog therapy activities affect mental, emotional, social and physical zone of man. Constant element of these activities is stroking, cuddling and feeding the dog during plays which are prepared individually for each patient. An important element that should not be omitted is relaxation which may consist in laying patient by a dog and listening to calm music or a story [2, 9].

Two methods are used when carrying occupations with autistic children. The first is a "free contact' during which a child is not forced to close contact with an animal. In this case the trainer plays retrieving or jumping with a dog and encourages the child to play with. Occupations last c. 45 minutes and their aim is to open the child to world and to break its fear from the contact with the dog. This approach has to be the first step for future cooperation with therapist – psychologist [2]. The second methods relies on traditional rehabilitation exercises together with a dog and persons close to the child. Contact therapy gives autistic children many positive impressions. After getting used to and getting rid of fear of animals, children gained confidence and were able to relax in animals' company thanks to which they easier responded to therapists' orders.

The next group in which dog therapy brings visible effects is the children with the Down's syndrome. Basic task of dog therapy is to break fear of animals in children and to initiate contact with them. The therapy releases in such children a greater physical activity and independence, enlarges vocabulary, affects concentration and develops all senses in a little child [3, 5]. The animals have to fulfil some conditions and must possess the features like: absolute obedience, intelligence, calmness and protectiveness.

The races which possess such features and have appropriate body building are Labrador retrievers, Alsatian sheepdogs, Australian sheepdogs, Scottish collie and sometimes more calm Dobermann. Well trained dogs, apart from responding to basic commands (lifting the dropped items, opening and closing door), are able to react to c. 80 orders given under different conditions by different people. This is a result of long systematic training supervised by instructors [18].

The next group of dogs assisting people is the animals of rather specific abilities which warn people against near epileptic fit. They were discovered in the 1980s and now the organisation "Support Dogs" performs observations and audio-visual records of dog's behaviour before epileptic fits in their charges. In the Institute of Epilepsy in the USA the EEG records are used to check how a dog knows of the coming fit. It appears that individual dog's features like its concentration on a person and sensitivity to human behaviour and smell count most. Dogs are able to recognise the coming attack based on very subtle changes in human behaviour or smell. Practically all races fit for this type of work but the best are golden retrievers, setters, samoyeds, border collie and mongrels [1, 9, 18].

The use of dogs' or cats' smell becomes still more important in the prophylaxis of cancer diseases. The animals with a keen nose are able to find pathological changes in the skin or in deeper situated organs of human body. Studies on this very useful animal feature are carried out in the Institute of Genetics and Animal Breeding PAS in Jastrzębiec, Poland. Professor Tadeusz Jezierski makes tests on the recognition of lung, breast and skin cancers in which "his" dogs obtain 60–97 % accuracy in the identification of samples taken from a group of both healthy and ill persons [19].

Feline therapy

The name of the youngest branch of animal therapy is a combination of two words: Latin felis (cat) and Greek therapeia (care, treatment). Feline therapy usually assumes two forms: when a cat lives in a given institution and its presence affects patients or when it is brought by its carer (most often a volunteer) to weekly meetings with residents of the nursing home 1, 7].

Feline therapy is a form of contact therapy based on similar principles as dog therapy but applied to people who are afraid of dogs or have an allergy to their coat. Despite its independent nature, cat often becomes a catalyser of social ties since it provides the subject of a conversation. As a rule it is an animal of even-tempered character and pleasant to the touch which makes children like to play with, talk to and confide in cat. Such a company develops empathy and care over a small and helpless kitty sensitizes children to the needs of the others and teaches responsibility and gentleness. This is particularly important for children with emotional disorders [3, 7, 11].

Cat may substitute other persons for elderly and lonely people, may divert their attention from suffering and loneliness and initiate contacts with the surrounding. Feline therapy is efficient in supporting the treatment of such diseases as: arthritis, Alzheimer's disease, muscle atrophy, multiple sclerosis, eyesight and hearing disorders, cardiovascular diseases or depression [2, 7, 9, 14].

Now, feline therapy is applied in more and more medical institutions all over Europe. In Great Britain and the United States feline therapy has become popular for the last several years. It was noted that stroking a cat has a soothing effect on the psyche of the diseased and moreover, it reduces stress and relaxes [8, 10].

In Poland such a supporting therapy is in the initial phase and functions in a few institutions. One of them is a school for disabled children in Toruń where occupations with a cat-therapist Gucio have taken place for 3 years.

Every programme of animal therapy is based on cooperation between a person and properly trained animal. Training dog, cat or any other animal requires the engagement, patience and devotion from the owner. As in the case of dogs, cats have also their own individual features which, irrespective of race, age or sex, predestine them for or excludes them from working with people. Therefore, organisations dealing with animal assisted therapy such as “Pets and People” established some requirements which have to be fulfilled by a cat-therapist. Such a cat has to be more than one year old which allows for checking whether the cat is sociable and able to easily develop contacts with a group of people. Moreover, its immune system has to be stable enough to cope with possible infections by hospital microorganisms. It has to be familiar with various situations like the presence of dogs or other cats, noise and crowd which guarantees that it will not react with fear or escape in a new situation and that occupations with its participation will not pose a threat for a person. It should not be afraid of travelling in the public transport means. To be allowed to visit hospitals and other medical institutions the cat should be well-groomed and clean so it has to be accustomed to frequent hygienic treatments: to hair brushing, washing and cutting claws. It is also suggested to keep health certificate, to observe the terms of injections and worm controls and to pay regular visits to a vet.

Ragdoll is the race most often used in the therapy supporting the treatment of disabled children. The race was raised from cross-breeding of Burma cats with white Persian cats. The name reflects cats’ nature since “when taken on hands they loosen their muscles, get flabby and resemble a plush toy”.

Summary

Company of animals gives people a great psychological support. Observation of their exuberant nature is an amusement and makes people smile leading to relaxation and mitigation of pain in people suffering from various diseases. Visits of four-legged pets in nursing homes and hospitals are the impulse to make contacts with other patients and personnel and withdrawn persons even start to confide in volunteers. A person having close contact with non-evaluating and non-judging creature raises his/her self-esteem and physical and mental state. Such contacts improve general health status of patients with cardiovascular problems, decrease the rate of heart-beat and the survival of patients after myocardial infarction or heart transplantation is higher under such circumstances.

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TERAPEUTYCZNA ROLA ZWIERZĄT W ŻYCIU CZŁOWIEKA NA PRZYKŁADZIE DOGO- I FELINOTERAPII

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Abstrakt: Zajęcia z animaloterapii skierowane są do ludzi zdrowych oraz osób z różnymi zaburzeniami rozwojowymi, dotykającymi sfery psychicznej, fizycznej i emocjonalnej. Najczęściej jednak terapie zwierzęce stosuje się dla wspomagania leczenia dzieci chorych na porażenie mózgowe, zaburzenia rozwoju motorycznego, niedowład kończyn, zanik mięśni, autyzm, zespół nadpobudliwości psychoruchowej, deficyt uwagi (ADHD, ADD), niestabilność emocjonalna, opóźnienie umysłowe. Celem niniejszej pracy jest opisanie roli, jaką zwierzęta odegrały w historii oraz nadal odgrywają w życiu współcześnie żyjącego człowieka. Ponadto zaprezentowanie programów terapeutycznych z udziałem psów i kotów oraz metod wspomagania leczenia różnych jednostek chorobowych u dzieci i u ludzi starszych.

Słowa kluczowe: terapie zwierzęce, animaloterapia, dogoterapia, felinoterapia

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REGRESSION MODELING OF AGRRICULTURE GREENHOUSE GASES EMISSIONS IN POLAND

REGRESYJNE MODELOWANIE ROLNICZYCH EMISJI GAZÓW CIEPLARNIANYCH W POLSCE

Abstract: Agricultural greenhouse gases emissions are mainly produced in direct emissions from plant and animal production as well as those associated with land use changes. Studies attempt to describe the variables correlated with agricultural greenhouse gas emissions using linear regression. The analysis covered two groups of independent variables such as the main crops and livestock. The analysis included the last 20 years and variables were set using Pearson's linear correlation. The resulting model concerns 87.5 % of the variability of agricultural greenhouse gases emissions, by cattle, horses, and rye. The study was conducted using the statistical package R-Project.

Keywords: greenhouse gases, agriculture emissions, linear regression, modeling, R-Project, livestock production, crops

Introduction

Agriculture constitutes a serious share in gas emissions. The dynamic growth of human population causes an increase in food demand, which in turn enforces an intensification of agricultural production and the associated environmental degradation.

Agricultural greenhouse gases (GHG) emissions are mainly produced in direct emissions from plant and animal production as well as those associated with land use changes. It should be emphasized that a significant indirect share in other economic sectors of manufacturing products for agriculture is a necessity. A significant negative contribution involved processes associated with livestock production [1]. As indicated by estimates the share of livestock production may be from 50 to 80 % [1]. Intensive livestock production has always been an excessive burden on the environment. 3/4 of

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the world's agricultural land is devoted either to the production of animal feed or for grazing. A significant threat is currently observed, the displacement of the traditional method of livestock and small commercial farms by advanced industrial systems, which only leads to the intensification of rapid environmental degradation and an increase in hazardous pollutants. About half of manufactured meat is produced using factory farming methods of animals [2].

The sources of greenhouse gas emissions from agriculture in Poland include: enteric fermentation of animals (CH_4), animal manure (CH_4, N_2O), agricultural soils (N_2O), and the burning of plant residues (CH_4, N_2O). These gases represent a much larger global warming potential than emitted in the greatest amounts the CO_2. Their concentration is increasing rapidly. These gases have the ability to persist in the atmosphere for many years – up to 12 years for CH_4 and N_2O up to 120 years [3]. Besides that they are characterized by a considerably higher rate than the CO_2 of global warming GWP (*Global Warming Potential*) and absorbing the heat 23 times more intensely in the case of CH_4 and about 300 times for N_2O [4]. The biggest changes in emission reduction in Poland occurred in 1989–1993 when economic transformations were taking place [5].

N_2O emissions in Poland are predominantly in agriculture and in 2009, 75 thousand Mg (tons) of N_2O was emitted, of which approximately 77 % is accounted as from agricultural soils and about 13 % from livestock manure. In the case of CH_4 – 587 thousand Mg (tons) of the gas was emitted, and around 75 % came from enteric fermentation and 15 % from livestock manure. A fractional part is attributed to the burning of agricultural waste [6].

The size of CH_4 and N_2O emissions in recent years is presented in Fig. 1 and 2.

The necessity for modeling possible greenhouse gases emissions in relation to current and future crop and livestock production has become particularly important in recent years. A major challenge at the present time is to adjust agriculture to evolving climatic conditions and to support actions to reduce these changes. Studies on the impact of agricultural production on the environment, especially greenhouse gas emissions from this sector, is currently the issue undertaken by scientists and policy makers around the world [7–14].

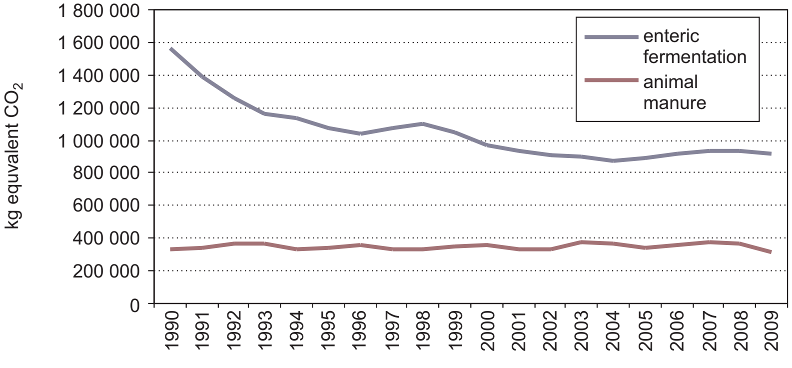

Fig. 1. CH_4 emissions from agriculture in the years 1990–2009 in CO_2 equivalent by major category

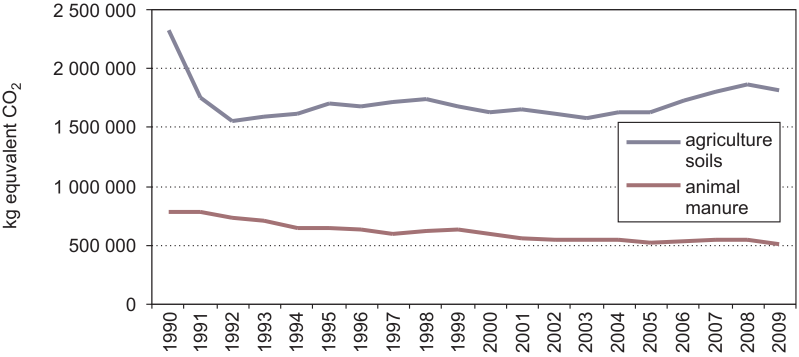

Fig. 2. N_2O emissions from agriculture in the years 1990–2009 in CO_2 equivalent by major category

Modeling is important both from a scientific perspective and practical, economic exploitation. In this context, efforts being made to bring additional information to this particular field of knowledge seem justified.

Only a comprehensive approach to land use and farming, including the principles of sustainable development, can bring a permanent solution to the problem of agricultural emissions.

Methodology and tools

This research attempts to describe the chosen factors responsible for agricultural GHG emissions (CH_4 and N_2O) using linear regression. Regression methods have been successfully used in similar studies which examined the influence of crops and husbandry and/or the participation of other factors connected with agricultural emissions [15]. The research was conducted at farm level. Testing covered, for example: the share of the green soil in the total area of agricultural land, livestock density, farmers education, the amount of nitrogen lodged with purchased fertilizers, the amount of nitrogen lodged with purchased feed, the amount of nitrogen lodged with purchased breeding animals [16] or the share of arable land in the area of farms, number of days of grazing, the livestock density [17].

There are a number of direct or indirect variables affecting the emissions. The study analyzes efforts to describe the impact of two important groups of independent variables such a major crops in Poland (wheat, barley, canola, rye, triticale, corn, oats, and use of mineral fertilizers) and livestock production (cattle, pigs, poultry, horses). The study used the Central Statistical Office [6], the Food and Agricultural Organization [18], the International Fertilizer Industry Association [19] and the United Nations Framework Convention on Climate Change [20] databases. The analysis included the last 20 years.

Correlations relative to the dependent variable (agricultural GHG emissions), were determined using Pearson's test. It is a commonly applicable linear correlation coefficient and is used in cases where both variables are measurable and have a distribution close to normal, and the relationship is linear. If the absolute value of the correlation coefficient is closer to 1, then the correlative relationship between the variables is stronger.

Linear regression analysis which was used in research assumes that between the input and output variables, there is a linear relationship. A straight line should reflect the best fit to the data set [21]. Most often the classical least squares method and its derivatives are used for this purpose. This method is the oldest and the easiest to implement [22]. The study was conducted using the statistical package R-Project.

Results and discussion

A sample result returned from the Pearson correlation test and generated by program R is given in Fig. 3. In case a), the p-value complies with the hypotheses and is lower than the established significance level, which is 0.05.

a)

```
cor.test(Cattle_Pcs,Agri.emission.GHG_Gg,method="pearson",
alternative = "greater")

        Pearson's product-moment correlation

data:  Cattle_Pcs and Agri.emission.GHG_Gg
t = 8.6262, df = 18, p-value = 4.122*10^-8
alternative hypothesis: true correlation is greater than 0
95 percent confidence interval:
 0.7854564 1.0000000
sample estimates:
      cor
0.8973402
```

b)

```
cor.test(Rapeseed,Agri.emission.GHG_Gg,method="pearson",
alternative = "greater")

        Pearson's product-moment correlation

data:  Rapeseed and Agri.emission.GHG_Gg
t = -0.3929, df = 18, p-value = 0.6505
alternative hypothesis: true correlation is greater than 0
95 percent confidence interval:
 -0.4553343  1.0000000
sample estimates:
        cor
-0.09221111
```

Fig. 3. The exemplary results of Pearson's test: a) fulfilling the assumptions, b) not fulfilling the assumptions, where: t – value of t statistics investigating the significance of the correlation coefficient, df – the size of the group, p-value – boundary of significance level

Table 1 presents the others linear correlation coefficients for the rest considered variables.

As follows from the p-value levels and correlations, the Pearson test showed positive correlations with the test variable for such variables like: cattle, horses, sugar beet, and oats. Other variables like poultry, fertilizer use, wheat, and rapeseed were not statistically significant and they have been omitted in the next step of studies. In the first

Table 1

Pearson test result for all variables

Variables	t	p-value	Correlations
Cattle	8.6262	$4.122 \cdot 10^{-8}$	0.897
Poultry	–1. 6888	0.9457	–0.37
Horses	4.8174	$6.903 \cdot 10^{-5}$	0.75
Pigs	2.2179	0.01983	0.463
Fertilizer use	–3. 0141	0.9963	–0.579
Pasture&meadows	1.7006	0.05311	0.372
Wheat	–0.1542	0.5604	–0.036
Barley	1.9099	0.03611	0.41
Triticale	–1.5996	0.9365	–0.353
Sugar beet	2.4844	0.01152	0.505
Oats	6.9028	$9.361 \cdot 10^{-7}$	0.852
Maize	–3.1109	0.997	–0.591
Rapeseed	–0.3929	0.6505	–0.092
Rye	2.3795	0.01430	0.489

approach a model for all variables satisfying the conditions was formulated. Some variables proved to be statistically insignificant in this regression model (Fig. 4a).

A rejection of statistically insignificant variables results in a higher coefficient of determination. In case 4b the Adjusted R-Squared takes into account the number of variables in the model, which is higher and amounts to 0.875.

The function lm (linear model) performs a linear model adjustment, appoint residua, adjusted coefficients of the model (where the Estimate – represents the assessment of the value of the regression coefficients, Std. Error – provides information about the standard error of this assessment, t-value is the value of statistics test for this factor and Pr(>|t|) – is a p value determined for the t-student test). Multiplate R-Squared coefficient of determination indicates a compatibility model fits to the actual data and a proportion of the total variability of the dependent variable is explained by the resulting model. The value of Adjusted R-Squared is usually smaller than the previous one. The estimated model can be written as:

$$Agricultural\ emission\ GHG = 1{,}740e{+}04{+}4{,}251e{-}03{*}cattle{-}6{,}911e{-}03 \\ {*}horses{-}2{,}598e{-}03{*}rye \tag{1}$$

The resulting model explains over 87.5 % of the variability of agricultural GHG emissions. The remaining 12.5 % can be explained by others variables not included in this study. The obtained results indicate the highest proportion of cattle. It is well known that, cattle-farming creates a substantial emission of mostly CH_4 and nitrogen compounds. The dominating source of emissions of ammonia NH_3 is livestock production [23]. Ruminant animals emit much more gases than those with single

a)

```
lm(Agri.emission.GHG_Gg~Cattle_Pcs+horses+pigs+pastures.meadows+Barley+
Sugar.beet+Oats+Rye_Ha,data=dane2)
summary(modelPP)

Call:
lm(formula = Agri.emission.GHG_Gg ~ Cattle_Pcs + horses + pigs +
pastures.meadows + Barley + Sugar.beet + Oats + Rye_Ha, data = dane2)

Residuals:
     Min       1Q   Median       3Q      Max
-2079.66  -868.08    46.62   748.24  2133.40

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)       2.113*10^-4 9.812*10^3 2.154  0.05427 .
Cattle_Pcs        4.397*10^-3 1.055*10^-3 4.169  0.00157 **
horses           -5.605*10^-3 4.873*10^-3 -1.150  0.27446
pigs             -2.304*10^-4 2.808*10^-4 -0.821  0.42931
pastures.meadows  6.165*10^-4 2.419*10^-3 0.255  0.80355
Barley           -1.971*10^-3 7.836*10^-3 -0.252  0.80603
Sugar.beet       -2.861*10^-3 1.307*10^-2 -0.219  0.83075
Oats             -5.096*10^-4 1.754*10^-2 -0.029  0.97734
Rye_Ha           -2.649*10^-3 2.846*10^-3 -0.931  0.37196
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1481 on 11 degrees of freedom
Multiple R-squared: 0.9055,     Adjusted R-squared: 0.8368
F-statistic: 13.18 on 8 and 11 DF,  p-value: 0.0001245
```

The following result was obtained after removing them from the model:

b)

```
modelPP<-
lm(Agri.emission.GHG_Gg~Cattle_Pcs+horses+Rye_Ha,data=dane2)
summary(modelPP)

Call:
lm(formula = Agri.emission.GHG_Gg ~ Cattle_Pcs + horses + Rye_Ha, data = dane2)

Residuals:
     Min       1Q   Median       3Q      Max
-2017.16  -748.16   -23.37   749.11  2505.53

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.740*104 2.648e+03  6.572 6.43*10-6 ***
Cattle_Pcs   4.251*10-3 6.191*10-4   6.867 3.79*10-6 ***
horses      -6.911*10-3    3.853*10-3  -1.794   0.0918 .
Rye_Ha      -2.598*10-3 1.083*10-3  -2.399   0.0290 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1296 on 16 degrees of freedom
Multiple R-squared: 0.8947,     Adjusted R-squared: 0.875
F-statistic: 45.32 on 3 and 16 DF,  p-value: 4.797*10-8
```

Fig. 4. The results of linear model

chamber stomach [24]. The largest amount of nitrogen excreted in manure comes from cattle in the first place, especially cows with high milk efficiency than from horses [23]. Estimates of greenhouse gas emissions in Poland are presented [25], and indicate that a high proportion of CH_4 emissions are from enteric fermentation of cattle – 79 %. The second source is anaerobic manure decay responsible for 10–15 % of the emissions [1]. The last one important factor in the model is the share of rye. Analysis of the rye crop in the last 20 years indicates that the beginning of the 90s was characterized by the highest areas cultivated in Poland. For instance the size of wheat areas in 1990 amounted to

2.28 million hectares and rye 2.31 million hectares [5]. Rye crop area over the last two decades has been declining. The share of this variable in the model can be justified by the highest CH_4 emission factor for this type of grain (Table 2) [26].

Table 2

CH_4 emission factor values for major crops in Poland

Grains	CH_4 emission factor in kg/TJ
Wheat	0.1816
Barley	0.1473
Maize	0.0367
Oats	0.1506
Rye	0.2004
Other grains	0.1618

Based on the modeling results the observed relationships between variables were presented. For this purpose, a scatterplot was prepared, which is a graphical representation of the correlation and facilitates visual assessment to determine the strength and type of relationships between variables. If the correlations of the test points are grouped along a hypothetical straight line – a regression line or a curve, this illustrates the existence of a relationship. When the increase of the independent feature value generates growth of the independent variable we can talk about the directly proportional connection. Otherwise, there is an inverse relationship. Using the scatterplot function it was possible to generate a graphical interpretation of the variables (Fig. 5).

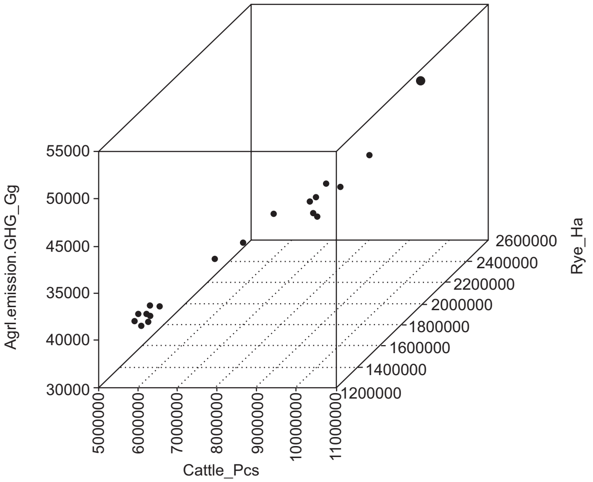

Fig. 5. Scatterplot 3D for GHG emissions vs cattle and rye

With the increase of both variables – cattle and rye, agricultural emissions increasing.

Conclusion

In order to properly shape environmental policy, its inventories and modeling the effects of efforts purposive to improve air quality, appear to be an adequate instrument of the assessment.

The variables provided by the Pearson test correlating with the dependent variable were used to build a linear regression model. Based on the coefficient of determination it can be concluded that the model explains 87.5 % of the variability of agricultural GHG emissions. The most important model variables are cattle and horse production, and also rye cultivation. The remaining 12.5 % can be attributed to the variables, which were not included in this study. The resulting form of the model appears to be justified because of high emissions, which is a breeding of these two groups of animals. Participation in the model of rye cultivation substantiated a high rate emission of CH_4 which is characteristic of this kind of grain.

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REGRESYJNE MODELOWANIE ROLNICZYCH EMISJI GAZÓW CIEPLARNIANYCH W POLSCE

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Abstrakt: Emisje rolnicze głównych gazów cieplarnianych to ważniejsze bezpośrednie emisje wytwarzane w produkcji roślinnej i zwierzęcej, jak również te związane ze zmianami w sposobie użytkowania gruntów. W badaniach podjęto próbę opisu zmiennych skorelowanych z rolniczymi emisjami gazów cieplarnianych z wykorzystaniem regresji liniowej. Do badań przyjęto dwie grupy zmiennych niezależnych, tj. główne uprawy oraz zwierzęta hodowlane. Analizie poddano okres ostatnich 20 lat. Korzystając z testu Pearsona, wyznaczono liniowe korelacje między zmiennymi. Otrzymany model w 87,5 % wyjaśnia zmienności rolniczych emisji GHG zmiennością udziału bydła, koni oraz żyta. Badania prowadzono z wykorzystaniem pakietu statystycznego R-Project.

Słowa kluczowe: gazy cieplarniane, rolnicze emisje, regresja liniowa, modelowanie, R-Project, produkcja zwierzęca, zboża

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EFFECT OF VISCOSITY CHANGES OF REACTION MIXTURE ON THE KINETICS OF FORMATION OF LINEAR LIVING POLYMER

WPŁYW WZROSTU LEPKOŚCI ŚRODOWISKA REAKCYJNEGO NA KINETYKĘ PROCESU POWSTAWANIA POLIMERU LINIOWEGO W POLIMERYZACJI ŻYJĄCEJ

Abstract: In the studies presented herein an attempt to include the impact of the increase in viscosity of the reaction mixture on the kinetics of living polymers formation was carried out and this effect should be included in the numerical calculations. The presented method of solving the equations system of mass balance allows a determination of values of the kinetic parameters of polymerization by introducing a correction factor and a partial reaction rate constant. This move enabled a simplification of the mathematical expressions describing the polymerization process and a possibility of connecting the kinetic parameters with the average molar mass of the system and thus – with the viscosity of the reaction mixture. The presented method enables studies on the polymerization processes in cases, when the rate of reaction does not depend on the viscosity increase or rate of reaction depends on the viscosity increase and on the average molecular mass.

Keywords: kinetics of polymerization, reaction rate constant, linear polymer, viscosity, living polymerization

Introduction

Mathematical modelling is a method of studying polymerization processes that is much cheaper than experimental studies. Therefore, mathematical models are often used both for analysis of the polymerization processes and in the design of chemical equipment. The bases of such a modelling are mass and heat balances. However, in the polymerization processes the heat balance is very often neglected because of the practically constant process temperature. So the mass balances of each component of the mixture have been derived to describe the polymerization process. The process of living polymerization has been taken into account in this paper.

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Results

The mathematical model presented in work allows a determination of the values of the kinetics parameters of polymerization reactions. We have assumed that only irreversible reactions of the $A + B \rightarrow C$ type [1, 2], rate was described by the second order kinetic equation of the form:

$$r_i = k_{AB} \cdot C_A \cdot C_B \tag{1}$$

have been taken into account in this paper.

We are interested in polymerization reactions in which the reaction rate depends on viscosity of the reaction mixture, *ie* increase of viscosity causes decrease of the reaction mixture [3, 4].

Mathematical models describing the polymerization processes are usually very complex because of a great number of unknown kinetic parameters. The most popular method of describing the polymerization processes bases on the assumption that the values of all reaction rate constants are identical [5–9]. This assumption makes possible to reduce the very complex set of balance equations to a rather simple form. Another simplification of the most frequent occurrence in the literature depends on the assumption that viscosity of the reaction mixture does not have any effect on the reaction rates.

Another way of solving the set of balance equations describing the polymerization process has been presented in this paper. We have got the significant simplification of the mathematical model by introducing in place of reaction rate constant k_{AB} the product of two partial reaction rate constants k_A and k_B [10–12]:

$$k_{AB} = k_A \cdot k_B \tag{2}$$

where k_A and k_B refer to the components A and B, respectively.

Although from a formal point of view the expressions k_{AB} and $k_A \cdot k_B$ means the same, the latter one has the form that is much more convenient for numerical calculations because it helps to present equation (1) in the form [13]:

$$r_{ij} = k_i \cdot kj \cdot C_i \cdot C_j \tag{3}$$

Let us consider the living polymerization process carried out in the batch type reactor. Assuming that all monomers B (activated monomers A*) have been created at the same time only the *propagation step* (PS) of the *living polymerization process* (LPP) have to be analysed. When the propagation step starts only monomer B and monomer A (inactive) are present in the reaction mixture, so the first reaction of the propagation step is of the form:

$$B + A \xrightarrow{k_B \cdot k_A} AB \tag{4}$$

The method of designating all the components of the reaction mixture with the corresponding partial reaction rate constants is presented in Table 1.

Table 1

Components of reaction mixture with the corresponding partial reaction rate constants numbers of monomers in polymer chains

Components of reaction mixture	Partial reaction rate constants	Numbers of monomers
$[A] = C_1$	k_1	1
$[B] = C_2$	k_2	1
$[AB] = C_3$	k_3	2
$[A_2B] = C_4$	k_4	3
$[A_3B] = C_5$	k_5	4
...	...	...
$[A_iB] = C_{i+2}$	k_{i+2}	$i + 1$
...	...	...

According to Table 1 the living polymerization process has been described by the following system of the stoichiometric equations:

$$\begin{aligned}
&B + A \xrightarrow{k_2 \cdot k_1} AB \\
&AB + A \xrightarrow{k_3 \cdot k_1} A_2B \\
&A_2B + A \xrightarrow{k_4 \cdot k_1} A_3B \\
&A_3B + A \xrightarrow{k_5 \cdot k_1} A_4B \\
&A_4B + A \xrightarrow{k_6 \cdot k_1} A_5B \\
&\dots \\
&A_{n-3}B + A \xrightarrow{k_{n-1} \cdot k_1} A_{n-2}B \\
&A_{n-2}B + A \xrightarrow{k_n \cdot k_1} A_{n-1}B \\
&\dots
\end{aligned} \tag{5}$$

where: k_j – partial reaction rate constant of component j.

Using the equation (5) and remembering about earlier assumptions we have established the rate of reaction for all components of the process in the following form:

$$\frac{d[B]}{dt} \equiv \frac{dC_2}{dt} = -k_1C_1k_2C_2 \tag{6}$$

$$\frac{dC_1}{dt} = -k_1C_1\sum_{i=2}^{n} k_iC_i \tag{7}$$

$$\frac{dC_3}{dt} = -k_1 C_1 (k_2 C_2 - k_3 C_3) \tag{8}$$

$$\frac{dC_4}{dt} = -k_1 C_1 (k_3 C_3 - k_4 C_4) \tag{9}$$

...

$$\frac{dC_{n-1}}{dt} = -k_1 C_1 (k_{n-2} C_{n-2} - k_{n-1} C_{n-1}) \tag{10}$$

...

And the general equation for *m*-component was described using the following expression:

$$\frac{dC_m}{dt} = -k_1 C_1 (k_{m-1} C_{m-1} - k_m C_m) \quad \text{for } 2 < m \tag{11}$$

Using the formulas (5–10) for particular odd fractions, the general mathematical models of analyzed polymerizations process for odd fractions has been introduced:

$$\begin{cases} \dfrac{dC_1}{dt} = -k_1 C_1 \sum\limits_{i=2}^{n} k_i C_i \\ \dfrac{dC_2}{dt} = k_1 C_1 k_2 C_2 \\ \dfrac{dC_m}{dt} = k_2 C_2 (k_{m-1} C_{m-1} - k_m C_m) \end{cases} \qquad (m = 3, 4, ..., n) \tag{12}$$

Bearing in mind the dependencies describing viscosity changes with the progress of polymerization, expressing viscosity as a function of the average molar mass M_n of the reaction mixture, we included an impact of viscosity increase on the rate of polymerization by introducing a correction factor Z_i.

This factor allows expressing a partial reaction rate constant as a function of the average molar mass of the reaction mixture:

$$k_i = k_1 \cdot Z_i(M_n) \tag{13}$$

Correction factor Z_i is a function of the average molar mass of the reaction mixture M_n. To determine $Z_i(M_n)$ function we used the simplest form of correction factor Z_i:

$$Z_i(M_n) = \gamma \cdot \left(\frac{M_n}{M_0} \right)^{-p} \tag{14}$$

where: p – a parameter,
γ – a constant factor characterized for the polymerization process;
M_0 – the molar mass of the monomer.

The range of the linear increase of viscosity as a function of number average molecular weight, has been described by the following equation [1]:

$$\eta = \text{const} \cdot M_n \tag{15}$$

Equations (13), (14) and (15) allow expression of viscosity as a function of kinetic parameters k_i:

$$\eta = \text{const} \cdot M_0 \left(\frac{k_1 \cdot \gamma}{k_i} \right)^{\frac{1}{p}} \tag{16}$$

and then:

$$k_i = \text{const} \cdot k_1 \gamma \left(\frac{\eta}{M_0} \right)^{-p} \tag{17}$$

The above equation makes it possible for both simplification of the mathematical expressions describing the polymerization process and connecting the kinetic parameters k_i with the average molar mass of the system M_n, and thus – with the viscosity of the reaction mixture. Estimation of kinetic parameters k_i based on experimental results given in the literature and depended on minimization the objective function of the form:

$$S = \sum_{i=1}^{b} (O_i - E_i)^2 \to \min \tag{18}$$

where b is a number of empirical data, E_i is the empirical value, O_i is the calculated value.

We have done numerical calculations for living polymerization of 2-phenyl-2--oxazoline including increase of viscosity followed by the increase of the average molar mass in each calculation step.

The ring-opening polymerization of 2-oxazolines in acetonitrile was investigated under pressure conditions with conventional heating. The investigated polymerizations were performed at 120 °C and 140 °C in acetonitrile under 1.5 MPa external nitrogen pressure with methyl tosylate as initiator as depicted in Fig. 1. When all monomer is consumed, a second monomer can be added to synthesize block copolymers or a

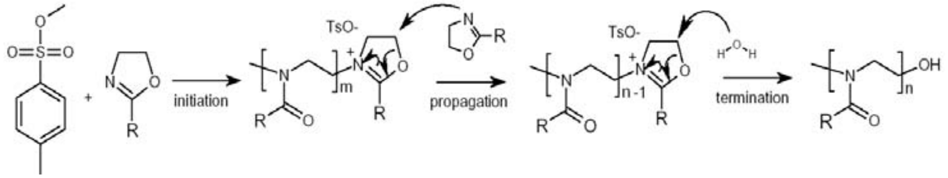

Fig. 1. Reaction mechanism for the cationic ring-opening polymerization of 2-oxazolines utilizing methyl tosylate as initiator

nucleophile can be added to introduce a functional end-group (Fig. 1 depicts the addition of water as terminating agent).

Two reactors were filled with 2-phenyl-2oxazoline (1.1 g, 7.5 mmol) and 1.4 cm^3 of a methyl tosylate as initiator stock solution (0.091 M; 0.41 g methyl tosylate in 19 g acetonitrile), resulting in 2.5 cm^3 reaction mixtures with 3 M 2-phenyl-2oxazoline (45 w%) and a monomer to initiator ratio of 60. After applying a pressure of 15 bar to the reactors, the polymerization mixtures were heated to 120 ^{o}C or 140 ^{o}C for a predefined time. Subsequently, the reactors were cooled to 60 ^{o}C, the pressure was released and the polymerizations were finished with water (100 cm^3). This procedure

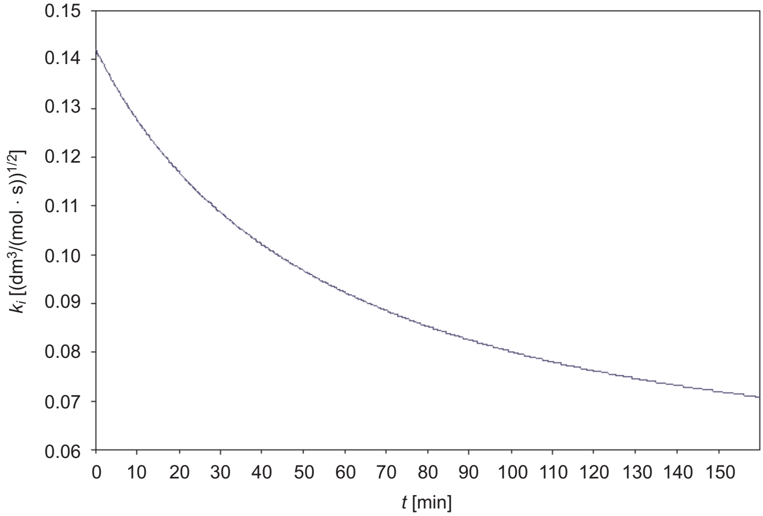

Fig. 2. Dependence of the reaction rate constants k_i [(dm^3/(mol · s))$^{1/2}$] in time t [min] during living polymerization process of 2-phenyl-2oxazoline at 120 ^{o}C calculated according model (12)

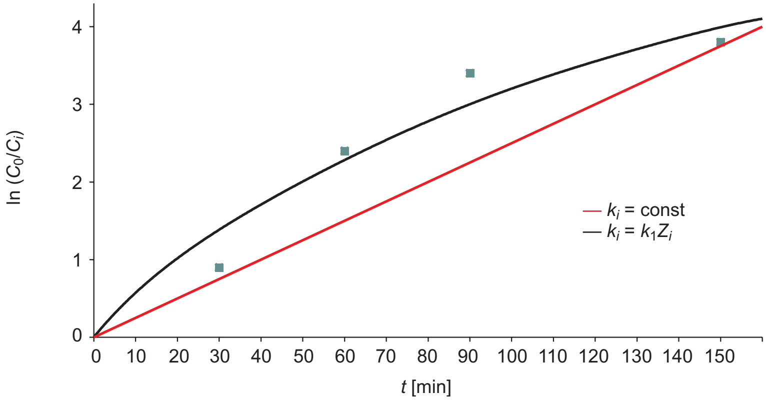

Fig. 3. Dependence of the ln(C_0/C_i) in time t [min] during living polymerization process of 2-phenyl-2-oxazoline at 120 ^{o}C: (–) theoretical line, when k = const; (–) calculations using model (12), when k_i = k_1Z_i; (■) experimental data [14]

was repeated for four different reaction times (120 °C: 15, 25, 95 and 155 min; 140 °C: 15, 25, 35 and 50 min) after which all mixtures were characterized by ^{1}H NMR and GPC. As a consequence, the first quenched polymerization mixtures were heated three additional times to 120 °C before analysis [14].

On the basis of the experimental results available in [14] and own calculations, we have determined the values of both kinetic parameters (Fig. 2) and dependence $\ln(C_0/C_i)$ at 120 °C, which is shown in Fig. 3.

Estimation of kinetic parameters k_i and equation (17) allowed for the calculation of the objective function *S*, which is presented in Table 2 and Table 3.

Table 2

Comparison of the calculated values of $\ln(C_0/C_i)$ with those obtained from experimental data

t [min]	Polymerization of 2-oxazoline at 120 °C $\ln(C_0/C_i)$	
	Literature data k_i = const	The calculated values according model (12) $k_i = k_1Z_\mathrm{i}$
30	0.90	1.377
55	2.25	2.146
87	3.35	2.898
155	3.90	4.092
	$S_c = 1.912$	$S_\eta = 0.480$

Table 3

Comparison of the calculated values of $\ln(C_0/C_i)$ with those obtained from experimental data

t [min]	Polymerization of 2-oxazoline at 140 °C $\ln(C_0/C_i)$	
	Literature data k_i = const	The calculated values according model (12) $k_i = k_1Z_\mathrm{i}$
15	1.8	2.223
25	3.8	3.078
35	4.1	3.752
50	4.3	4.596
	$S_c = 2.956$	$S_\eta = 0.909$

On the next step we have determined the values of both kinetic parameters (Fig. 4) and dependence $\ln(C_0/C_i)$ in time during living polymerization process of 2-phenyl-2-oxazoline at 140 °C, which is shown in Fig. 5.

Estimation of kinetic parameters k_i and equation (17) allowed for the calculation of the correlating factor, which is presented in Table 4.

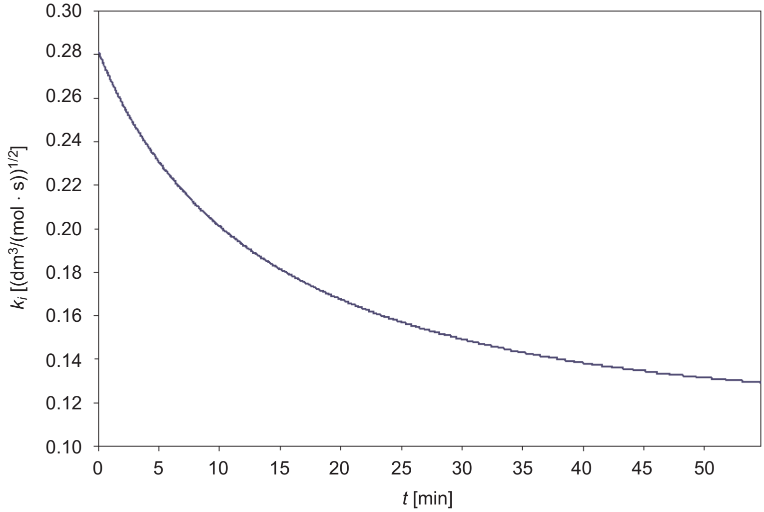

Fig. 4. Dependence of the reaction rate constants k_i [(dm^3/(mol · s))$^{1/2}$] in time t [min] during living polymerization process of 2-phenyl-2oxazoline at 140 °C calculated according model (12)

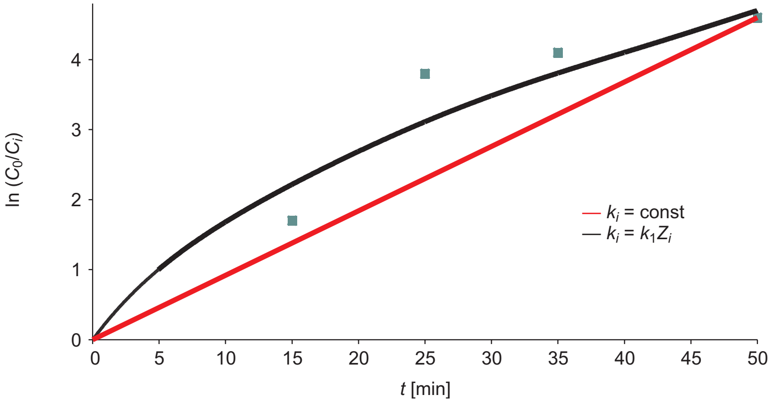

Fig. 5. Values of the ln(C_0/C_i) in time t [min] during living polymerization process of 2-phenyl-2-oxazoline at 140 °C: (–) theoretical line, when k = const; (–) calculations using model (12), when $k_i = k_1Z_i$; (■) experimental data [14]

Table 4

Value of the objective function (18) and of the correlating factor $\beta(\eta)$

Investigated process	S_c (k = const)	S_η ($k_i = k_1Z_i$)	$\beta(\eta)$
Polymerizations of 2-phenyl-2oxazoline in 120 °C	1.912	0.480	0.749
Polymerizations of 2-phenyl-2oxazoline in 140 °C	2.956	0.909	0.692

where $\beta(\eta)$ correlating factor, $\beta(\eta) \in (0,1)$, described by the equation:

$$\beta(\eta) = \frac{S_c - S_\eta}{S_c} \tag{19}$$

where: $\beta(\eta)$ – correlating factor, $\beta(\eta) \in (0,1)$,
S_c – minimum value of the objective function (18) when η = const,
S_η – minimum value of the objective function (18) when $\eta \neq$ const.

This shifts are respectively equal to 10 min for the propagation step at temperature 120 °C and 7 min for the propagation step at temperature 140 °C. The results are shown

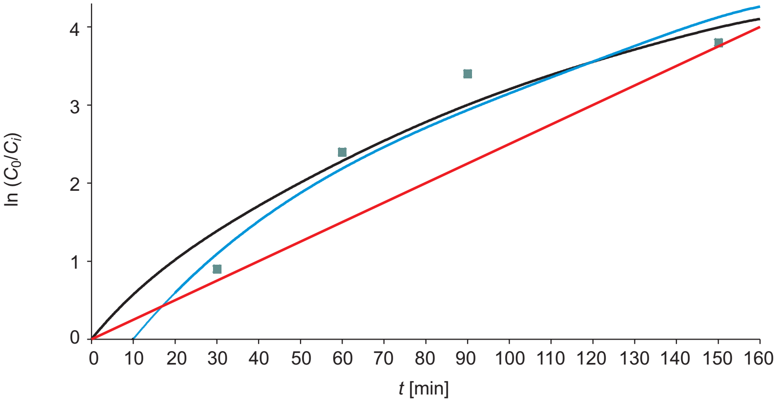

Fig. 6. Dependence of the $\ln(C_0/C_i)$ in time t [min] during living polymerization process of 2-phenyl-2--oxazoline at 120 °C: (–) theoretical line, when k = const; (–) calculations using model (12), when $k_i = k_1 Z_i$, $t_0 = 0$ min; (–) calculations using model (11), when $k_i = k_1 Z_i$, $t_0 = 10$ min; (■) experimental data [14]

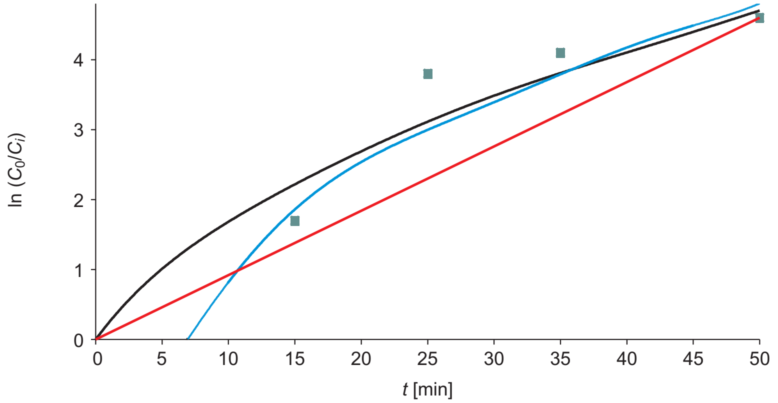

Fig. 7. Dependence of the $\ln(C_0/C_i)$ in time t [min] during living polymerization process of 2-phenyl-2--oxazoline at 140 °C: (–) theoretical line, when k = const; (–) calculations using model (12), when $k_i = k_1 Z_i$, $t_0 = 0$ min; (–) calculations using model (11), when $k_i = k_1 Z_i$, $t_0 = 7$ min; (■) experimental data [14]

in Fig. 6 and 7 and the Table 5. The delay of the start of the propagation step may be caused by the imperfection of mixing of the reaction mixture.

Table 5

Value of equation (18) and correlating factor β

Investigated process	S_c when k = const)	S_η (when $k_i = k_1 Z_i$)	$\beta(\eta)$
Polymerizations of 2-phenyl-2oxazoline at temperature 120 °C	1.912	0.480	0.749
Polymerizations of 2-phenyl-2oxazoline at temperature 120 °C – shift in time t_0 = 10 min	1.912	0.409	0.786
Polymerizations of 2-phenyl-2oxazoline at temperature 140 °C	2.956	0.909	0.692
Polymerizations of 2-phenyl-2oxazoline at temperature 140 °C – shift in time t_0 = 7 min	2.956	0.768	0.740

Summary

The method of solving the set of mass balance equations describing the propagation step of the living polymerization process has been presented in this paper.

Two cases have been taken into account:

– the reaction rate does not depend on viscosity changes of the reaction mixture,

– the reaction rate depends on viscosity changes and decreases with increasing viscosity of the reaction mixture

In this paper we have the significant simplification of the mathematical model of living polymerization process by introducing in place of the reaction rate constant k_{ij} the product of two partial reaction rate constants presented in form (2). Using partial reaction rate constants, the number of mass balance equations have been reduced to the number of components in the reaction mixture.

The introduction of partial reaction rate constant also makes it relatively easy to connect kinetics parameters k_i with average molar mass of polymer M_n and consequently with viscosity of reaction mixture η.

On the basis of the experimental results and own calculations, we have determined the values of kinetics parameters of living polymerization process and the influence of viscosity changes on rate of living polymerization process reaction by setting the correction factor Z_i described by the equation (14). In order to find the minimum value of the objective function (18) the simplex optimisation method has been used. As the result, the correlating factor $\beta(\eta)$ was calculated. Correlating factor $\beta(\eta)$ allowed to define experimental values of the degree of representation.

Each experimental case described theoretically in this paper is nearer to the reality when changes in viscosity have been taken into account.

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WPŁYW WZROSTU LEPKOŚCI ŚRODOWISKA REAKCYJNEGO NA KINETYKĘ PROCESU POWSTAWANIA POLIMERU LINIOWEGO W POLIMERYZACJI ŻYJĄCEJ

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Streszczenie: Praca prezentuje wpływ wzrostu lepkości mieszaniny reakcyjnej na kinetykę tworzenia polimeru liniowego w procesie polimeryzacji żyjącej 2-fenylo-2-oksazoliny. Efekt wzrostu lepkości mieszaniny reakcyjnej wskutek procesu polimeryzacji uwzględniono w obliczeniach numerycznych. W pracy przedstawiono metodę rozwiązywania układu równań bilansu masy procesu polimeryzacji żyjącej 2-fenylo-2-oksazoliny, pozwalającą na wyznaczenie parametrów kinetycznych procesu polimeryzacji. Możliwość wyznaczenia parametrów kinetycznych reakcji polimeryzacji osiągnięto przez wprowadzenie tzw. cząstkowej stałej szybkości reakcji. Wprowadzenie cząstkowej stałej szybkości reakcji pozwoliło na znaczne uproszczenie wyrażeń matematycznych opisujących proces polimeryzacji oraz dało możliwość powiązania parametrów kinetycznych ze średnią masą molową mieszaniny, a przez to z lepkością układu reakcyjnego. Przedstawiona metoda pozwala analizować proces polimeryzacji żyjącej polimerów liniowych, nie tylko gdy zmienia się lepkość mieszaniny reakcyjnej, ale również gdy lepkość jest stała lub gdy parametry kinetyczne nie zależą od lepkości, a zależą od mas molekularnych poszczególnych reagentów.

Słowa kluczowe: kinetyka polimeryzacji, stała szybkości reakcji, polimer liniowy, lepkość, polimeryzacja żyjąca

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Elwira TOMCZAK[1] and Dominika SZCZERKOWSKA

EFFECT OF COIR CHEMICAL MODIFICATION ON THEIR SORPTION OF SELECTED HEAVY METALS

WPŁYW MODYFIKACJI CHEMICZNEJ WŁÓKNA KOKOSOWEGO NA ZDOLNOŚĆ SORPCYJNĄ WYBRANYCH METALI CIĘŻKICH

Abstract: The paper presents the results of removal of heavy metals ions from aqueous solution by adsorption. Zn(II), Cd(II), Co(II), Cu(II) and Ni(II) were adsorbed on lignocellulosic sorbent, a natural polymer (concentration range: 20–50 mg/dm^3). Sorption capacity was determined for raw and chemically modified coir fibres. Fibre samples activated with 30 % H_2O_2 solution and 5 % NaOH solution were tested. Fibres modified with sodium hydroxide were found to have the highest sorption capacity. Samples treated with H_2O_2 were found the least effectiveness. The results showed that the time required to reach equilibrium for most of the metal ions was approximately 5 hours. The equilibrium sorption data for test ions were analyzed using three widely applied isotherms: Redlich–Peterson, Freundlich and Langmuir. Assessment of statistical parameters confirmed that a good fit to experimental data for most of cases was obtained. The test results confirmed that coir is an efficient sorbent for the removal of heavy metals ions from water and that it may be used as an alternative biosorbent for the treatment of contaminated water.

Keywords: coir, chemical modification, sorption equilibrium, heavy metal ions

Introduction

Regions dominated by industry with large mining, metallurgy and galvanizing plants are the main sources of heavy metals emission and environment contamination. Heavy metals are characterized by high toxicity and persistence in the environment due to the ability to change their chemical structure *ie* type of chemical bonds. Chemical bonds between certain metals result in environment exposure to a whole group of these elements: *eg* zinc ores often contain lead, copper and cadmium [1, 2].

Water is one of the prime elements responsible for life on earth so the biggest threat are compounds with good water solubility that easily pass through barriers such as cell walls, damaging them as they migrate. The amount of metals that may be dissolved in a solution depends on its reaction, presence of other substances, concentration of anions and organic and inorganic chelating ligands [3].

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Many authors stress that increasing level of heavy metals in different components of the environment is a global problem [4, 5]. At present it is difficult to define the risk quantitatively as there is no data concerning risk identification and assessment, and information on the impact of individual elements is fragmentary.

Application of cost-intensive methods of heavy metals separation has led to the search for available and renewable ion exchange materials. Novel studies are designed to investigate unconventional plant/natural lignocellulosic products and adsorbents such as: corncobs [6], banana fibers [7], scobs [8], chitosan [9] or buckwheat hull [10].

Materials and method

The aim of the study was to evaluate the process of heavy metals sorption onto fibrous coir as an alternative method of water treatment, allowing for the removal of harmful contaminants. The studies were performed using a raw and chemically modified sorbent. The results have been analyzed by most commonly used Langmuir, Freundlich and Redlich–Peterson isotherms.

Materials

Coir is one of the environment-friendly sorbents that is a by-product of coconut processing. Fibrous mass is extracted from the outer shell of a coconut (*Cocos nucifera*). The palm is grown in all tropical regions, but most of the fibers used in the industry have their origin in India and Sri Lanka. The plant is grown for food.

Coir is a hygroscopic material, highly resistant to abrasion, harsh weather conditions and salt water. It is made of fibrous bundles of 10–35 cm in length and 12–25 μm in diameter. The fiber is composed of cellulose (*ca* 28 %), hemicellulose polysaccharides (38 %) and lignin (35 %) that makes the material strong and stiff [11]. The fibers are able to complex and adsorb metals as a result of ion exchange and chemical processes, which makes them a cost effective adsorbent and allows for the use of waste materials obtained as a result of the plant material processing.

Raw coir was boiled at 90 ^{o}C and washed with distilled water to remove dirt. Then the fibers were cut to obtain 3–5 cm long fibers and modified chemically:

- K1 – fibers boiled at 90 ^{o}C,
- K2 – fibers boiled at 90 ^{o}C + 30 % H_2O_2 (t = 3 h, T = 25 ^{o}C),
- K3 – fibers boiled at 90 ^{o}C + 30 % H_2O_2 (t = 3 h, T = 90 ^{o}C),
- K4 – fibers boiled at 90 ^{o}C + 5 % NaOH (t = 3 h, T = 25 ^{o}C),

The obtained fibrous samples were washed with distilled water and dried until solid mass was obtained.

Experimental

Five batches of aqueous solutions were prepared of specific analyte concentration (10–50 mg/dm^3) and uniform composition ($ZnSO_4$, $CdSO_4$, $CoSO_4$, $CuSO_4$, $NiSO_4$) – Fluka, Germany. In a multicomponent solution concentration of each component of the mixture was the same, which means that each of the cations in the solution was concentrated *eg* 50 mg/dm^3. Samples of coir (5 g) were placed into conical flasks and 200 cm^3 of test solution was added (pH = 5). The mixture was then mechanically shaken on a water bath until adsorption equilibrium was achieved ($T = 25\ ^{o}$C). Heavy metals concentration was determined by IC (ICS-1000, IonPac AS5A, Dionex).

Mathematical description of equilibrium

Based on the initial C_0 [mg/dm^3] and current concentration C_t [mg/dm^3] in the analyzed systems, the sorption capacity of adsorbed ions was calculated using the following formula (1):

$$q = \frac{V}{m} \cdot (C_0 - C_t) \tag{1}$$

where: q – sorption capacity [mg/g],
C_0 – initial concentration of heavy metal ions [mg /dm^3],
C_t – concentration of heavy metal ions after time t [mg/dm^3],
m – mass of adsorbent [g],
V – solution volume [dm^3].

Langmuir, Freundlich and Redlich–Peterson classic sorption formulas were used to analyze the equilibrium experiment.

Adsorption isotherm was developed by Langmuir in 1916. It owes its popularity to a relatively simple form and satisfactory conformity of experimental and theoretical values.

$$\frac{q_e}{q_m} = \frac{K_L \cdot C_e}{1 + K_L \cdot C_e} \tag{2}$$

where: q_e – equilibrium ion concentration in the sorbent [mg/g d.m. coir],
C_e – equilibrium ion concentration in the solution [mg/dm^3],
K_L, q_m – Langmuir constans describing sorption equilibrium.

As stated in the monolayer adsorption theory, adsorption equilibrium in the solid-liquid system may be described by an empirical equation called Freundlich isotherm.

$$q_e = K_F \cdot C_e^n \tag{3}$$

K_F, n – Freundlich constans describing sorption equilibrium.

Redlich–Peterson model was developed as a result of compilation of Langmuir and Freundlich equations. The above relationships is in the following form:

$$q_e = \frac{K_{RP} \cdot C_e}{1 + B \cdot C_e^a} \tag{4}$$

K_{RP}, B, a – Redlich–Peterson constans describing sorption equilibrium.

Results and discussion

The results of experiments and calculations are presented in the Figures below. Figures 1 and 2 present the concentration profile change for Cu(II) and Cd(II) ions as a function of time. In both cases, best results of metal recovery were obtained for samples modified with 5 % NaOH (K4). Raw fibers (K1) had smaller sorption capacity. In the case of the two other modifications (K2 and K3) lower values of adsorbed ions were obtained, even compared with unmodified material.

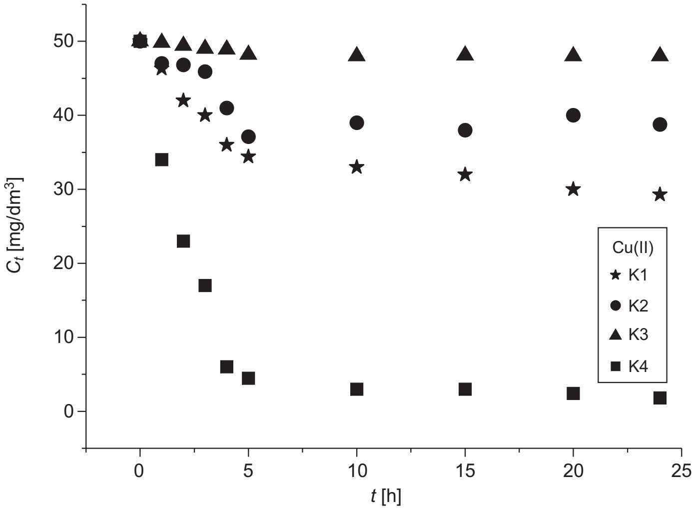

Fig. 1. Adsorption kinetics of Cu(II) onto coir K1, K2, K3, K4 (C_0 = 50 mg/dm^3)

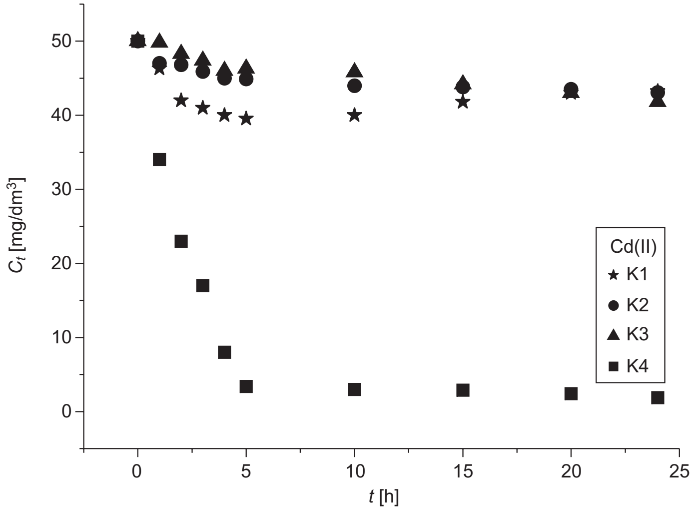

Fig. 2. Adsorption kinetics of Cd(II) onto coir K1, K2, K3, K4 (C_0 = 50 mg/dm^3)

This was probably due to too aggressive chemical modification with an oxidant (H_2O_2) and high temperature. A similar tendency was also observed for Zn(II), Ni(II) and Co(II) ions within the whole range of analyzed concentrations.

Therefore, further experiments were carried out for K1 and K4 fibers. K4 material showed a comparable adsorption capacity for all of the analyzed metals (q_{Cu} = 1.93 mg/g, q_{Ni} = 1.91 mg/g, q_{Zn} = 1.92 mg/g, q_{Co} = 1.89 mg/g, q_{Cd} = 1.93 mg/g).

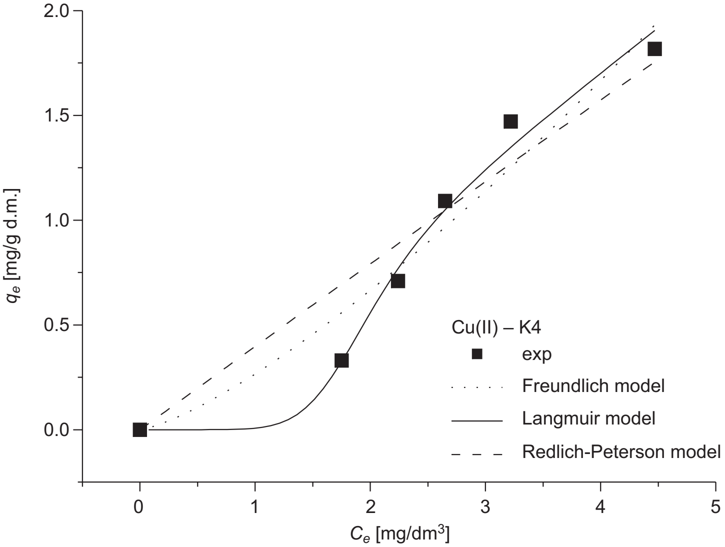

Fig. 3. Redlich–Peterson, Langmuir and Freundlich isotherms for Cu(II) sorption onto K4

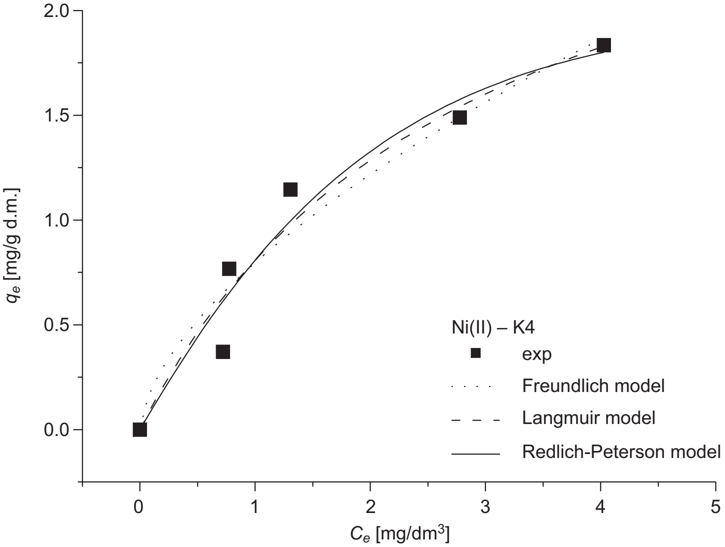

Fig. 4. Redlich–Peterson, Langmuir and Freundlich isotherms for Ni(II) sorption onto K4

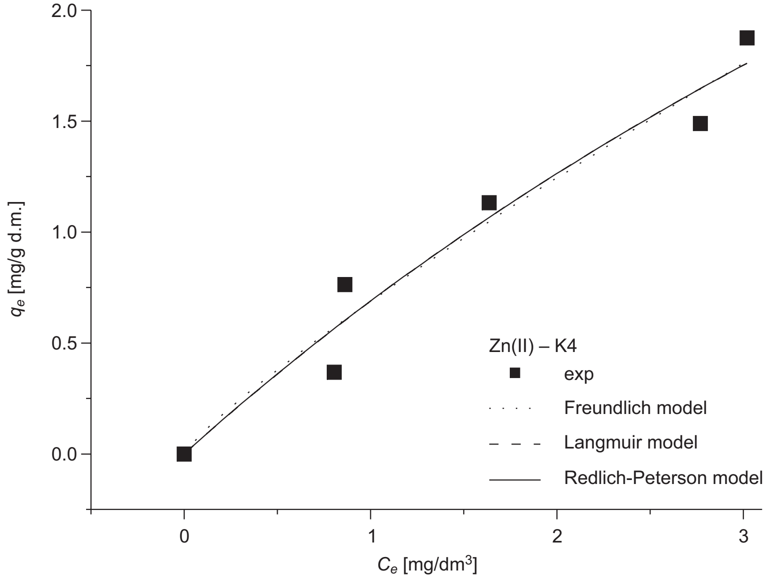

Fig. 5. Redlich–Peterson, Langmuir and Freundlich isotherms for Zn(II) sorption onto K4

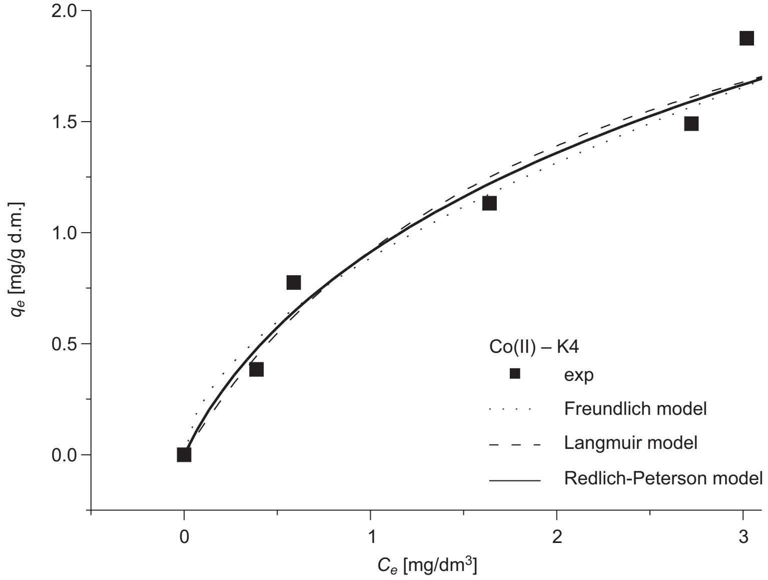

Fig. 6. Redlich–Peterson, Langmuir and Freundlich isotherms for Co(II) sorption onto K4

Characteristics parameters for the discussed models are presented in Table 1. The sorption isotherm models showed the square determination coefficient of $R^2 = 0.912–0.988$, which confirms that sorption models are well suited to analyze experimental data.

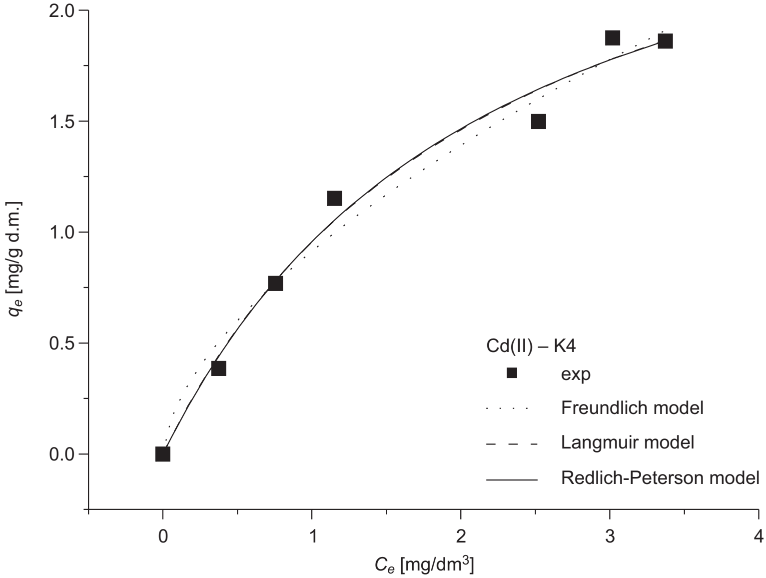

Fig. 7. Redlich–Peterson, Langmuir and Freundlich isotherms for Cd(II) sorption onto K4

As stated above, lower adsorption values were obtained with K1 material. Raw fibers had the highest sorption capacity toward copper ions (q_{Cu} = 1.38 mg/g) and showed the lowest affinity for cobalt (q_{Co} = 0.15 mg/g).

Table 1

Specification of Redlich–Peterson, Langmuir and Freundlich models for coir K4

Model	Parameter	Cu	Ni	Zn	Co	Cd
Freundlich	K_F [mg/g] [dm^3/mg]$^{1/n}$	0.266	0.796	0.687	0.886	0.914
	n	1.323	0.613	0.857	0.568	0.605
	R^2	0.944	0.945	0.956	0.969	0.981
Langmuir	q_m [mg/g]	117.6	3.14	7.683	2.857	3.109
	K_L [dm^3/mg]	0.003	0.347	0.099	0.474	0.444
	R^2	0.912	0.955	0.956	0.970	0.986
Redlich– –Peterson	K_{RP} [dm^3/g]	58.06	0.154	0.105	1.032	0.411
	a [dm^3/mg]$^{1/n}$	–6.78	1.405	0.969	0.743	1.036
	B	0.007	6.076	7.288	1.796	3.288
	R^2	0.988	0.957	0.956	0.972	0.986

Chemical modification with H_2O_2 (K2) significantly reduced sorption capacity toward all ions as shown in Figures 8 and 9. The application of hydrogen peroxide as a modifying factor resulted in significantly lower sorption capacity.

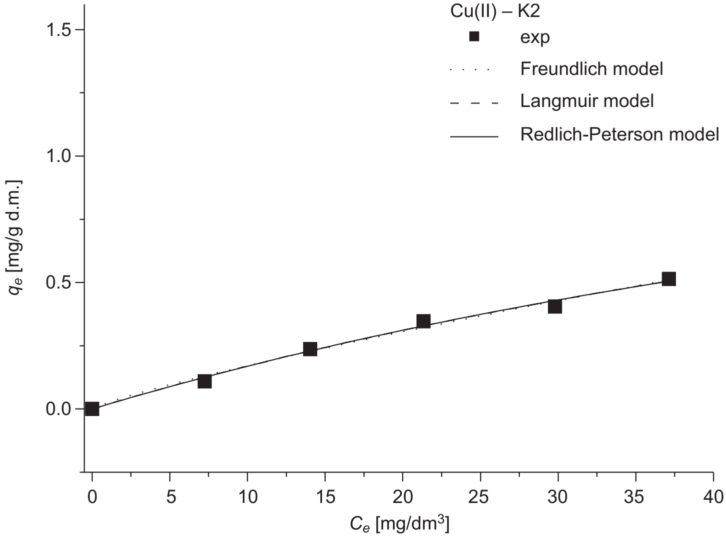

Fig. 8. Redlich–Peterson, Langmuir and Freundlich isotherms for Cu(II) sorption onto K2

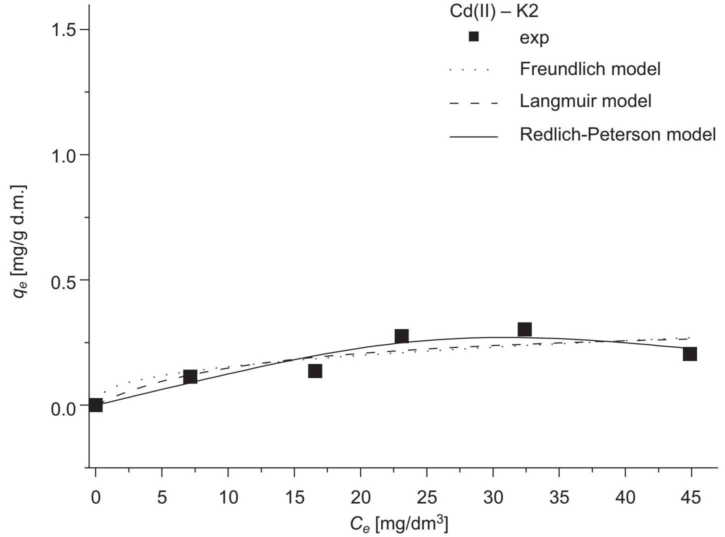

Fig. 9. Redlich–Peterson, Langmuir and Freundlich isotherms for Cd(II) sorption onto K2

Conclusions

1. Four methods of coir modification were tested. It was expected that modification with H_2O_2 and NaOH would improve sorption capacity. The results shown that only

modification with 5 % NaOH improved sorption capacity. Affinity for heavy metals sorption on K4 fiber was much higher than for other materials used in the experiment. Fibers modified with H_2O_2 had the lowest, and indeed very small, sorption capacity.

2. It was shown however, that accordingly modified coir is an effective material for the removal of heavy metals from aqueous solutions and it may serve as an alternative to more expensive, conventional adsorbents.

3. Sorption capacity of a given material depends on modification method. Additionally, it may be assumed that adsorbate location and transfer mechanism may be dependent on the number of mixture components *ie* ion competitiveness in binding to the active sites.

4. Langmuir, Freundlich and Redlich–Peterson models described the experimental data well within the whole range of analyzed concentrations.

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WPŁYW MODYFIKACJI CHEMICZNEJ WŁÓKNA KOKOSOWEGO NA ZDOLNOŚĆ SORPCYJNĄ WYBRANYCH METALI CIĘŻKICH

Wydział Inżynierii Procesowej i Ochrony Środowiska
Politechnika Łódzka

Abstrakt: W pracy zaprezentowano wyniki badań adsorpcyjnego usuwania jonów metali ciężkich z roztworu wodnego. Adsorpcja Zn(II), Cd(II), Co(II), Cu (II) oraz Ni (II) była prowadzona na roślinnym sorbencie – włóknach orzecha kokosowego (zakres stężeń 20–50 mg/dm^3). Określono wydajność sorpcyjną dla surowych i modyfikowanych chemicznie włókien. Włókna aktywowano 30 % roztworem H_2O_2 oraz 5 % roztworem NaOH. Najwyższą pojemność sorpcyjną wykazały włókna modyfikowane NaOH, zaś najmniejszą traktowane H_2O_2. Równowagę procesu adsorpcji dla większości jonów metali osiągnięto po około 5 h. Do opisu równowagi sorpcji badanych jonów przetestowano trzy równania: Redlicha–Petersona, Freundlicha i Langmuira.

Analiza parametrów oceny statystycznej potwierdziła, że we wszystkich przypadkach uzyskano dobre dopasowanie do danych eksperymentalnych. Uzyskane wyniki badań potwierdzają użyteczność włókna kokosowego w procesie usuwania metali ciężkich z wody oraz jego przydatność jako alternatywnego biosorbentu uzdatniającego wodę.

Słowa kluczowe: włókno kokosowe, modyfikacja chemiczna, równowaga sorpcyjna, metale ciężkie

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AQUATIC PLANTS – A SOURCE OF INFORMATION ABOUT SURFACE WATER POLLUTION WITH HEAVY METALS

ROŚLINY WODNE – ŹRÓDŁO INFORMACJI O ZANIECZYSZCZENIU WÓD POWIERZCHNIOWYCH METALAMI CIĘŻKIMI

Abstract: The objective of our studies was to evaluate the applicability of selected aquatic plants to water biomonitoring. The algae *Spirogyra* sp. and *Elodea canadensis* Michx. collected in the littoral zone of the "Lesna Przystan" bathing site in Glebocko (southern Poland) were used in the studies. The concentrations of Mn, Fe, Cu, Zn, Cd and Pb were determined in the biota by means of the Atomic Absorption Spectrometry (AAS). Based on the studies, the pollution of the bathing site water was preliminary assessed. Furthermore, possible correlations between concentrations of heavy metals in selected elements of the biota were suggested.

Keywords: *Spirogyra* sp., *Elodea Canadensis* Michx., heavy metals, biomonitoring

Some organisms that inhabit a given ecosystem can accumulate pollutants, such as heavy metals, in proportion to their concentration in the environment. Thus, the living matter becomes a convenient indicator that accumulates analytes polluting the environment. The samples obtained from these organisms are an example of integrated samples and determine their incorporation into the *Biological Early Warning System* – BEWS) [1].

Biomonitoring is gaining popularity as a water pollution assessment method. Aquatic plants, including algae [2] and Canadian pondweed (*Elodea Canadensis* Michx.) [3] – organisms that display measurable morphological changes in response to physical and chemical variations in the ecosystems they inhabit, are used in these studies. These organisms accumulate large amounts of pollutants, particularly heavy metals [4, 5].

The studies, conducted using the aquatic plants, reported in literature show that they are a practical tool to be successfully used for biomonitoring of surface water pollution [6–12] and in phytoremediation [13, 14].

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Sample freshwater ecosystem biomonitoring studies using algae include studies conducted in Poland (the Large Turawa Lake) [2], the Czech Republic (the Thaya River) [15], the Russian Federation (12 rivers of the Kola Peninsula, the Yenisei River in Siberia, Lake Baikal) [16–18], Scotland (a lake in the mountain range Lochnagar) [19], Greece (the Evros River Delta) [20], Argentina (Nahuel Huapi, Gutirrez and Moscardi Lakes in the Andes) [21], and Australia (4 rivers near Sydney: Hawkesbury, Parramatta, Cooks and Clyde) [22].

The biomonitoring studies using *Elodea canadensis* were conducted, *inter alia*, in Poland (the rivers that feed into the Oder River were monitored) [3], the Czech Republic (rivers that feed into the Morava River were monitored in the period between 1992 and 1994) [15], Moldova (the Dniester River pollution with mercury was assessed) [23] and in the New Zealand (the Taupo Volcanic Zone rivers and lakes were monitored) [24].

The results of these studies show that both, the algae and *Elodea canadensis* accumulate large amounts of heavy metals and are a good indicator to be used in the assessment of the surface water pollution with these analytes.

The objective of the studies, whose results are presented in this paper, apart from the preliminary biomonitoring of water of the "Lesna Przystan" bathing site in Glebocko, was to demonstrate correlations between the sorption properties of *Spirogyra* sp. and *Elodea canadensis* Michx.

Characteristics of the site

The "Lesna Przystan" bathing site in Glebocko, a former sand and gravel pit, is located in the south-western Poland (N 50°59′ N, 17°48′ E), Grodkow commune, Brzeg

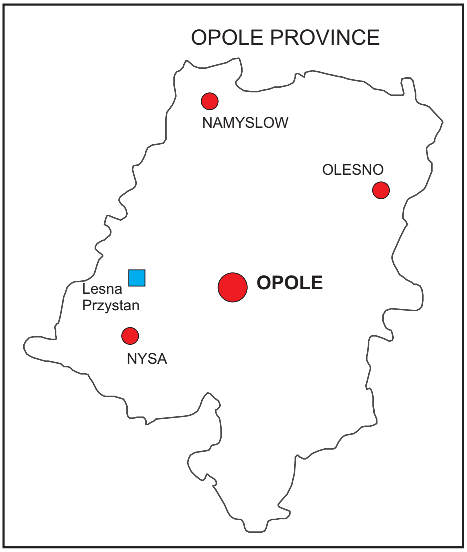

Fig. 1. Location of the "Lesna Przystan" bathing site

district, Opole province. The area of the main pond (reservoir I), where the bating site is located, is 40.37 ha. The area of the smaller pond (reservoir II), which is not used, is 8.59 ha.

The "Lesna Przystan" bathing site is a summer holiday destination for inhabitants of neighboring communes. The location of the "Lesna Przystan" bathing site is shown on the map in Fig. 1.

Sampling and sample preparation methods

The samples of the biota: algae *Spirogyra* sp. and *Elodea canadensis* Michx. were collected in July 2011, up to approximately 0.5 m from the lake shore, in the littoral zone of the reservoir [25]. The biota sampling sites are shown on the map in Fig. 2.

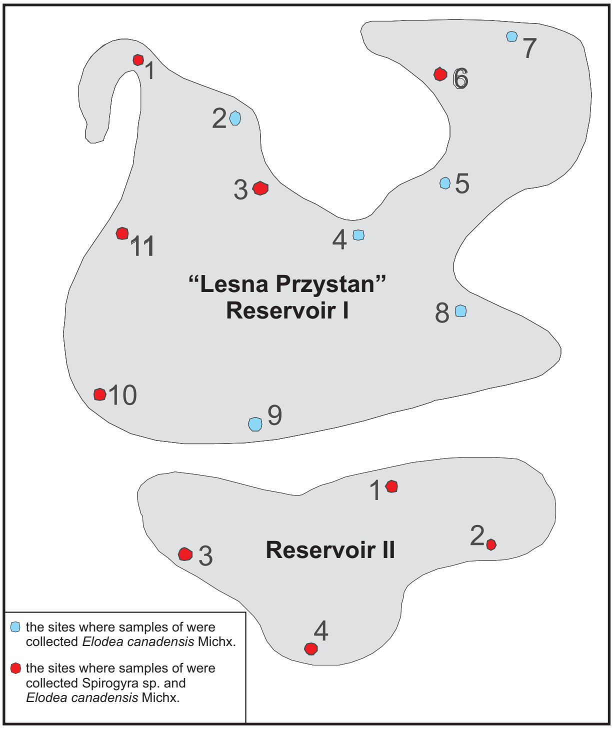

Fig. 2. The biota sampling sites

The samples for analyses were cleaned of mechanical impurities and dried at temperatures not exceeding 303 K. The 1.0 g biota samples were homogenized in an agate mortar and stored in appropriately labeled polyethylene containers.

Then, the 0.5 g portion of each sample was mineralized in the mixture of hydrochloric acid and nitric acid (3:1 v/v) using the CEM's MARS-X microwave system [26].

The conductivity and pH of water were determined at each biota sampling site by means of the CX-401 multifunction meter manufactured by Elmetron from Zabrze (Poland), whose absolute error was ΔpH = 0.01 and Δκ = 0.1 μS/cm, respectively.

Quality control

The determination of heavy metals in solutions after mineralization was carried out using an atomic absorption spectrometer UNICAM Model SOLAAR 969 (prod. Thermo Electron Corporation, USA). Table 1 shows the instrumental detection and quantification limits for heavy metals, characteristic for this spectrometer [27].

Table 1

The instrumental detection and quantification limits
for the atomic absorption spectrometer UNICAM-SOLAAR 969 [4]

Metal	Instrumental detection limit *IDL* [mg/dm^3]	Instrumental quantification limit *IQL* [mg/dm^3]
Mn	0.0016	0.029
Fe	0.0043	0.06
Cu	0.0045	0.041
Zn	0.0033	0.013
Cd	0.0028	0.032
Pb	0.013	0.10

The spectrometer was calibrated against a standard solution from ANALYTIKA Ltd. (Czech Republic). Table 2 shows the concentrations of metal elements determined with the spectrometer in the reference material (prod. Institute for Reference Materials and Measurements, Belgium) BCR 414 (plankton) and BCR 482 (lichen).

Table 2

Comparision of measured (AAS) and certified data
in the reference BCR 414 plankton and BCR 482 lichen

Element	AAS		BCR 414 plankton		*Dev.**
	Mean	±*SD*	Certified value	±Uncertainty	
	[mg/kg d.m.]				[%]
Mn	276	15	299	12	–7.7
Fe	1.74	0.21	1.85	0.19	–5.9
Cu	27.8	1.9	29.5	1.3	–5.8
Zn	103	4	112	3	–8.0
Cd	n.d.	n.d.	0.383	0.014	n.d.
Pb	3.7	0.3	3.97	0.19	–6.8

Table 2 contd.

Element	AAS		BCR 482 lichen		*Dev.**
	Mean	±*SD*	Certified value	±Uncertainty	
	[mg/kg d.m.]				[%]
Mn	31.2	0.8	33.0	0.5	–5.5
Fe	n.d.	n.d.	804	160	n.d.
Cu	6.54	0.18	7.03	0.19	–7.0
Zn	93.9	2.5	100.6	2.2	–6.7
Cd	0.52	0.04	0.56	0.02	–7.1
Pb	37.6	0.9	40.9	1.4	–7.6

* Deviation – the relative difference between measured and certified concentrations.

Biomonitoring study results

The studies involved samples of aquatic plants collected from 15 sites (Fig. 2) located along the shoreline of the reservoirs. The mean concentrations of heavy metals (Mn, Fe, Cu, Zn, Cd and Pb) determined in the biota samples are shown in Tables 3 and 4.

Table 3

Heavy metal concentrations in the biota samples collected from the "Lesna Przystan" bathing site – reservoir I [mg/kg d.m.]

Site no.	Mn	Fe	Cu	Zn	Cd	Pb
Spirogyra sp.						
1	967	1628	11.96	64.2	1.96	18.9
3	1729	1715	13.96	67.7	3.35	8.4
6	1493	1823	12.82	51.3	2.69	43.3
10	1194	1520	11.81	82.7	3.40	6.7
11	1207	1881	18.93	80.9	1.73	28.7
Average	1318	1713	13.90	69.3	2.63	21.1
Elodea canadensis Michx.						
1	805	1127	8.63	56.5	1.85	30.2
2	720	768	7.60	41.3	1.53	16.7
3	582	983	5.84	30.3	1.31	5.76
4	756	984	5.00	37.1	1.38	24.9
5	409	706	4.27	26.7	1.21	3.38
6	582	950	5.25	39.7	0.53	9.91
7	434	661	4.44	22.9	1.32	15.9
8	660	1171	6.85	40.9	0.66	9.59
9	468	509	5.10	33.2	1.28	7.75
10	443	296	4.70	23.7	0.83	5.35
11	587	1071	6.30	35.3	0.94	12.6
Average	586	839	5.81	35.2	1.17	12.9

Table 4

Heavy metal concentrations in the biota samples collected from the "Lesna Przystan" bathing site – reservoir II [mg/kg d.m.]

Site no.	Mn	Fe	Cu	Zn	Cd	Pb
Spirogyra sp.						
1	970	1669	5.12	30.9	1.45	41.3
2	985	801	2.02	23.8	1.79	16.5
3	1384	1853	6.57	26.4	5.04	20.1
4	2847	3886	8.71	64.8	16.84	23.5
Average	1546	2047	5.61	36.5	6.28	25.4
Elodea canadensis Michx.						
1	564	1034	7.22	85.1	0.66	30.8
2	517	923	7.99	28.6	0.51	8.20
3	391	393	0.91	7.06	1.22	3.87
4	549	1098	9.86	44.4	3.57	20.8
Average	505	862	6.49	41.3	1.49	15.9

Interpretation of the biomonitoring study results

Based on the biomonitoring studies conducted using *Spirogyra* sp. and *Elodea canadensis* Michx., the distribution of pollutants in the "Lesna Przystan" bathing site basin was preliminary assessed. The study results are shown on diagrams in Fig. 3–10.

Despite the small size of the reservoirs, there are differences in heavy metal concentrations in the plants that inhabit these reservoirs. The mean concentrations of metals accumulated in the Canadian pondweed and alga samples decrease as follows:

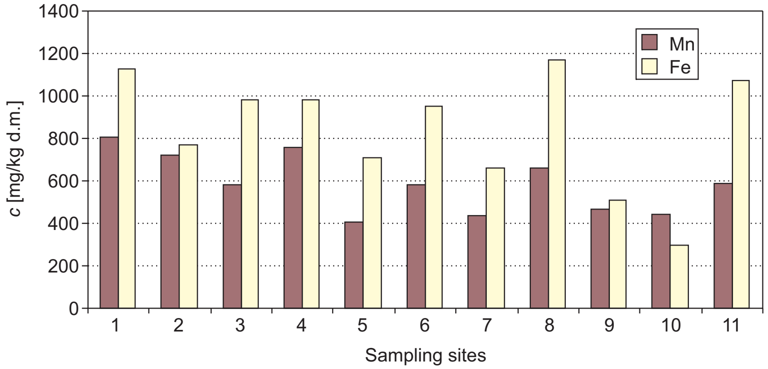

Fig. 3. The content of iron and manganese in the *Elodea canadensis* Michx. samples from sites 1–11 (reservoir I)

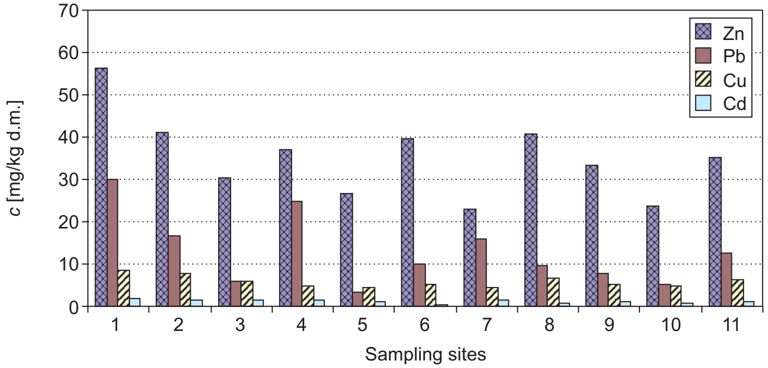

Fig. 4. The content of copper, cadmium, zinc and lead in the *Elodea canadensis* Michx. samples from sites 1–11 (reservoir I)

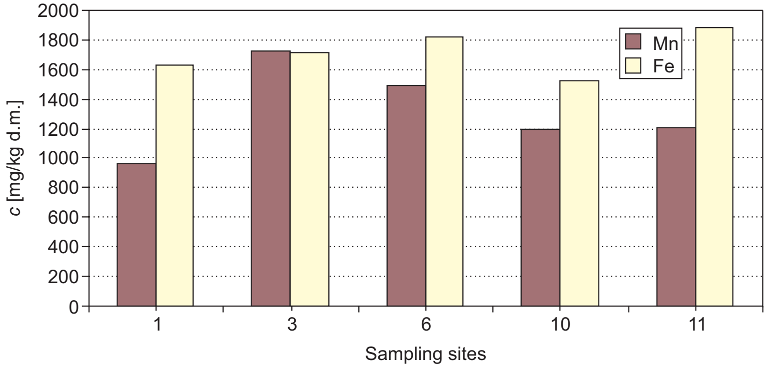

Fig. 5. The content of iron and manganese in the algae *Spirogyra* sp. samples from reservoir I

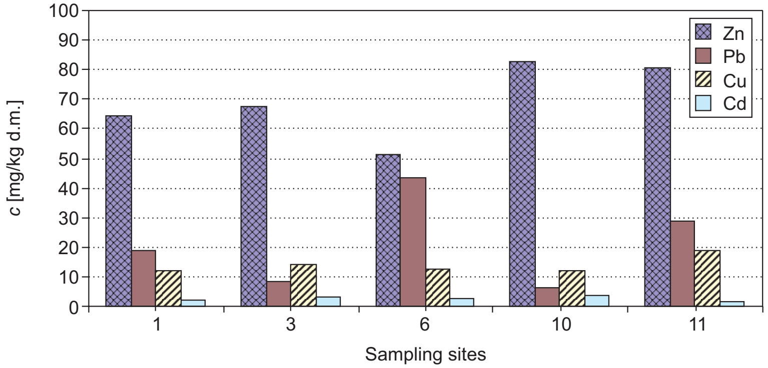

Fig. 6. The content of copper, cadmium, zinc and lead in the algae *Spirogyra* sp. samples from reservoir I

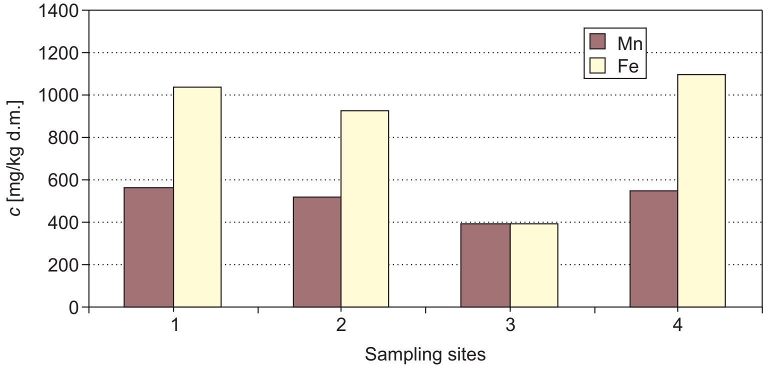

Fig. 7. The content of iron and manganese in the *Elodea canadensis* Michx. samples from reservoir II

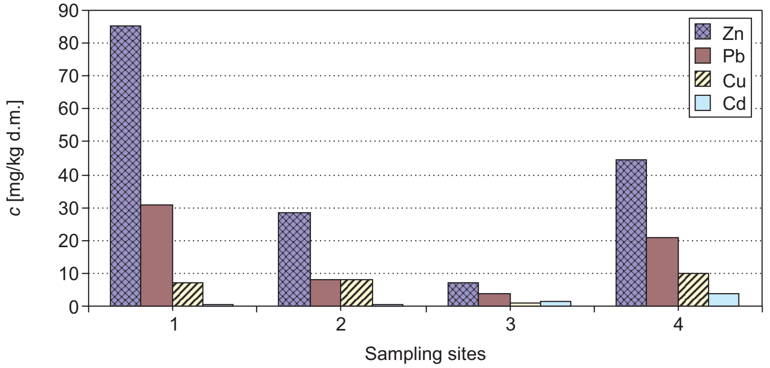

Fig. 8. The content of copper, cadmium, zinc and lead in the *Elodea canadensis* Michx. samples from reservoir II

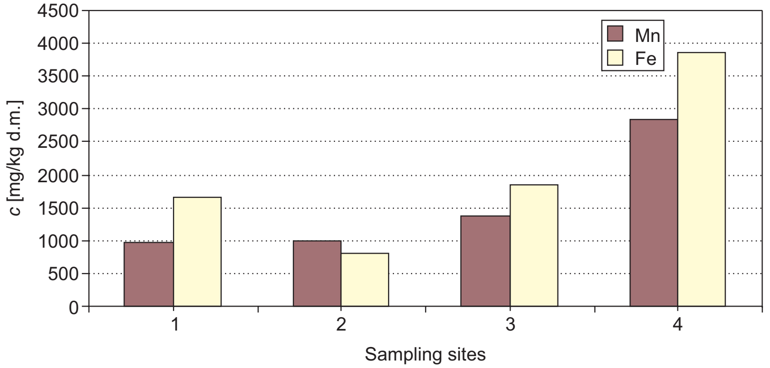

Fig. 9. The content of iron and manganese in the algae *Spirogyra* sp. samples from reservoir II

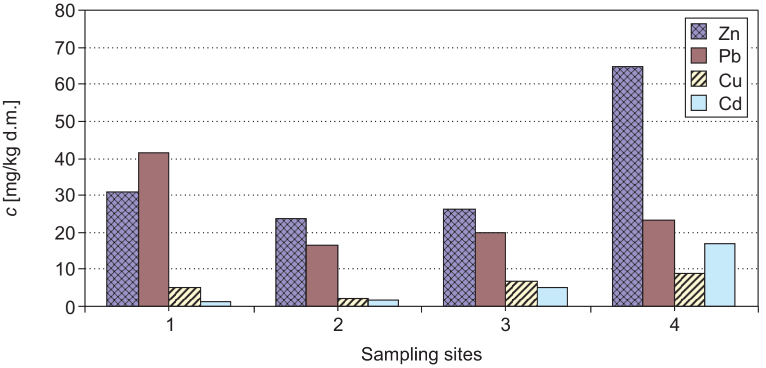

Fig. 10. The content of copper, cadmium, zinc and lead in the algae *Spirogyra* sp. samples from reservoir II

Fe > Mn > Zn > Pb > Cu > Cd (algae) and Fe > Mn > Pb > Zn > Cu > Cd (*Elodea canadensis* Michx.).

In order to assess the distribution of concentrations of metals accumulated in the analyzed plants from reservoirs I and II, the variation coefficients $RVC = 100\ \% \cdot SD/M$ were determined, where: SD – standard deviation, M – mean metal concentration in samples collected from one reservoir. The results are shown in Table 5.

Table 5

Variation coefficients demonstrating the diversity of distribution of metal concentrations in reservoirs I and II

SD [%]					
Mn	Fe	Cu	Zn	Cd	Pb
RESERVOIRS I					
Spirogyra sp.					
22	8	21	19	29	72
Elodea canadensis Michx.					
23	33	24	27	33	65
RESERVOIRS II					
Spirogyra sp.					
63	77	61	63	126	14
Elodea canadensis Michx.					
17	43	73	45	107	55

As the parameters shown in Table 5 demonstrate, in a single reservoir, there are significant differences in concentrations of metals accumulated in the analyzed samples. The level of accumulation of metals is affected by the concentration of their bioavailable forms in water, affinity to the sorption structures of plants and a number of abiotic and biotic factors affecting the sorption processes, including the time of exposure, cellular metal distribution, metal storage and detoxification forms, interactions with other compounds present in the cell, species properties (*eg* adaptability) and the presence of other ions in the reservoir water [29]. No statistically significant correlations between concentrations of the analyzed metals in algae and *Elodea canadensis* Michx. were identified in the individual samples. However, such correlations were found with respect to mean concentrations of metals accumulated in the analyzed plants. The results are shown on diagrams in Fig. 11 and 12.

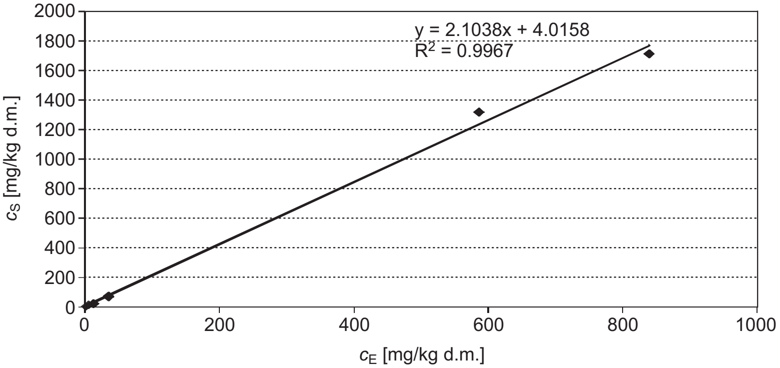

Fig. 11. Comparison of mean concentrations of heavy metals accumulated in the algae (S) and *Elodea canadensis* Michx. (E) samples from reservoir I

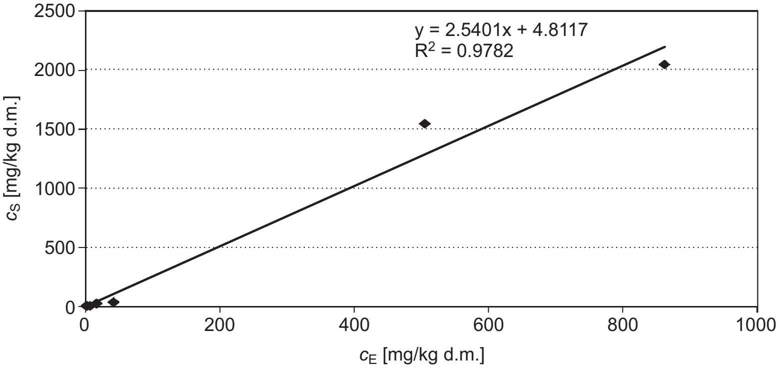

Fig. 12. Comparison of mean concentrations of heavy metals accumulated in the algae (S) and *Elodea canadensis* Michx. (E) samples from reservoir II

The coefficients of determination R^2 determined for the linear functions shown in Fig. 11 and 12 indicate that there are correlations between the mean concentrations of analytes in the samples of algae and *Elodea canadensis* Michx. Furthermore, the results shown on the diagrams indicate that as far as the heavy metal bioaccumulation is concerned, *Spirogyra* sp. is a better accumulator than *Elodea canadensis* Michx.

Summary and conclusions

Biomonitoring is gaining popularity as a method for assessing the pollution of aquatic ecosystems. The biomonitors demonstrate different sensitivity and a characteristic reaction to the environmental factors and have good sorption properties.

The analyzed plants *Spirogyra* sp. and *Elodea canadensis* Michx. demonstrate the ability to accumulate significant amounts of heavy metals despite their low concentration in the environment.

The results of the biomonitoring studies conducted on the selected biota elements suggest diverse pollution with heavy metals of the "Lesna Przystan" bathing site in Glebocko. The mean concentrations of metals accumulated in the analyzed samples of algae and *Elodea canadensis* Michx. decreased as follows: Fe > Mn > Zn > Pb > Cu > Cd (algae) and Fe > Mn > Pb > Zn > Cu > Cd (*Elodea canadensis* Michx.). No statistically significant correlations between concentrations of analytes accumulated in the analyzed plants were identified. Such correlations were found with respect to the mean values determined for samples collected from a single reservoir.

The pollution of the analyzed reservoirs with the analyzed analytes is probably due to the activities of the holiday resort located near reservoir I and leachate from fields in the vicinity of the analyzed reservoirs.

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ROŚLINY WODNE – ŹRÓDŁO INFORMACJI O ZANIECZYSZCZENIU WÓD POWIERZCHNIOWYCH METALAMI CIĘŻKIMI

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Abstrakt: Celem przeprowadzonych przez nas badań była ocena możliwości wykorzystania wybranych roślin wodnych w biomonitoringu wód. Do badań wykorzystano glony *Spirogyra* sp. (Skrętnica) i *Elodea Cana-*

densis Michx., (Moczarka kanadyjska) zebrane w strefie litoralnej kąpieliska „Leśna Przystań” w Głębocku (południowa Polska). W biocie, metodą absorpcyjnej spektrometrii atomowej (AAS), oznaczono stężenia: Mn, Fe, Cu, Zn, Cd i Pb. Na podstawie przeprowadzonych badań dokonano wstępnej oceny zanieczyszczenia wód kąpieliska. Wskazano również na możliwe korelacje pomiędzy stężeniem metali ciężkich w wybranych elementach bioty.

Słowa kluczowe: *Spirogyra* sp., *Elodea Canadensis* Michx., metale ciężkie, biomonitoring

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HEAVY METALS IN BOTTOM DEPOSITS OF “KORYTOW” RESERVOIR

METALE CIĘŻKIE W OSADACH DENNYCH ZBIORNIKA KORYTÓW

Abstract: The aim of this study was to determine and to evaluate heavy metals (Cd, Cu, Pb, Zn) concentrations in sediments of “Korytow” reservoir, located on Pisia-Gagolina river. In analyzed sediments concentrations of heavy metals was diversified due to their natural concentrations. Concentrations of Zn, Cu, Cd in upper layers were lower than concentrations in lower layers which means, that mean concentration of this metals in sediments is reducing. Concentration of Pb was balanced (117.1 mg/kg) in each sample core. Mean Pb concentration is much higher than natural geochemical background. Results of this work shows that concentration of toxic metals (Cd and Pb) in sediments was high. It confirms that water environment is polluted by heavy metals.

Keywords: heavy metals, bottom sediments, surface waters reservoirs

Development of civilisation has led to introducing heavy metals into the natural environment in amounts remarkably excessing the natural load. Bottom sediments accumulate many elements, some of which are heavy metals, hazardous for natural environment, as well as animals and people [1]. Their amount might be several orders of *eg* 15 magnitude higher than in the water phase [2–5]. The examining of metals in lake sediment has been used for long years in observing the environmental effects [6–8]. Human activities (such as industrial wastes, settlement wastes etc.) as well as geological structure constitute the main source of metals in aquatic ecosystems [9–12]. Metals cannot be biologically degraded like organic contaminants and thus they accumulate especially in the sediment by being absorbed in complex structures. Metals which accumulate in the sediment may turn into factors threatening the ecosystem

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well-being and may constitute a danger and risk factor for the environment [13–14]. Heavy metals in surface waters does not stay in water phase for a long time [14–15]. As a result of various environmental processes heavy metals are removed from waters into sediments. In polluted deposits, concentrations of past and present widely industrial--used heavy metals are usually magnified [16–18]. Sediments with high concentration of toxic and harmful components are also potential source of pollution. Heavy metals are not permanently fixed on sediments and can be released back to the water, as a result of chemical and biochemical processes which takes place in deposits, as a result of changes in environmental conditions [19]. Heavy metals accumulated in sediments in changeable conditions can migrate to water phase [16, 18] or be absorbed by plants [20]. It is very important to obtain reliable information about the toxicity of the lake's sediments. Different bioavailability of forms in which pollutants exists in the environment results in their different biological activities [21, 22].

The aim of this study was to determine and to evaluate heavy metals (Cd, Cu, Pb, Zn) concentrations in sediments of "Korytow" reservoir, collected from 3 sample points located on Pisia-Gagolina river in Zyrardów district.

Study area

The "Korytow" reservoir is located in the village of Korytow, 3.5 km far from the centre of Zyrardow (Fig. 1). Its front barrage, with the other at the top, is situated on the

Fig. 1. The "Korytow" reservoir

38th km of the course of the Pisia-Gagolina river, and it closes the catchment area covering 73.0 square km. The body was created in 1910 by pile-up of waters of the river, by a front earth dam with a weir having abutments made of bricks. It is located in a natural valley, without side dams. The water body is not eguipped with any other devices to let flood water in, the whole flow goes through the main section of the weir [19, 23, 24]. Due to significant surface (3.4 ha) and natural shape of the shoreline as well as little depth in the area, the body is a natural refuge for animals and plays a role of bird sanctuary [19, 23].

Methods

Sediments were sampled from 3 sample points from "Korytow" reservoir by scooping up sediment with a plastic spade. Sample points are shown in Fig. 2.

Fig. 2. Location of sampling sites in the Korytow reservoir (source: © użytkownicy OpenStreetMap http://www.openstreetmap.org/, CC BY-SA, http://creativecommons.org/licenses/by-sa/2.0/.)

Sediments were sampled in autumn 2006 and in winter 2007. Sediment cores from each sample point were partitioned into 5 cm lengths. The collected sediment samples were packed into polyethylene bottles and transferred to the laboratory, where they were dried at room temperature. In dry sediments grain size fractions and heavy metals were analyzed. For determining the relationship between grain size and metal contents, the sediment samples were fractionated into six grain sizes by an controlled sieve shaker.

From samples collected in autumn 2006 < 250 μm grain size of bed sediments has been used. It was caused by a fact that those grain size fraction is dominant part of bottom sediment and does not have accidental pollutions [13]. From samples collected in winter 2007 < 63 μm and 100–250 μm grain size of bed sediments has been used. It was caused by influence of grain size on result of analysis.

From dried and sieved samples, 1 g of sediments was weighed out to mineralization with mixture of HNO_3 + $HClO_4$ (4 : 1) acids in a Teflon bomb. In obtained solutions the total concentration of Zn, Cu, Pb and Cd was determined using *flame atomic absorption spectrometer* (FAAS) of Philips company, England. The same procedure without samples was used as a control. Three measurements were conducted for each sample. Quality assurance and quality control (QA/QC) for metals in sediment samples were estimated by determining metal concentrations in the Merck Standard solutions (Merck, Darmstadt, Germany). The detection limit was calculated based on the estimated instrumental detection limit assuming that 1 g of a sample is digested or diluted to 100 cm^3. Detection limits (mg/kg of dry matter) for Cu, Pb, Cd and Zn were: 0.01; 0.03, 0.002 and 0.01, respectively.

Results and discussion

In samples from autumn 2006 the dominant was grain size fractions 250–500 μm and 100–250 μm. Amount of grain size fraction < 63 μm was low. The distributions of grain size fractions within different depths, was similar. The lowest amount of grain size fraction < 63 μm was in sample point next to the dam (S) and on the right side of reservoir (P) in top layer of sediments. Low concentration of smallest parts (< 63 μm) may be caused by high flow in this part of reservoir or lack of costal vegetation. In deeper layer of sediments percentage amount of fraction < 63 μm was higher, and its percentage part was close to concentration in sample point L (on the left side of reservoir). Sample point L was close to coast of reservoir, next costal vegetation what caused higher accumulation of < 63 μm parts. In samples from winter 2007 the distributions of grain size fractions was similar. The dominant was grain size fractions 250–500 μm and 500–1000 μm. Amount of grain size fraction < 63 μm was higher than in autumn 2006, and similar in each sample point. This is probably caused by low winter flow.

Comparing grain size < 63 μm and 100–250 μm it is important to know, that proportion between all four heavy metals (zinc, copper, lead, cadmium) are the same for those fractions. What is more in grain size fraction < 63 μm there is much more heavy metals that 100–250 μm fraction. The highest differences was observed for Pb. Analysis shows that dominant metals in sediments were Pb and Zn. The highest concentration of Pb was 117.1 mg/kg, and Zn was 103.3 mg/kg. The highest concentration, over natural geochemical, was observed for Cd. Its highest concentration was 21.8 mg/kg, which shows high anthropogenic pollution [20, 21]. Concentrations of Zn, Pb, Cu was similar to natural geochemical concentrations, or a little higher.

Mean concentration of zinc in analyzed sediments was 52 mg/kg. The highest concentration from all results was 103.3 mg/kg. The highest concentration in upper layer of bottom sediments was 84 mg/kg. Because of outreaching of natural, geochemical concentration of zinc, 100 mg/kg [21, 22], was only in deeper layers of sediments (5–15 cm), it is possible to say, that in upper layer of sediments concentration of zinc was close to natural. Horizontal distribution of zinc was almost the same in each sample point, but a little higher concentrations was observed in sample point on the right side of reservoir (P). The lowest concentrations was observed in sample point on the left side of reservoir (L). Concentration of Zn in upper layer of bottom sediments was higher in autumn than in winter (25 % difference). Analysis of vertical distribution shows that zinc pollution is decreasing for about 10 %.

Table 1

Mean concentrations of Zn, Cu, Pb, Cd in sediments of "Korytow" reservoir

Concentration [mg/kg d.m.]		Zn	Cu	Pb	Cd
Total		52.6	27.8	67.9	2.9
Autumn		59.1	21.9	79.4	2.1
Winter	Mean	49.4	30.8	62.1	3.4
	< 63 μm	54.3	33.9	68.1	4.3
	100–250 μm	44.3	28.5	56.4	2.4

Mean concentration of cadmium in analyzed sediments was 2.9 mg/kg. The highest concentration from all results was 2.73 mg/kg. The highest concentration in upper layer of bottom sediments was 2.17 mg/kg. In both cases it is strong anthropogenic pollution [18]. Horizontal distribution of cadmium was almost the same in each sample point, but a little higher concentrations was observed in sample point next to the dam (S), were lower flows causes sedimentation of suspensions. Concentration of cadmium in upper layer of bottom sediments was higher in winter than in autumn (10 % difference). Analysis of vertical distribution shows that cadmium pollution is decreasing for about 10 %, but historical and present cadmium pollution was and still is very high.

Mean concentration of copper in analyzed sediments was 27.85 mg/kg. The highest concentration from all results was 63.61 mg/kg. The highest concentration in upper layer of bottom sediments was 49.92 mg/kg. In both cases it concentration is higher then natural [19]. Horizontal distribution of copper was almost the same in each sample point. It is similar to Zn and cadmium distribution. Differences of concentrations between three points are low, but the highest concentrations was observed in sample point next to the dam (S). The lowest concentrations was observed in sample point on the left side of reservoir (L). Concentration of Cu in upper layer of bottom sediments was higher in winter than in autumn (30 % difference). Analysis of vertical distribution shows that copper pollution is decreasing for about 25–80 %. That shows that historical concentration was much higher than present and pollution is decreasing.

Mean concentration of lead in analyzed sediments was 68 mg/kg. The highest concentration from all results was 117.1 mg/kg. Concentration of lead is higher then natural [19]. Horizontal distribution of lead was almost the same in each sample point. It is similar to Zn, Cd and Cu distribution in sediments of "Korytow" reservoir. The highest concentrations was observed in sample on the right side of reservoir (P). Concentration of lead in upper layer of bottom sediments was higher in autumn than in winter (20 % difference). Analysis of vertical distribution shows that lead concentrations, excluding sample point P, are almost the same for all depths, which means that historical and present concentrations of this metal are still the same.

Difference of metals contents in the sediment "Korytow" reservoir resulted mainly from the character of the surrounding catchment. Lead and cadmium in sediment of river Pisia-Gagolina and "Korytow" reservoir are characterized by high and different levels to geochemical background, which is determined by geochemical structure of that area as well as its management status. It seems that sediments from those flows are not genetically closely associated with the material of direct catchment of studied rivers.

In Poland there are not legally biding regulations referring to bottom sediments classification, which would comply with commonly used categorization of polluting elements. The first Polish geochemical classification of riverbed sediments and lake bed sediments was made by Polish Geological Institute and is used in State Environmental Monitoring. Sediments were divided into 3 classes according to their metal content, based on geochemical criteria [25].

Table 2

Classification of water sediments based on geochemical criteria [25]

Mark	Description	Unit	Cd	Cu	Pb	Zn
Geochemical background	—	mg/kg	< 0.5	6	10	48
Class I	slightly polluted sediments	mg/kg	< 1	< 20	< 50	< 200
Class II	moderately polluted sediments	mg/kg	< 5	< 100	< 200	< 1000
Class III	polluted sediments	mg/kg	< 20	< 200	< 500	< 2000

Conclusions

1. The dominant grain size fraction in sediments was 250–500 μm and 100–250 μm. The distributions of grain size fractions within different depths, was similar.
2. Grain size fraction < 63μm accumulated much more Zn, Cu, Pb and Cd than other fractions.
3. Total concentrations of analyzed metals were higher than natural geochemical background.
4. Results of this work shows the following order Cd > Pb > Zn > Cu of danger caused by heavy metals.
5. Analysis of vertical distribution shows that Zn and Cd pollution is decreasing for about 10 %, Cu pollution is decreasing for about 25–80 %, Pb concentrations are almost the same for all depths.
6. The biggest danger for water environment is Cd and Pb. Results of this work shows that there is a relationship between concentrations of Zn and Pb and between concentrations of Cu and Cd.
7. Metals collected in sediments of "Korytow" reservoir could be dangerous for local ecosystem.
8. Metals collected in sediments of "Korytow" reservoir have anthropogenic origin.
9. Sediments collected on bottom of the reservoir are covered by a layer of quite clean (unpolluted) sediments, but they may be a source of secondary pollution of water samples from autumn 2006 the dominant was grain size fractions 250–500 μm and

100–250 μm. Amount of grain size fraction < 63 μm was low. The distributions of grain size fractions within different depths, was similar.

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METALE CIĘŻKIE W OSADACH DENNYCH ZBIORNIKA KORYTÓW

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Abstrakt: Celem badań było oznaczenie zawartości Zn, Cu, Pb i Cd w osadach dennych zbiornika Korytów, zlokalizowanego na rzece Pisi-Gągolinie. W prowadzonych badaniach obserwowano duże zróżnicowanie stężeń czterech metali w odniesieniu do ich naturalnej zawartości. Stężenia Zn, Cu i Cd w warstwie powierzchniowej osadów były niższe niż w głębszych warstwach, co świadczy o redukcji średniej zawartości metali w osadach. Zawartość ołowiu była wyrównana w głąb osadów (117,1 mg/kg), przekraczając znacznie poziom tła geochemicznego środowiska.. Na podstawie uzyskanych wyników można stwierdzić, że osady charakteryzowały się wysoką zawartością toksycznych metali (Cd i Pb). Potwierdza to znaczne zanieczyszczenie środowiska wodnego metalami

Słowa kluczowe: metale ciężkie, osady denne, zbiorniki wód powierzchniowych

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INFLUENCE OF MANY YEARS' AGROUTILIZATION OF WASTEWATERS FROM PRODUCTION OF FATS OF ANIMAL ORIGIN ON CHANGEABILITY OF HEAVY METAL SPECIATION FORMS IN SOIL

WPŁYW WIELOLETNIEJ AGROUTYLIZACJI ŚCIEKÓW Z PRODUKCJI TŁUSZCZÓW ZWIERZĘCYCH NA ZMIENNOŚĆ FORM SPECJACYJNYCH METALI CIĘŻKICH ZAWARTYCH W GLEBIE

Abstract: The paper presents the results of four-year research on changeability of the fractional composition (of five fractions: exchangeable, carbonate, related to hydrated oxides Fe/Mn, organic and so-called residual one) of heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) determined in soil being subject to agroutilization of pretreated wastewaters from animal fat production within the time.

The pollutants load directed to soil mainly contained polysaccharides and proteins, and the process of dosing wastewaters took place gradually, at regular intervals during the whole experimental period. On the basis of the research it was found that the total average metal concentrations did not change significantly within the time. In case of total cadmium, mercury, lead or chromium, an insignificant growth trend was registered for the total concentration in relation to initial concentrations (C_0). Analyzing the fractional content of the individual metals in the soil within the experimental period (determined in accordance with the Tessier's method), the limited phenomenon of concentrations changeability of the soluble, carbonate and organic fractional loads was found in the case of cadmium, mercury and lead.

Keywords: heavy metals, heavy metal fractional content, agro-utilization of wastewaters from animal fat production

Pretreated post-production waste from animal fat processing can be agroutilized for irrigation and as fertilizers [1–5]. The doses and the frequency of these operations are limited by numerous environmental regulations, which result in threshold values for a number of substances, among others the level of the heavy metal content in pretreated wastewaters and soils where agroutilization is carried out [2, 3, 6]. In the load of residual substances after pretreatment, there are mainly dissolved and colloidal protein

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substances and polysaccharides, which, after being introduced to soil, are subject to complex chemical and biochemical processes [6, 7]. Literature describes various aspects related to the application of wastewaters from animal fat production to agro-utilization [2–13]. However, there is a lack of problems concerning metal speciation changeability within the many-year agroutilization. These aspects seem to be significant from the point of view of individual metals' mobility and transporting some of their forms in a vegetable system during the vegetation, which can be significant for their practical use in the case of plants cultivated on such irrigated acreage [14–17].

Material and methods

Post-production wastewaters from animal fat production (from food industry and technological ones) were pretreated with the use of two-stage coagulation [2, 3]:

a) stage I^o (acidic coagulant PAX 25 (Kemipol Police [19]) in dose to reaction pH = 4.8 ± 0.2;

b) stage II^o (reaction coagulant SAX 25 (Kemipol Police [19]) in dose to pH = 7.4 ± 0.2).

Coagulation was aided with flocculation (Praestol 859 BC Stockhausen) in dose 25.0–35.0 g/m^3 of wastewaters and with *pressurized flotation* (PF) (saturation time 15.0 min, saturation pressure 500 kPa, recirculation level 15 %) aided with hydrogen peroxide oxidation (in dose 250.0 g/m^3 wastewaters). Pretreated wastewater parameters [mg/dm^3] dosed to the experimental field during the time of carrying out the research were as follows [2]: *total nitrogen* (TN) 34.7–103.6, *total phosphorus* (TP) 15.3–43.1, *etheric extract* (EE) 5.9–48.9, *total suspensions* (TSS) 20.1–85.0, *chemical oxygen demand* (COD) 849.2–3166.3, *five-day biochemical oxygen demand* (BOD_5) 322.4–1300.4 and reaction (pH) 7.2–7.7. The content analysis of the individual, leached heavy metal fractions and changeability of individual fractional loads over the four-year analyzes were carried by sampling from the experimental field (of the area of 15.0 ares) in equal time slots – every six months (collected in April and October) in accordance with the standard PN-ISO 10381-2, taking samples from the depth of the surface layer 0.0–20.0 cm. Soil samples air-dried to solid mass (100.0 g) were subject to extraction and mineralization, and the metal concentrations in individual fractions were determined with *atomic absorption spectrometry* AAS with the use of BUCK Scientific 210 VGP apparatus. In order to determine the individual fractional loads, Tessier's methodology was used as in the detailed extension given above [17, 18]:

a) Exchangeable (fraction I). Soil samples were treated with 400.0 cm^3 (1.0 M) $CaCl_2$ and after intensive mixing (at pH 7.0) shaken for 1.0 hour at room temperature. The analyses were made after separating solid components with the use of filtration.

b) Carbonate (fraction II). Washed and dried soil samples which remained after carrying out the a) procedure were subject to extraction by adding 800.0 cm^3 (1.0 M) CH_3COONH_4 and acidating it with 80 % CH_3COOH to pH 7.0. After mixing, the mixture was shaken for 5 hours at room temperature. During this operation, the reaction was controlled: when pH > 7.0 – it was acidified. The analyses were made after separating solid components with the use of filtration.

c) Related to Fe/Mn oxides (fraction III). 800.0 cm^3 (0.04 M) of $NH_2OH \cdot HCl$ solution in 25 % CH_3COOH was added to b) and mixed to the washed and dried sample of the solid phase which remained after applying the procedure. Samples (of pH 2.0) were shaken for 5.0 hours at the temperature 96 ± 3 ^oC. The analyses were made after separating solid components with the use of filtration.

d) Organic (fraction IV). 200.0 cm^3 (0.02 M) HNO_3 and 200.0 cm^3 30 % of the aqueous solution of H_2O_2 were added to the washed and dried sample, then mixed (pH 2.0) and shaken for 135.0 min at the temperature 85 ± 3 ^oC. Next, 200.0 cm^3 30 % H_2O_2 was added and shaken for 135.0 min. After that time, 400.0 cm^3 (3.0 M) CH_3COONH_4 in 20 % HNO_3 (v/v) was added and shaken for 30.0 min at room temperature (pH 2.0). The filtrate was subject to analysis, and the residual solid component was washed and dried.

e) The residual one (fraction V). It was shaken for 1.0 hour at boiling point, adding HNO_3 and H_2O_2 twice during the time until liberation white ashes. Next 400.0 cm^3 30 % H_2O_2 was added and shaken for 30.0 min at boiling point, and at the same time the analyses of heavy metal content were made for the filtrate.

Discussion of the results

The aim of the paper was to compare the level of the content and exchangeability of five heavy metal fractions (Cd, Cr, Cu, Hg, Ni, Pb, Zn) (I – exchangeable, II – carbonate, III – related to Fe/Mn oxides, IV – organic and V – so-called the residual one), during four-year experimenting period carried on soils from the irrigated and fertilized with pretreated wastewaters from the animal fat production field (Table 1). Dosing wastewaters and carrying out agrotechnical operations on the subject field were carried out as given in [2, 3], and the present paper elaborates the problems described in this publication. In literature, there were numerous publications concerning the problem of agroutilization of wastewaters from the animal fat production, among others the ones concerning the aspect of changes in heavy metal concentrations triggered by agroutilization [2–13]. However, there are no reports on the topic of changeability of the fractional fields during many years of experiments with fertilizing and irrigating soils with these wastewaters. This problem seems to be particularly essential since the total metals concentration does not reflect the transformations which take place with the use of metals and wastewaters components, if necessary their soil transformation products during many years of providing soil with these substances [14, 16].

On the basis of the research conducted within four years of the experiments, it was found that total, average metals concentrations within this period of time were not the subject to significant changes [2]. In the case of cadmium, mercury or copper, an insignificant growth trend for the total concentration in relation to initial concentrations (C_0) was found [2]. Analyzing the fractional content of individual metals in the subject soil during the experiments, the limited phenomenon of percentage fluctuation in fractional loads share was found: soluble (fraction I), carbonate (fraction II) and organic (fraction IV) in the case of cadmium, mercury and lead (Table 1).

Table 1

Changeability of the individual fractional loads determined during 4-year experiments

No.	Heavy metal	Fractions	% participation of fractions in subsequent measurements								
			P0	P1	P2	P3	P4	P5	P6	P7	P8
1	Chromium (Cr)	I	1.3	1.2	1.8	1.4	0.2	2.8	1.5	3.4	1.9
		II	1.2	1.3	1.1	2.7	3.1	3.7	4.9	4.1	2.9
		III	7.6	6.6	5.3	5.9	6.9	4.5	3.3	2.1	4.2
		IV	4.5	5.3	6.4	3.7	5.1	3.9	4.2	5.5	5.8
		V	85.4	85.6	85.4	86.3	84.7	85.1	86.1	84.9	85.2
2	Cadmium (Cd)	I	0.5	1.3	3.3	3.6	4.2	3.5	4.8	4.3	4.1
		II	8.2	6.9	7.3	4.1	4.7	5.7	4.4	4.7	4.4
		III	6.7	8.8	9.5	9.2	9.4	6.6	9.1	7.4	8.9
		IV	6.7	11.5	8.5	7.7	10.6	9.1	9.8	9.5	8.8
		V	77.9	71.5	71.4	75.4	71.1	74.1	71.9	70.1	70.8
3	Nickel (Ni)	I	1.5	1.8	1.8	1.4	0.2	2.8	1.5	1.4	3.9
		II	1.7	3.4	1.7	2.7	3.3	3.7	4.9	2.4	2.9
		III	7.6	6.6	5.1	5.9	6.9	4.5	8.8	10.9	8.2
		IV	3.7	2.7	4.7	3.2	2.1	3.3	4.2	5.5	1.8
		V	85.5	85.5	85.7	86.8	87.7	85.7	86.6	79.8	83.2
4	Lead (Pb)	I	0.7	4.5	4.5	1.6	2.6	1.7	0.9	3.5	3.7
		II	6.0	3.7	4.7	6.6	6.6	3.4	5.0	5.4	5.5
		III	12.9	10.0	9.5	8.0	8.8	9.5	12.7	10.4	8.8
		IV	7.2	8.0	6.5	9.9	10.2	8.2	10.8	10.0	10.5
		V	73.9	73.8	75.8	73.9	71.8	77.2	70.6	70.7	71.5
5	Copper (Cu)	I	1.0	1.1	1.0	0.7	0.5	1.9	0.9	0.6	0.9
		II	0.9	0.8	0.7	2.5	1.5	1.5	0.5	0.9	1.2
		III	4.4	2.7	0.7	1.4	2.5	1.5	3.2	2.6	4.8
		IV	4.6	5.2	11.7	2.3	8.5	5.2	5.5	7.1	7.5
		V	89.1	90.2	85.9	93.1	87.0	89.9	89.9	88.8	85.6
6	Mercury (Hg)	I	2.2	4.5	4.5	1.6	2.6	2.7	0.9	3.5	3.7
		II	6.0	3.7	4.7	6.6	6.6	5.4	5.0	5.4	5.5
		III	12.9	10.0	9.5	8.0	8.8	10.5	12.7	10.4	8.8
		IV	8.0	8.0	10.5	9.9	10.2	10.2	10.8	10.0	10.5
		V	70.9	73.8	70.8	73.9	71.8	71.2	70.6	70.7	71.5
7	Zinc (Zn)	I	0.9	1.1	1.0	0.7	1.9	1.3	0.6	1.7	1.1
		II	3.2	3.3	3.2	2.3	0.9	4.9	2.2	2.9	1.5
		III	7.9	9.0	7.0	6.6	8.5	6.6	7.7	7.6	9.0
		IV	1.2	1.6	0.6	1.3	1.7	1.2	4.0	2.2	3.2
		V	86.8	85.0	88.2	89.1	87.0	86.0	85.5	85.6	85.2

Where: P0 – zero sample (before agro-utilization of pretreated wastes) – total initial concentrations (C_0) were, respectively [mg/kg dry mass]: Cd – 0.31, Hg – 0.27, Cr – 2.08, Ni – 3.66, Cu – 6.87, Pb – 8.84 and Zn – 28.28; P1 – P8 measurement series carried out every 6 months within four years of analyzes.

This phenomenon can be interpreted by creating conditions for releasing soluble forms, probably in the form of aqua complexes ($[M(H_2O)]^{+m}$ and organic soil transformation products, mainly the low molecular acids during vegetation periods (originating form polysaccharides, *ie* glycogen provided with wastewaters). This group contains both complex systems $(M(R)]^{+m+n}$: where: R – organic ligand (*ie* products of polysaccharides hydrolysis: monosaccharides; products of soil metabolism: low molecular acids, polyacids, low molecular condensation products etc., m and n – metal and organic ligand loads) as well as organic insoluble compounds [20, 21]. Forming metal-organic bonds is justified, also by the continuous supply of organic substance transferred to soil and consequently by the transformation processes with participation of these substances and their decomposition products, especially during the vegetation period [15, 16, 20, 21]. In the case of cadmium and mercury, the phenomenon of significant percentage diversity of fractional loads shares within the time should be also assessed from the point of view of these values random distribution which results from the relatively low concentrations of these elements in soil: for Cd – $C_0 = 0.31$ (± 0.15), and for Hg – $C_0 = 0.27$ (± 0.20) mg/kg dry mass.

In the case of the other elements, the changeability of the individual fractions with the time did not prove any regularities, which can be interpreted by the character of the determined, stable phenomenon. While analyzing fractional composition, definitely the highest share was registered in the case of the so-called residual fraction, for cadmium, mercury and lead exceeding 70 %, and for the rest of the metals 85 % (fraction V, Table 1). The soluble fraction (fraction I) was registered for each metal below 5 %, similarly as in the case of carbonate fraction (fraction II). In the case of the fraction related to hydrated oxides Fe/Mn, the most stable tendency was observed to keep a similar content of this fraction for all examined metals within the time of the experiment. The organic fraction (fraction IV) was characterized by significant changes in its percentage share within the time, especially in the case of cadmium, mercury and lead. The lack of evidence of a significant change in quantitative relations of the individual fractional loads also resulted from a significant fact, that is from buffer properties of the examined soil, for which no significant reaction changes were registered (apart from periodical fluctuations), and this parameter is the essential one in the case of almost all chemical and biochemical soil transformations.

Conclusions

On the basis of the present research it was found that the total average metal concentrations did not significantly change within four years of experiments. In the case of cadmium, mercury or copper, an insignificant growth trend in their total concentration was registered in relation to output concentrations. Analyzing the fractional composition of the individual metals in the examined soil within the time of the experiment, a limited phenomenon of percentage fluctuation of the soluble fraction load, carbonate or organic was found in the case of cadmium, mercury and lead.

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WPŁYW WIELOLETNIEJ AGROUTYLIZACJI ŚCIEKÓW Z PRODUKCJI TŁUSZCZÓW ZWIERZĘCYCH NA ZMIENNOŚĆ FORM SPECJACYJNYCH METALI CIĘŻKICH ZAWARTYCH W GLEBIE

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Abstrakt: Przedstawiono wyniki czteroletnich badań nad zmiennością w czasie składu frakcyjnego (pięciu frakcji: wymywalnej, węglanowej, związanej z uwodnionymi tlenkami Fe/Mn, organicznej i tzw. pozostałej) metali ciężkich (Cd, Cr, Cu, Hg, Ni, Pb, Zn) oznaczanych w glebie poddanej agroutylizacji podczyszczonych ścieków z produkcji tłuszczów zwierzęcych. W ładunku zanieczyszczeń kierowanych do gleby zawarte były głównie policukry oraz białka, a proces dawkowania ścieków był prowadzony sukcesywnie, w regularnych odstępach czasu przez cały okres eksperymentu. Na podstawie badań stwierdzono że całkowite, średnie stężenia metali w tym okresie nie ulegały istotnym zmianom. W przypadku kadmu, rtęci, ołowiu czy chromu ogółem zarejestrowano nieznaczny trend wzrostu całkowitego stężenia w relacji do stężeń wyjściowych (C_0). Analizując w okresie doświadczalnym skład frakcyjny (oznaczany zgodnie z metodyką Tessiera) poszczególnych metali w przedmiotowej glebie, stwierdzono występowanie ograniczonego zjawiska zmienności stężeń pul frakcji rozpuszczalnych, węglanowych oraz organicznej w przypadku kadmu, rtęci i ołowiu.

Słowa kluczowe: metale ciężkie, skład frakcyjny metali ciężkich, agroutylizacja ścieków z produkcji tłuszczów zwierzęcych

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EFFECTIVENESS OF METHANE FERMENTATION OF VIRGINIA FANPETALS (*Sida hermaphrodita* Rusby) UNDER MESOPHILIC CONDITIONS

EFEKTYWNOŚĆ PROCESU FERMENTACJI METANOWEJ ŚLAZOWCA PENSYLWAŃSKIEGO (*Sida hermaphrodita* Rusby) W WARUNKACH MEZOFILOWYCH

Abstract: This study was aimed at determining the technological effectiveness of methane fermentation process of plant biomass from Virginia fanpetals (*Sida hermaphrodita* Rusby) in continuous reactors at a temperature of 37 °C. The experiment was divided into four experimental series differing in the feeding of reactor's volume with a load of *organic dry matter* in the range of 1.0 kg o.d.m./m^3 · d to 4.0 kg o.d.m./m^3 · d. The highest technological effectiveness, including the quantity and qualitative composition of the biogas produced, was reached at substrate loading range of 1.0 kg o.d.m./m^3 · d to 2.0 kg o.d.m./m^3 · d. The reactors' loading with the higher feedstocks of biomass resulted in diminished effects of the fermentation process.

Keywords: virginia fanpetals, methane fermentation, biogas, energetic biomass

Introduction

In the Member States of the EU, including Poland, a rapid advance is being observed in technologies that enable the practical exploitation of renewable energy sources. One of the key elements of a system based on pure energy is the utilization of biomass, including plants that originate from dedicated energetic crops. Taking into account the currently applied technical and technological solutions for biomass processing, including combustion, gasification, and pyrolysis, a very prospective solution in this respect is the process of methane fermentation. The outcome of this technology is the production of biogas with a high concentration of methane that may be exploited in production processes of electric energy and heat [1].

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One of the restrictions to the extensive application of agricultural gas-works systems is the availability and price of organic substrate which is a feed to bioreactors. Very often, biogas is produced from biodegradable organic wastes originating from the municipal, industrial and agricultural sectors. A number of installations have been operating based on slurry, sewage sludges, wastewaters from the food industry or the organic fraction of municipal wastes. Nevertheless, the quantity of those wastes is not equivalent to the demand for organic substrate and therefore, it is necessary to seek for corresponding alternative amongst energetic crops. The greatest success so far has been achieved from maize silage fermentation, however other plants with a similar energetic potential are still being searched after [2–4].

Biomass Research and the intensive farming of trees, herbaceous plants and aquatic species indicate the specific development. Some of these species appear to be economically viable as energy crops. Commercialization of biomass and waste incineration systems are becoming more popular as a potential replacement of conventional energy sources [2–4].

This study was aimed at determining the technological effectiveness of methane fermentation process of plant biomass from Virginia fanpetals (*Sida hermaphrodita* Rusby) in continuous reactors in terms of the volume of biogas produced and its methane content.

Materials and methods

The experiment was conducted at the Department of Environmental Protection Engineering, University of Warmia and Mazury in Olsztyn. The plant material used in the process of methane fermentation was fresh, pretreated (mechanically disintegrated) biomass of Virginia fanpetals (*Sida hermaphrodita* Rusby). The basic characteristics of the plant substrate was provided in Table 1.

Table 1

Characteristics of biomass applied in the experiment

Parameter	Unit	Virginia fanpetals
Dry matter	mg/g fresh weight	449.0
Mineral matter	mg/g fresh weight	61.4
Organic matter	mg/g fresh weight	337.6

In the experiment, the inoculum to be used in model anaerobic reactors originated from fermentation tanks of an agricultural gas-works fermenting swine slurry and maize silage. The characteristics of the anaerobic sludge applied was presented in Table 2.

The experiment was divided into four experimental series differing in the feeding of reactor's volume with a load of organic dry matter:

Series I – 1.0 kg o.d.m./$m^3 \cdot d$,
Series II – 2.0 kg o.d.m./$m^3 \cdot d$,
Series III – 3.0 kg o.d.m./$m^3 \cdot d$,
Series IV – 4.0 kg o.d.m./$m^3 \cdot d$.

Table 2

Characteristics of anaerobic sludge applied in the experiment

Parameter	Unit	Min. value	Max. value	Mean	Standard deviation
pH	—	7.89	8.08	7.98	0.10
Hydration	[%]	96.40	96.80	96.60	0.20
Dry matter	[%]	3.20	3.60	3.40	0.20
Volatile substances	[% d.m.]	47.32	51.04	49.18	2.63
Ash	[% d.m.]	48.96	52.68	50.82	1.86
CST	[s]	466	479	472.5	9.2

In all the series, the tested plant biomass was prehomogenized using a device for mechanical destruction of organic substrate structures and thereafter, hydrated to a respective level with tap water. The degree of hydration resulted from the adopted technological solutions of the experiment, namely:

– hydraulic retention time: 40 days,

– the feeding of reactor's tank with organic substrate load: in the range of 1.0 to 4.0 kg o.d.m./$m^3 \cdot d$,

– temperature of the process: 37 ^oC.

The basic experimental data of the conducted study are collected in Table 3.

Table 3

Study design under dynamic conditions

Experimental series	Reactor's volume [dm^3]	Expected feedstock of o.d.m. [g o.d.m./$dm^3 \cdot d$]	Biomass quantity [g/d]	Dry matter quantity [g/d]	Organic dry matter quantity [g/d]	Hydration applied [%]	Volume of hydrated biomass [cm^3/d]
Series I	4.0	1.0	10.3	4.83	4.03	95.17	100
Series II	4.0	2.0	20.6	9.66	8.06	90.34	100
Series III	4.0	3.0	30.9	14.49	12.09	85.51	100
Series IV	4.0	4.0	41.2	19.32	16.12	80.68	100

The experiment was carried out with continuous-stirred anaerobic reactors with the active volume of 4.0 dm^3 (total volume of 5.0 dm^3). The initial concentration of anaerobic sludge in the exploited anaerobic tanks was kept at the level of 3.40 g d.m./dm^3. The characteristics of the sludge applied in the experiment was presented in Table 2. The reactors were fixed in a thermo-isolated tank equipped in a heating system and a hot air circulation system (Fig. 1). The reactors were additionally equipped in temperature sensors, stirring system, substrate feeding system, process products discharge system and system for biogas quantity measurements and biogas analysis. In the exploited reactors, the time of heating was remaining in the function of temperature. As a result of heating system operation, the temperature inside the exploited anaerobic reactors was increasing. Following the experimental assumptions, the study was

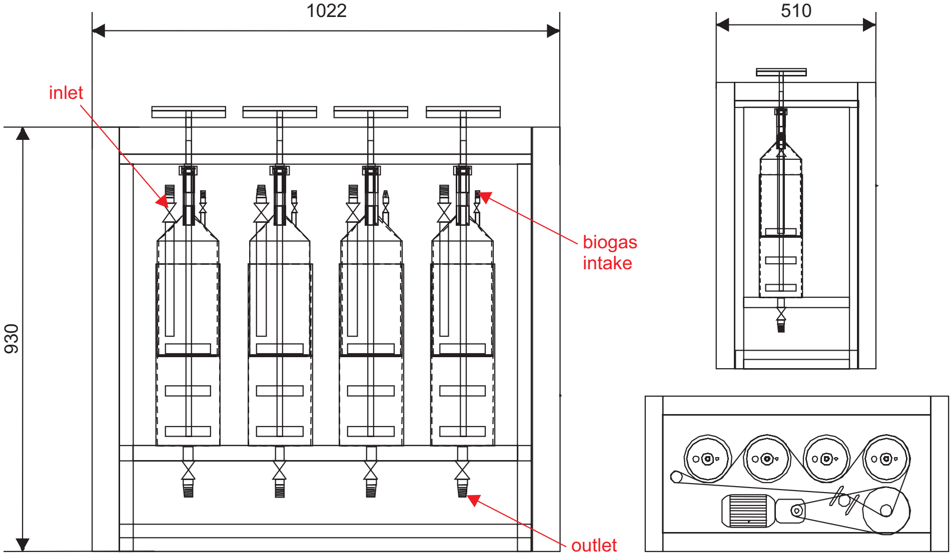

Fig. 1. Scheme of experimental installation used for analyses under dynamic conditions

conducted at a temperature of 37 °C. The heaters' operation was initiated by a thermal controller in direct response to indications of the temperature sensors located inside the exploited model reactor. Once the temperature dropped below the assumed value of 37 °C, the heating systems were initiated. Once the temperature sensor fixed inside the reactor displayed the accurate value of temperature, the supply of heaters was cut off automatically. The hysteresis was adopted as ±1 °C.

The substrate's hydraulic retention time in the reactor reached 40 days in all experimental variants. The loading with a feedstock of organic compounds ranged from 1.0 kg o.d.m./$m^3 \cdot$ d to 4.0 kg o.d.m./$m^3 \cdot$ d. Once a day, 100 cm^3 of fermented substrate were collected and subjected to physicochemical analyses. With the same frequency, the tested organic substrate (Virginia fanpetals, sorghum silage) was fed to the technological system in the quantity of 100 cm^3/day. At the moment of reactor's feeding, the flow in the valve discharging biogas was cut off in order to eliminate adulteration in the quantity of biogas produced.

A temporary or total flow of biogas was measured in a continuous mode using a flowmeter by Allborg SS-Body company. Simultaneously, the qualitative composition of biogas was evaluated daily by means of an Lxi analyzer by GasData company. The inflow and outflow of the substrate was always below the level of sludge in the reactor, so as to avoid introduction of accidental air into the reactor. To this end, the inlet was additionally equipped in a gate. The applied method of reactor's content stirring was based on vertical stirrers operating with the yield of 50 rpm.

The study was focused on determining the effectiveness of biogas production as affected by the technological variant applied. The conducted analyses involved the determination of methane content in gaseous metabolites of anaerobic bacteria as well as effectiveness of biogas production from the plant biomass fed.

Results

In the course of the experiment, the effectiveness of biogas production expressed per Mg (ton) of organic matter fed to the technological system was observed to diminish along with the increasing loading of carbonic compounds feedstock. In the first series, in which reactor's loading reached 1.0 kg o.d.m./$m^3 \cdot d$, the mean quantity of biogas produced accounted for 470 m^3/Mg o.d.m.. This part of the study demonstrated also that a stable level of biogas production effectiveness was achieved after 50 days of technological system exploitation. From the beginning of the experiment, the production of biogas was observed to increase successively from the initial value of *ca* 290 m^3/Mg o.d.m. to *ca* 470 m^3/Mg o.d.m. (Fig. 2). The content of methane in biogas produced ranged from 65 % at the beginning of the experiment to the value of 47 % reached after 50 days of feeding the model fermentation tanks (Fig. 3). The mean yield of methane production in that part of the study accounted for *ca* 230 m^3CH_4/Mg o.d.m. (Fig. 5).

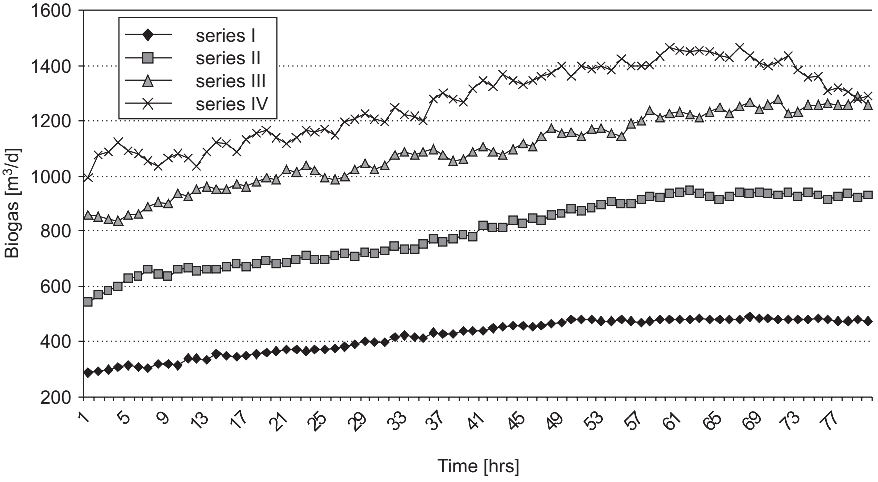

Fig. 2. The efficiency of biogas per Mg (ton) of input to the technological system of Sida organic matter depending on the experiment series

In the second experimental series, in which the system was fed with 2.0 kg o.d.m./$m^3 \cdot d$ of organic substrate, the production of biogas ranged from 280 m^3/Mg o.d.m. at the beginning of the experimental cycle to 460 m^3/Mg o.d.m. at the end of the experiment. This effectiveness of the fermentation process enabled biogas production at a level of *ca* 920 m^3/d (Fig. 2). The ultimate effectiveness of biogas production was achieved after *ca* 60 days of the process. It was found that in this experimental series, the content of methane in the gaseous metabolites of fermentation bacteria was comparable with the results noted in the first series of this stage of the study (Fig. 3). The daily yield of methane production was at a level of 450 m^3CH_4/d (Fig. 4).

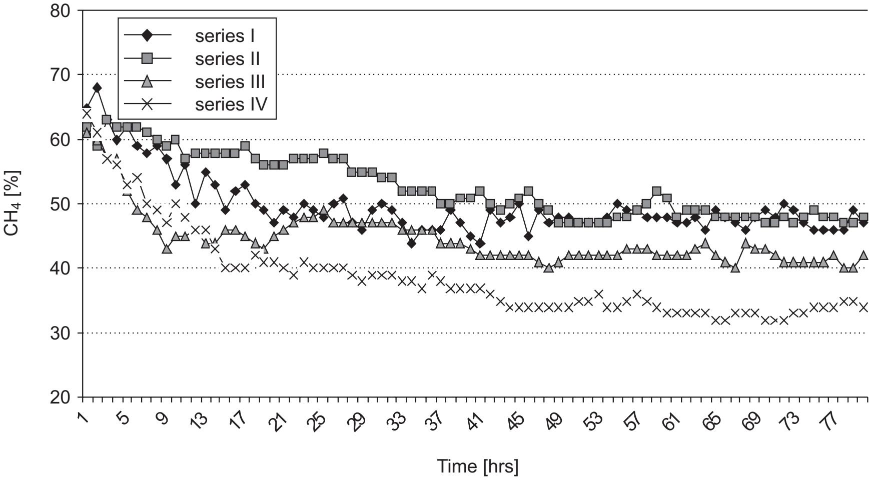

Fig. 3. The content of methane in the biogas produced, depending on the series of experiments

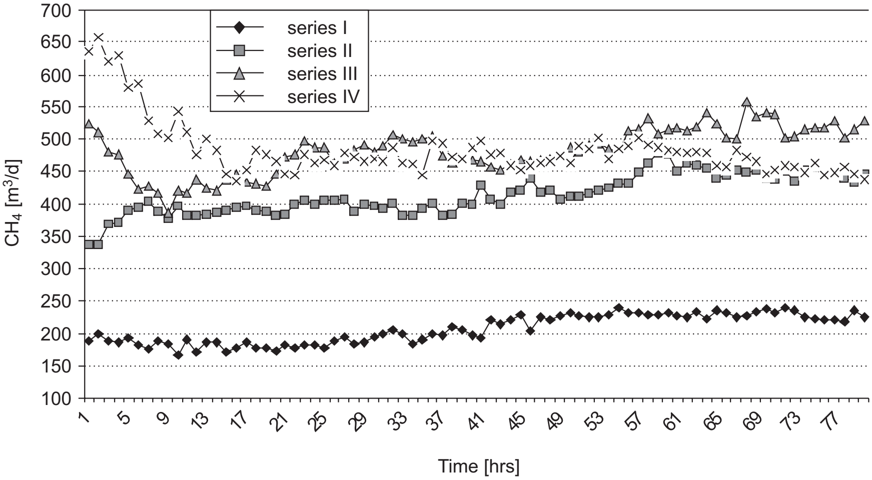

Fig. 4. The efficiency of biogas production per day of biomass of Sida, depending on the experiment series

The lower final effects including the intensity of biogas production and its qualitative composition were observed when the tank's loading with a feedstock of organic compounds reached 3.0 kg o.d.m./$m^3 \cdot$ d. The application of this technological variant was demonstrated to assure biogas production at a level of *ca* 420 m^3/Mg o.d.m., which resulted in reaching the daily production of sewage gas at the level of 1260 m^3/d (Fig. 2). The content of methane in the gaseous phase was negligibly over 40 % (Fig. 3).

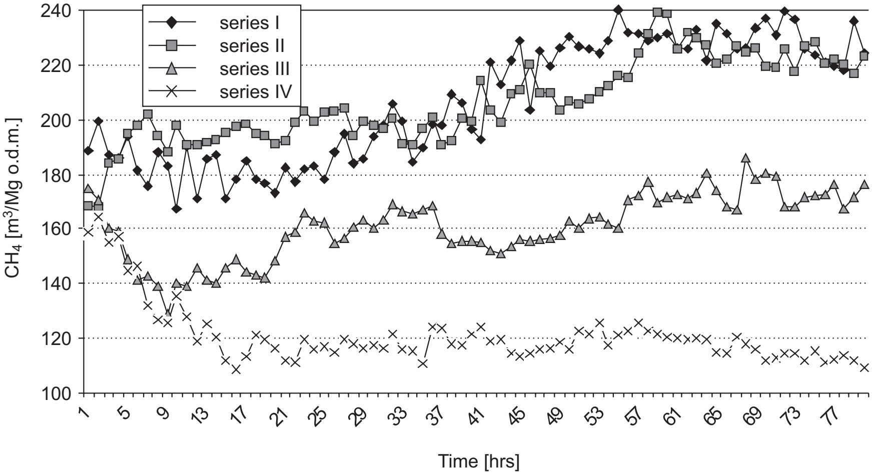

Fig. 5. The efficiency of production of methane per tonne of input to the technological system of Sida organic matter depending on the experiment series

A remarkably lower effectiveness of sewage gas production was obtained in the fourth series with the model reactor's loading of 4.0 kg o.d.m./m^3 · d. In this technological solution, the final effects of methane fermentation were as follows: effectiveness of biogas production at the end of the experiment – 320 m^3/Mg o.d.m., and methane concentration in the gaseous phase – 35 %. In this part of the experiment, the daily effectiveness of methane production accounted for *ca* 450 m^{3}CH$_4$/d and for as little as around 110 m^{3}CH$_4$/Mg o.d.m. (Fig. 4, 5).

Discussion

In many publications are presented experiments in which the authors present the possibility of processing of biomass energy with different characteristics in the process of methane fermentation. In one of the experiments were used corn stalks, oat straw and wheat straw. Tests were conducted under anaerobic conditions at 37 ^{o}C. Biomass used has undergone a process of anaerobic digestion of manure and swine. The best results were achieved for corn stalks where the amount of produced biogas was 12 dm^3/d gives the percentage of methane in the biogas 68 %. For the oat straw and biogas production yield obtained oscillate around the level of 9 dm^3/d at 57 % methane content. The results achieved in the fermentation of Sida, which recorded the best performance of biogas of approximately 650 m^3/d at a load of 1.0 kg · d smo/m^3 during the first 15 days of process [5].

Weiland presents the possibility of obtaining biogas from plants such as ryegrass, clover or alfalfa. The amount of harvested biogas ranges from 300 to 400 m^{3}CH$_4$/Mg of tar depending on technological solutions and the type of raw material. In the case of an investigation to determine the efficiency of biogas production values are oscillating at a level lower than 230 m^3CH_4/Mg of tar in the first series with a load of 1.0 kg · smo/m^3 [6].

Dinuccio fermenting biomass in the form of corn, straw, rice, grapes, whether obtained in the biogas methane content at 50–60 %. The content of methane obtained from Sida as a result of anaerobic fermentation in this study ranges on average from 30 to nearly 70 % taking into account the whole experiment. However, focusing on the series I and II, which gave the best quality parameters of biogas can be compared with results obtained by the earlier article [7].

Zheng and others used in the process of anaerobic digestion of two types namely wild rygrass and Leymus triticoides. The use of hydraulic retention time of 33 days obtained 251 m^3 of biogas/Mg tar methane content of 65 % [8]. These figures are lower than those obtained by the authors in the fermentation process Sida, where the load of 1.0 kg · d smo/dm^3 reported productivity 470 m^3/Mg of tar the highest methane content of 65 % and its average production rate was very comparable and amounted to 230 m^3CH_4/Mg tar.

Fermentation of leafy grass (Alterniflora Spartina) at 35 ^oC and retention time of 60 days allowed to obtain 80 % efficiency of biogas. At the end of the experiment the total biogas yield was 358 dm^3/kg^{-1} solids. The content of methane has increased from 53 to 61 % from 3 to 13 days to the end of the process and remained practically at the level of 62 % [9].

Others of the authors took into account the corn, cereals (triticale, rye and wheat) and grass. Plants were grown on 60 ha and the parameters that were determined in the experiment were the biogas and methane yield, quality, biogas, biomass processing and energy, biogas and coal for energy. Duration of the study was 6 weeks, during which time biogas was tested 10 times, capacity was 1 dm^3 chambers and the temperature of process 38 ^oC. Research has shown failed to reach an average methane yield of 398 dm^3 CH_4/kg^{-1} corn solids. In the case of mixtures of cereals were the values from 140 to 343 dm^3 CH_4/kg^{-1} solids. However, for grass, this value was in the range 190–392 dm^3 CH_4/kg^{-1} [10].

Conclusions

The study demonstrated that the achieved technological effects including the quantity of biogas produced and its methane content were directly affected by the applied loading of the anaerobic tank with a feedstock of organic compounds. It is especially tangible in the case of biogas production yield and in respect of the substrate organic matter fed to the tank. The highest level of methane production expressed per Mg of organic matter fed to the reactor, *ie* 240 m^3CH_4/Mg o.d.m., was noted with the loading range of 1.0 kg o.d.m. /m^3 · d to 2.0 kg o.d.m./m^3 · d. The application of the higher values of this technological parameter affected directly a decrease in methane production. Analogous results were obtained for the percentage content of methane in biogas produced. Values approximating 50 % were achieved in the first and second experimental series. In the case of the higher loading of the tank with the feedstock of organic matter, a significant decrease was observed in the content of methane in biogas.

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EFEKTYWNOŚĆ PROCESU FERMENTACJI METANOWEJ ŚLAZOWCA PENSYLWAŃSKIEGO (*Sida hermaphrodita* Rusby) W WARUNKACH MEZOFILOWYCH

Katedra Inżynierii Ochrony Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

Abstrakt: Celem badań było określenie efektywności technologicznej procesu fermentacji metanowej biomasy roślinnej ślazowca pensylwańskiego (*Sida hermaphrodita* Rusby) w reaktorach o pracy ciągłej w temperaturze 37 °C. Eksperyment podzielono na cztery serie badawcze różniące się wielkością obciążenia objętości reaktora ładunkiem *suchej masy organicznej* w zakresie od 1,0 kg s.m.o./m^3 · d do 4,0 kg s.m.o./m^3 · d. Najwyższe efekty technologiczne związane z ilością oraz składem jakościowym powstającego biogazu obserwowano w zakresie obciążeń od 1,0 kg s.m.o./m^3 · d do 2,0 kg s.m.o./m^3 · d. Wprowadzenie do reaktorów większych ilości biomasy skutkowało obniżeniem uzyskiwanych efektów procesu fermentacji.

Słowa kluczowe: ślazowiec pensylwański, fermentacja metanowa, biogaz, biomasa energetyczna

Varia

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