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CONTENTS

VARIA

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VARIA

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Łukasz BĄK[1*], Jarosław GÓRSKI[1]
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CONTENT OF HEAVY METAL COMPOUNDS IN BOTTOM SEDIMENTS OF THE SUCHEDNIOW WATER RESERVOIR

ZAWARTOŚĆ ZWIĄZKÓW METALI CIĘŻKICH W OSADACH DENNYCH ZBIORNIKA SUCHEDNIÓW

Abstract: Water reservoirs become silted at various intensity levels. Within the reservoir bowls, both allochtonic (built up outside the sedimentation area) and autochtonic (built up in the sedimentation area) matter is accumulated. As a result, reservoirs need desilting after a while. Then a problem arises how to manage the sludge removed from the reservoir bottom. The chemical properties of the bottom sludge, and particularly the content of heavy metals, decide whether it will be possible to use the sludge, and in what way. The chemical properties of the bottom sludge depend, to a far extent, on the character of the reservoir basin, the level of its urbanisation, and also on the climatic conditions. The paper presents the results of investigations into the content of heavy metals in the bottom sediments in the Suchedniow water reservoir. This water body is characterised by small mean depth of 1.05 m and mean annual flow across the dam profile of 0.63 $m^3 \cdot s^{-1}$. Forests dominate in the reservoir basin covering 45 % of its area, arable land constitutes 18 %, and the percentage of built-up area does not exceed 5 %. In recent years (2009–2011), the water reservoir has become much silted because of storing large soil masses near the local watercourses during the construction of S7 expressway. The amount of stored soil is estimated at 7.8 thousand m^3. For investigations, nine bottom sediments samples were collected, in which the content of the following heavy metals: Pb, Cr, Cd, Cu, Ni, Zn, Fe, and Mn was determined. Quasi-undisturbed sludge was taken into transparent cylinders with Eijkelkamp sampler, which made it possible to conduct analysis in sediment layers 20 cm in height. In order to evaluate the sediment pollution with heavy metals, the geoaccumulation index, the pollution coefficient and level were calculated. On the basis of admissible soil chemical pollution tables, the possibility of the sludge use in agriculture after extracting it from the reservoir bowl was assessed.

Keywords: reservoir, reservoir basin, bottom sediments, heavy metals

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Mineral, organic material and chemical pollutants transported by water are accumulated in water reservoirs. The amount of sediment deposits delivered and permanently retained in the reservoir, and also amount and type of pollution are determined by many factors. Those include: the hydrological regime of the basin, erosion processes, soil types, management and use of the basin area, and anthropogenic activity. Heavy metals, transported by river waters, are accumulated mainly in the bottom deposits due to sedimentation and sorption processes [1]. Heavy metals can penetrate to the aquatic environment with industrial and municipal sewage, and also with precipitation wastewater – as runoff from fields and meadows (containing chemical fertilisers, plant protection chemicals), transportation infrastructure, or the products of dust emission into the atmosphere [2, 3]. Precipitation wastewater contains zinc and lead, which occur in highest concentrations, copper and chromium, which have slightly lower concentrations, and trace amounts of cadmium and nickel. Transportation routes are the main source of pollution with heavy metals [4–7]. Uncontrolled introduction of heavy metals into the aquatic environment poses a particular hazard, even if they are delivered periodically and in small amounts. The threat results from their stability (they do not undergo biodegradation) and ability to accumulate in sediments and living organisms (plants, animals) [8]. Conducting tests on the content of heavy metals in bottom sediments is especially important for small water reservoirs, which are usually not covered by the State Environmental Monitoring, and in which the rate of silting is high enough to cause problems related to the management of removed silt.

One of the first studies on the chemical composition of bottom sediments was presented by Pasternak and Glinski [9]. Basing on the tests carried out on samples from twelve largest dammed reservoirs in southern Poland, the authors found out that the Turawa Reservoir on the river Mala Panew was the most polluted water body. The content of vanadium and zinc in its bottom sediments was 371 ppm and 135 ppm, respectively. Investigations into bottom sediments in one of Poland's largest reservoirs (Wloclawek Reservoir) indicate that the bottom material is strongly polluted with heavy metals (Table 1). Cadmium, chromium, copper, lead, nickel contents exceed geochemical background values many times. The calculations made by Bojakowska et al [10] show that the reservoir fine grained sediments contain approx. 190 Mg of cadmium, 7.600 Mg of chromium, 4.200 Mg of copper and 2.200 Mg of lead. The presence of caesium, uranium and radium is also detected in the sediments [11, 12].

For small dammed reservoirs located in the basins where forests and arable land take up a substantial part of the area (*eg* Krempna-2, Niedzwiadek, Cierpisz reservoirs) generally lower levels of trace elements are observed in the bottom material than it is the case with large water bodies (Table 1). Small reservoirs located in urban areas, in contrast, can be characterised by elevated content of some heavy metals. That is caused by pollutants that come from roads, residential areas, tourist facilities and are discharged with the surface runoff. The Kielce Reservoir, in whose vicinity busy transportation routes are located, is an example of such category. Bottom deposits of this reservoir show a high content of lead and cadmium (lead – 151.9 $\mathrm{mg \cdot kg^{-1}}$, cadmium – 14.5 $\mathrm{mg \cdot kg^{-1}}$), the source of which definitely lies in transportation. A high

Table 1

Content of heavy metals in bottom sediments in for selected dammed reservoirs

Sample collection site	Pb	Cr	Cu	Mn	Ni	Zn	Cd
	[mg · kg^{-1}]						
Mlyny Reservoir [13]	18.2	—	32.1	—	7.0	90.7	—
Kielce Reservoir [14]	151.9	34.9	14.4	—		41.1	14.5
Krempna-2 Reservoir [15]	1.7	10.4	50.8	—	52.9	76.5	0.3
Cierpisz Reservoir [15]	5.4	17.3	1.9	—	7.4	64.6	0.9
Maziarnia Reservoir [15]	9.5	11.5	5.2	—	9.3	24.6	0.3
Niedzwiadek Reservoir [15]	23.8	29.9	27.6	—	23.7	157.8	1.3
Besko Reservoir [16]	41.6	55.2	30.6	0.6	49.7	116.2	0.8
Psurow Reservoir [1]	18.2	—	32.1	—	7.0	90.7	—
Dzierzno Male Reservoir [17]	106.6	—	27.1	—	22.9	560.0	—
Wloclawek Reservoir [18]	5.5–50.1	—	—	114–3024	—	—	—
Reservoirs in Saxony, Germany [19]	130.0	—	75.0	—	57.0	490.0	—
Flumendosa Lake, Sardinia [20]	202.6	—	136.8	—	—	—	4.8
Guan-Ting Reservoir, China [21]	129.7	—	42.4	—	—	117.8	2.5

content of zinc (560.0 mg · kg^{-1}) and lead (106.6 mg · kg^{-1}) is noticeable in the sediments of the Dzierzno Male Reservoir (Table 1). That can be caused by discharge of industrial sewage from plants located in the basin area, which has continued for many years [17].

In the case of the Suchedniow reservoir, anthropogenic pollution sources include mainly domestic sewage carried from unsewered areas, and also agricultural runoffs. In the years 2009–2010, works related to the construction of S7 expressway were conducted in the Kamionka river catchment. During construction works, a significant increase in the volume of silt deposited in the reservoir bowl (7828 m^3 in total) and water quality deterioration (high content of mineral suspension) were observed [22]. The investigations aim to determine the content of heavy metals in the bottom sediments and to map their spatial distribution in the Suchedniow reservoir bowl.

Material and methods

Object description

The Suchedniow reservoir is located in the Suchedniow commune, Skarzysko county, Świętokrzyskie province. It was constructed in the years 1965–1974 by damming the river Kamionka (right-bank tributary of the river Kamienna) with earth-filled structures km 7 + 754. The reservoir has not been de-silted since when it was put in service. The thickness of sediments deposited in the reservoir ranges from 0.15 m (near the reservoir discharge system) to 1.2 m in the upper part of the reservoir. This water body is characterised by small mean depth of 1.05 m and mean annual flow across the dam profile of 0.63 m^3 · s^{-1}. At the normal water damming level of 258.0 m above sea level, the surface area of the water table is 21.40 ha, and the volume – 226000 m^3 [23].

The area of the Kamionka river basin, closed with the Suchedniow dam, is 83 km^2. Forests dominate in the reservoir basin covering 45 % of its area and arable land constitutes 18 %. Although the percentage of built-up area is small and does not exceed 5 %, because of a very elementary sewerage level, domestic sewage constitutes a potential source of pollution for the river Kamionka. Low-fertility soils within the basin are represented by: brown soils of light and medium boulder clays, podzolic soils of sands and loose gravels, poorly clayey and clayey soils of aquagenic loams [23]. The annual mean precipitation in the basin is 750 mm [24].

Investigation methodology

Samples of bottom sediments from the Suchedniow reservoir were collected in June 2012, in the thirty-eighth year of its service. With the use of boats, the samples were collected into the transparent cylinders with Ejkelkamp sampler, which allows silt sampling at quasi-undisturbed state. The samples to be studied were extracted from the following sites: dam-adjacent reservoir part (sample No. 1), along the mainstream running to the right of the island (samples No. 2, 6, 7), a tributary (sample No. 8), and from the south-western part of the bowl (samples No. 3–5, 9), where marina for water sports equipment and a beach are situated. Fig. 1 shows the location of sites, at which sediment samples were collected. After transporting the cylinders to the laboratory, the collected material was divided into layers 20 cm in thickness, from which identical sediment volumes were taken in order to average them. For each averaged sediment sample, the total content of selected heavy metals (Pb, Cr, Cd, Cu, Ni, Zn, Fe, Mn) was determined.

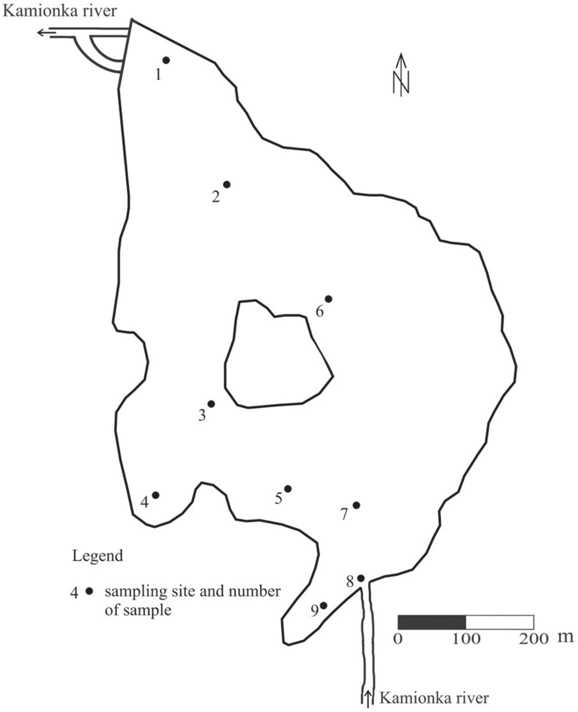

Fig. 1. Location of sites of bottom sediment sample collection

The samples thus prepared were dried to constant mass at 60 ^{o}C to prevent the growth of mould and microorganisms, which leads to the redistribution of chemical components and changes in isotopic composition. Dried samples were ground using an automatic mill until < 0.063 mm size fraction was obtained. Then they were subjected to microwave-assisted total mineralisation, using multi-stand Multiwave 3000 microwave mineraliser. The content of trace elements, depending on the concentration levels and the matrix obtained, was determined with AAS techniques with the use of SavantAA Sigma and SavantAA Zeeman atomic absorption spectrometers.

In accordance with the adopted geochemical method [25], the following indicators were employed: geoaccumulation index (I_{geo}), pollution coefficient (C^{i}_{f}) and pollution level (C_{deg}) to assess the pollution of the bottom sediments with heavy metals. The geoaccumulation index (I_{geo}) makes it possible to evaluate pollution by comparing current amounts of heavy metals in bottom sediments with the so-called pre-industrial concentrations [26]:

$$I_{geo} = \log_{10}\left(\frac{C_n}{1.5B_n}\right) \tag{1}$$

The following contents of trace elements in sediments were assumed to constitute the geochemical background: lead – 15 mg · kg^{-1}, chromium – 6 mg · kg^{-1}, cadmium – 0.5 mg · kg^{-1}, copper – 7 mg · kg^{-1}, nickel – 5 mg · kg^{-1}, zinc – 73 mg · kg^{-1}.

The pollution of the bottom sediments was assessed using such parameters as: pollution coefficient (C^i_f) and pollution level (C_{deg}) [27], which were calculated from the following dependence:

$$C^i_f = \frac{C^i_{0-1}}{B_n} \tag{2}$$

The pollution level (C_{deg}) of a site/area is expressed as the sum of individual coefficients C^i_f. Also, the possibility of the sediment use for farming, following its extraction from the reservoir bowl, was examined on the basis of tables of permissible level of soil pollution with chemicals.

As all the series of results concerning the content of the studied metals in sediments did not have normal distribution, the assessment of the strength of statistical relations between examined variables (averaged values of pollution indicators) was made on the basis of Spearman rank correlation.

Results and discussion

Results of investigations into the content of selected trace elements in averaged samples of bottom sediments collected from the Suchedniow reservoir are presented in Table 2.

Table 2

Heavy metal content in the Suchedniow reservoir bottom sediments collected in June 2012

No.	Pb	Cr	Cd	Cu	Mn	Ni	Zn	Fe
	[mg · kg^{-1}]							
1	75.68	63.92	10.75	42.97	621.80	29.38	216.94	20835
2	101.23	58.39	6.47	28.95	606.30	31.41	203.35	10911
3	86.92	37.36	8.24	26.39	499.76	31.38	196.38	19827
4	59.39	39.01	8.41	29.03	522.96	29.11	184.35	14298
5	72.22	40.02	9.28	33.25	674.23	29.01	195.29	11004
6	110.02	38.41	5.24	36.07	696.34	27.34	177.49	18005
7	96.23	49.35	5.02	29.11	557.27	29.34	196.35	21948
8	105.49	48.02	8.14	29.57	535.91	30.06	204.42	17324
9	104.24	45.11	10.47	32.95	698.45	31.21	209.56	17907

The analysis of the amounts of heavy metals in averaged samples indicates that the lowest variation of the obtained results is observed for nickel. The content of this element in the sediments did not exceed 32.00 mg $\cdot$ kg^{-1}. For chromium and copper, the values ranged 26.39–63.92 mg $\cdot$ kg^{-1}. Lead content ranged 59.38–110.02 mg $\cdot$ kg^{-1}, and that of zinc and manganese – 177.49–698.45 mg $\cdot$ kg^{-1}. The highest variation in the studied metals was observed for cadmium and iron. The content of these elements ranged substantially – from 5.02 to 10.75 mg $\cdot$ kg^{-1} for cadmium and 10911–21948 mg $\cdot$ kg^{-1} for iron.

As regards the content of heavy metals under consideration, the bottom sediments in the Suchedniow reservoir are characterised by high spatial differentiation (Fig. 2 and 3). The largest amounts of chromium, zinc, copper and cadmium were found in sample No. 1, collected at the front of the reservoir dam. The highest content of iron was revealed in samples No. 1 and 7 (the lower and the upper part of the reservoir, respectively), of nickel – in sample No. 3, and those of manganese and lead – in sample No. 6 (middle part of the reservoir). The lowest values of chromium, manganese and copper were found in sample No. 3, of zinc and nickel – in sample No. 6, of lead at stand No. 4, and those of cadmium and iron in samples No. 7 and 2, respectively.

The content of lead, chromium, cadmium, nickel and zinc in the bottom sediments of the Suchedniow Reservoir is much higher than the content of those metals in a majority of small storage reservoirs (Cierpisz, Maziarnia, Niedzwiadek, Mlyny), at the same time it is comparable with the values recorded for the Kielce Reservoir (Table 1). Such concentration values may indicate an occurrence of additional pollution sources in the basin, resulting in elevated heavy metal content. Municipal sewage discharged into the river Kamionka or rain wastewater from the street drainage of the town of Suchedniow may be such a source. During the site inspection, in a number of locations, the authors revealed illegal municipal sewage discharge into the river Kamionka upstream the reservoir. However, it proved impossible to collect samples for the analysis of heavy metal content. Both Kielce and Suchedniow water bodies share some characteristics, they lie in urban areas, busy transportation routes, residential areas and recreational facilities are situated nearby, which can explain the congruence of the data obtained for both reservoirs. Only in comparison with large reservoirs, namely Flumendosa, Guan-Ting and Saxony, the sediments under consideration show better results as regards Pb, Cu and Ni. The content of manganese rarely becomes a subject of investigations, therefore it is difficult to draw any satisfactory conclusions concerning the pollution with this element. An average content of manganese in Suchedniow bottom sediments equals 601 mg $\cdot$ kg^{-1} (Table 2) and is comparable with the mean value (780 mg $\cdot$ kg^{-1}) obtained based on 15 samples collected in the years 2008–2009 from the Wloclawek Reservoir [18] and 1000 times higher than the manganese content in the Besko Reservoir sediments [16] – Table 1.

During the 32-year service time, 78000 m^3 of sediments were deposited in the reservoir bowl [22]. On the basis of the assumptions on average heavy metal content in sediments (Table 2), calculations were made for individual elements. The results (in Mg) amount to: iron – 1.529, chromium – 4.2, zinc – 17.9, nickel – 2.7, copper – 54.4, lead – 2.9, and cadmium – 0.7.

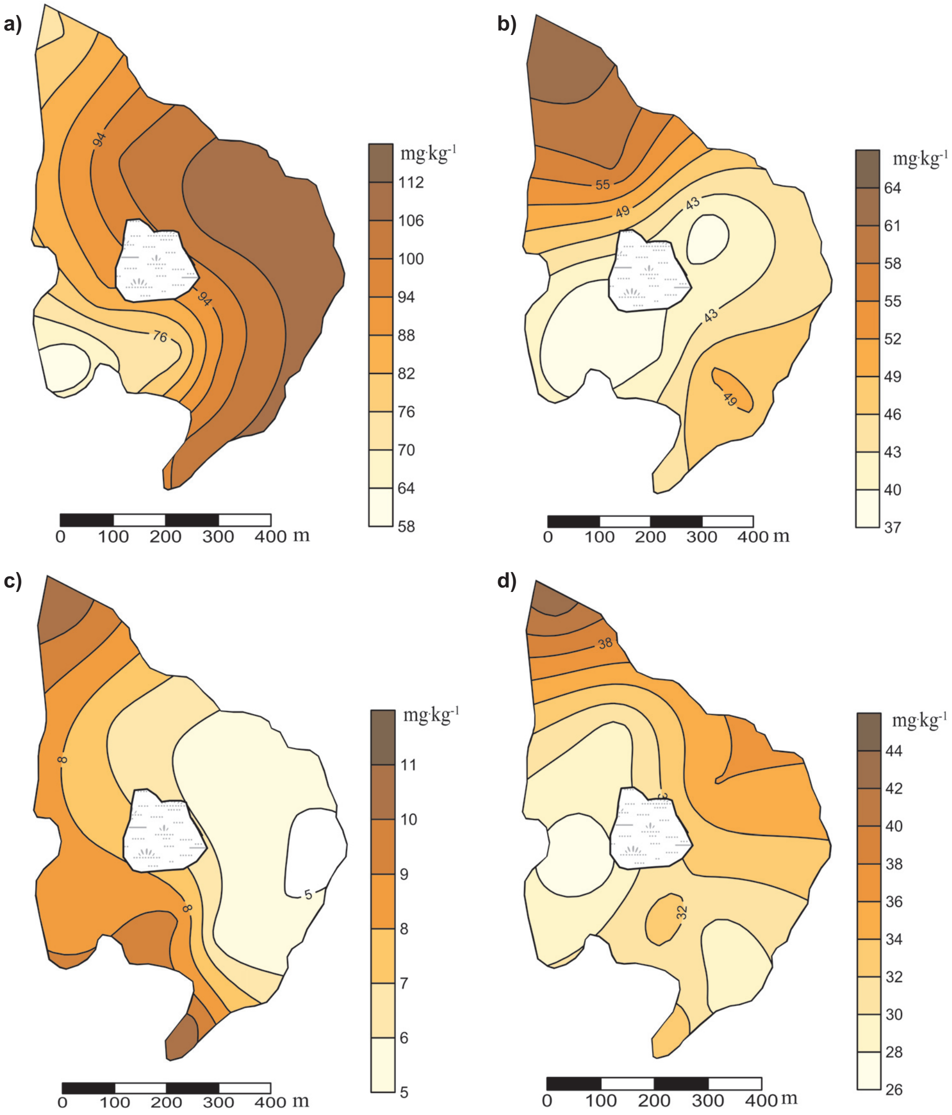

Fig. 2. Spatial distribution of heavy metal content in averaged samples of the Suchedniow reservoir bottom sediments: a) Pb, b) Cr, c) Cd, d) Cu

For all metals, except for zinc-nickel and manganese-copper pairs, no strong or very strong statistically significant relation at the level of $p = 0.05$ was found between results at individual points. For Zn-Ni and Mn-Cu pairs of metals, high correlation ($r = 0.68$–0.73) statistically significant at the level of $p = 0.05$ was observed.

The geoaccumulation index for studied metals, except for cadmium, ranged 0–1, which indicates low pollution of bottom sediments with those metals. As regards

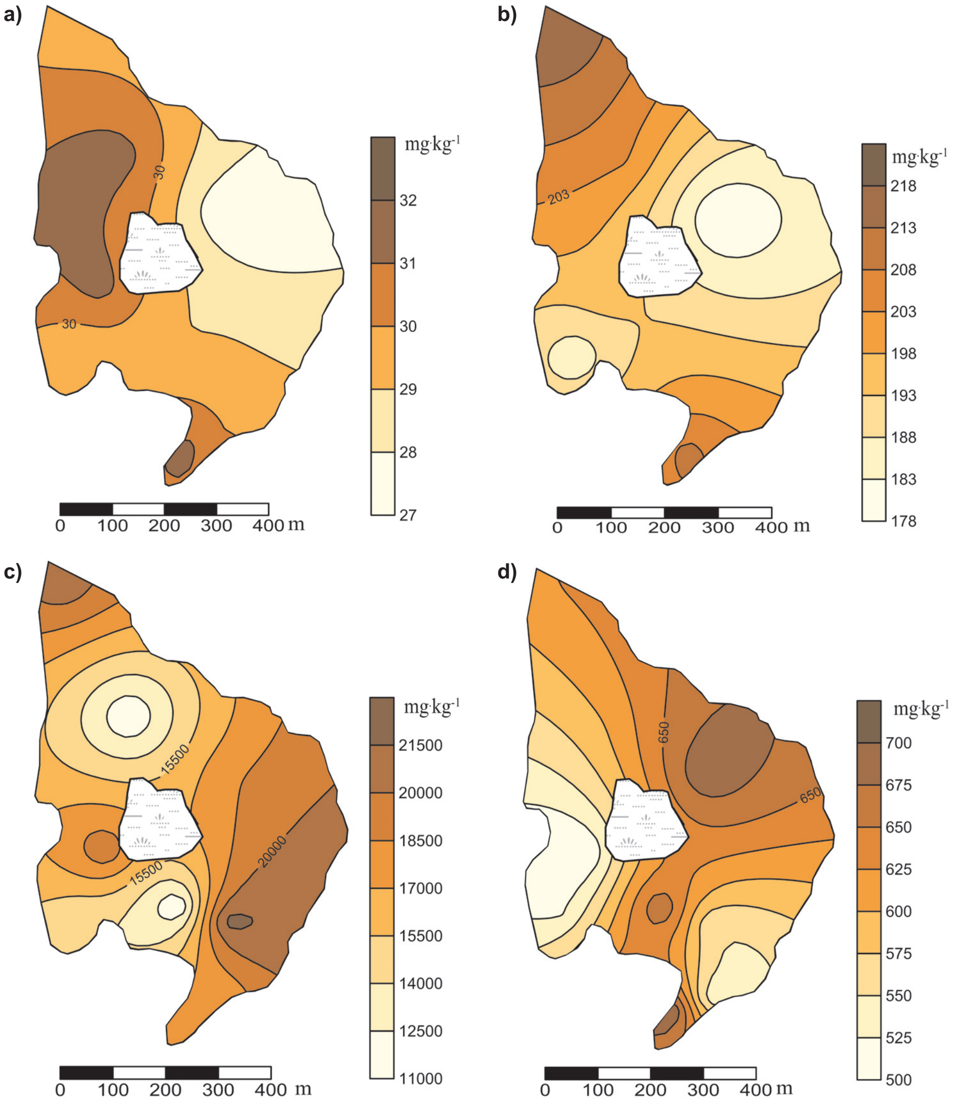

Fig. 3. Spatial distribution of heavy metal content in averaged samples of the Suchedniow reservoir bottom sediments: a) Ni, b) Zn, c) Fe, d) Mn

cadmium, the index exceeds the value 1 for samples No. 1, 3–5, 8, 9 (Table 3). That points to moderate sediment pollution with this element.

The pollution coefficient makes it possible to categorise sediments into groups depending on how many times the actual values are greater than those characteristic of the geochemical background. In accordance with the criterion established by Hakanson [27], the studied silts demonstrate a low pollution coefficient for Cr, Ni, Zn, and a moderate one for Pb, Cu. As regards Cd, the coefficient value is substantial, and very high in the case of sample No. 1. The pollution level ranges 6–12 (Table 3), which following the HÍkanson classification [27], indicates a moderate level of pollution with heavy metals.

Table 3

Geoaccumulation index, pollution coefficient and pollution level for individual trace elements in averaged samples of the Suchedniow reservoir bottom sediments

No.	Pb	Cr	Cd	Cu	Ni	Zn	Pb	Cr	Cd	Cu	Ni	Zn	C_{deg}
	I_{geo}						C_f^i						
1	0.53	0.85	1.16	0.61	0.59	0.30	1.76	0.34	6.57	1.22	0.61	0.77	11.27
2	0.65	0.81	0.94	0.44	0.62	0.27	1.77	0.18	5.98	1.69	0.59	0.33	10.55
3	0.59	0.62	1.04	0.40	0.62	0.25	1.35	0.17	2.08	2.18	0.20	0.46	6.44
4	0.42	0.64	1.05	0.44	0.59	0.23	1.22	0.16	3.16	1.04	0.21	0.53	6.32
5	0.51	0.65	1.09	0.50	0.59	0.25	1.20	0.19	3.45	1.29	0.39	0.64	7.16
6	0.69	0.63	0.84	0.54	0.56	0.21	1.29	0.23	3.25	1.25	0.30	0.86	7.18
7	0.63	0.74	0.83	0.44	0.59	0.25	1.41	0.16	4.02	1.20	0.19	0.61	7.60
8	0.67	0.73	1.04	0.45	0.60	0.27	1.89	0.17	5.68	2.12	0.21	0.66	10.73
9	0.67	0.70	1.14	0.50	0.62	0.28	2.42	0.21	5.38	2.23	0.34	0.92	11.50

In accordance with water sediment classification developed by the Polish Geological Institute (PIG) [28], bottom sediments of the Suchedniow reservoir were categorised as belonging to Class II (slightly polluted sediments) due to the content of Cr, Cu, Ni, Zn, to Class III (polluted sediments) because of the amount of Pb, and to Class IV (heavily polluted sediments) as a result of the content of Cd.

Analysing the quality of the bottom sediments in the Suchedniow reservoir with respect to the Regulation of the Minister of Environment of 16th April 2002 [29], it can be stated that in samples No. 1, 3, 4, 5, 8, 9, cadmium content exceeded the limit value. Consequently, the sediment should be treated as polluted. In accordance with the requirements set in the Regulation of the Minister of Environment of 9th Sept 2002 [30], however, it can be claimed the sediments, depending on the content of studied heavy metals, comply with the quality standards for A or B group soils. As a result, they are deemed to pose no threat to the soil environment, and therefore may be used for farming land, forest land and wastes.

Conclusions

1. The sediments in the Suchedniow reservoir are polluted with heavy metals, the content of which shows high spatial differentiation. In sediments collected near the dam front, the highest values were found for: Cr, Zn, Cu, Cd and Fe, whereas in the upper parts of the reservoir – for Pb, Cd, Ni and Fe.

2. The content of lead, chromium, cadmium, nickel and zinc in bottom sediments of the Suchedniow Reservoir is higher than in other Poland's small reservoirs with the Dzierzno Male Reservoir being the only exception, and comparable with that in the Kielce Reservoir. That indicates the presence of additional (municipal) source of pollution in the Kamionka River basin, which is largely agricultural in character.

3. The assessment of the bottom sediment pollution using the geoaccumulation index, pollution coefficient, pollution level, and the classification developed by the Polish Geological Institute and stated in the Regulation of the Minister of Environment demonstrate that with respect to the content of studied trace elements, the sediments are polluted to a different extent. Depending on the adopted criteria, bottom sediments can be classified as moderately to strongly polluted. Comparing the content of studied metals with adopted geochemical background it can be noted that Cr, Cu, Ni, Zn values do not exceed those of the background. Only Pb and Cd values are higher than those of the background. The share of the latter in the total pollution of the bottom sediments is the highest.

4. Only for Zn-Ni and Mn-Cu pairs of metals, the occurrence of strong or very strong correlation was found, which was statistically significant at the level of $p = 0.05$.

Nomenclature

I_{geo}	geoaccumulation index [-]
C_n	content of a given metal in the bottom sediment [$mg \cdot kg^{-1}$]
$1.5B_n$	geochemical background (B_n) and natural fluctuations in the content of a given metal in the environment, with small anthropogenic impact (1.5) [$mg \cdot kg^{-1}$]
C^i_f	pollution coefficient [-]
C^i_{0-1}	metal content in the bottom sediment [$mg \cdot kg^{-1}$]
C_{deg}	pollution level [-]

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ZAWARTOŚĆ ZWIĄZKÓW METALI CIĘŻKICH W OSADACH DENNYCH ZBIORNIKA SUCHEDNIÓW

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Abstrakt: Zbiorniki wodne ulegają zamuleniu z różną intensywnością. W obrębie ich czasz akumulowany jest materiał alochtoniczny (powstały poza obszarem sedymentacji) jak również autochtoniczny (utworzony w miejscu sedymentacji). W związku z powyższym po pewnym czasie wymagają one odmulenia. Powstaje wówczas problem zagospodarowania osadów wydobytych z dna zbiornika. Możliwość i sposób wykorzystania osadów dennych zależy od ich cech chemicznych, a zwłaszcza od zawartości metali ciężkich. Właściwości chemiczne osadów zależą w dużej mierze od charakteru zlewni zbiornika, stopnia jej zurbanizowania, jak również warunków klimatycznych. W pracy przedstawiono wyniki badań zawartości metali ciężkich w osadach dennych zbiornika Suchedniów. Akwen ten charakteryzuje się niewielką głębokością średnią, wynoszącą 1,05 m oraz średnim rocznym przepływem w profilu zapory równym 0,63 $m^3 \cdot s^{-1}$. Na obszarze zlewni dominują lasy – 45 % powierzchni zlewni, pola orne – 18 %, a udział terenów zabudowanych nie przekracza 5 %. W ostatnim okresie (2009–2011), na skutek składowania w pobliżu lokalnych cieków mas ziemnych, powstałych podczas budowy trasy ekspresowej S-7, doszło do jego intensywnego zamulenia, a ilość odłożonego materiału oszacowano na około 7,8 tys. m^3. Do badań pobrano 9 próbek osadów, w których oznaczono zawartość następujących metali ciężkich: Pb, Cr, Cd, Cu, Ni, Zn, Fe, Mn. Osad pobrano w stanie quasi-nienaruszonym, do przezroczystych cylindrów, za pomocą próbopobieraka „Eijkelkamp”, co umożliwiło wykonanie analiz w warstewkach osadu o wysokości 20 cm. W celu oceny stanu zanieczyszczeń osadów metalami ciężkimi obliczono indeks geoakumulacji, współczynnik oraz stopień ich zanieczyszczenia. Określono także (na podstawie tabel dopuszczalnego, chemicznego zanieczyszczenia gleb) możliwość rolniczego wykorzystania osadów po ich wydobyciu z misy zbiornika.

Słowa kluczowe: zbiornik, zlewnia, osady denne, metale ciężkie

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IMPACT OF THE WATER TABLE FLUCTUATIONS ON THE APPARENT THICKNESS OF LIGHT NON-AQUEOUS PHASE LIQUIDS

WPŁYW WAHAŃ POZIOMU ZWIERCIADŁA WODY PODZIEMNEJ NA MIĄŻSZOŚĆ POZORNĄ LEKKICH CIECZY ORGANICZNYCH

Abstract: The light non-aqueous phase liquids (LNAPLs) percolating into the subsurface from damaged underground storage tanks, pipelines cisterns and from the unsealed landfills present a threat to soil and groundwater. If a layer of LNAPL floats on the groundwater table the initial step of remediation should be its recovery. In order to a proper design of LNAPL recovery an assessment of the mobile LNAPL volume is required. This volume can be determined on the base of a thickness of LNAPL layer in the porous medium (the actual thickness) or on the base of LNAPL specific volume. Then the LNAPL actual thickness and specific volume are estimated on the base of the LNAPL thickness measured in the monitoring well (the apparent thickness). Unfortunately, the actual LNAPL thickness is always different from the apparent LNAPL thickness. This difference depends on the properties of soil and the amount and properties of LNAPL. Additional factors influencing significantly the difference between apparent and actual thicknesses are the fluctuations of the water table level (the hydraulic head fluctuations).

The major objectives of this study became to investigate the impact of hydraulic head fluctuations on the measured apparent and actual LNAPL thicknesses. Obtained results show that when the hydraulic head diminishes, the apparent thickness of LNAPL increases and the actual thickness decreases. Instead, when the hydraulic head again rises, the apparent thickness decreases, and the actual thickness increases. When the hydraulic head rises considerably it can take place that the free product don't be present in the observation well. The results affirm that hydraulic head fluctuations complicate considerably the estimation of the actual thickness and the mobile volume of LNAPL on the base of the apparent LNAPL thickness measured in the observation well.

Keywords: LNAPL, actual thickness, apparent thickness, hydraulic head, groundwater table fluctuations

The light non-aqueous phase liquids (LNAPL) percolate into the subsurface from damaged underground storage tanks, pipelines and cisterns [1]. A certain amount of hydrocarbons accesses the water environment with the leachates infiltrating from the

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unsealed landfills [2] and during the coal deposits exploitation [3]. In the case of the LNAPL spill its migration comprise three steps [1, 4]: (1) vertical infiltration through the vadose zone that goes ahead due to the forces of gravity and capillarity, (2) horizontal spread of LNAPL on the groundwater table and displacing of the capillary fringe; dissolving of some soluble constituents into the groundwater (3) stabilization of the lateral spread of free product and migration of the plume of dissolved contaminants in the direction of the hydraulic gradient. After stabilization, due to the water table level fluctuations can eventuate the vertical movement of the LNAPL lens [5]. At this stage both free product and residual phase are source of the soil and groundwater contamination with soluble constituents [6]. Water percolating through the residual LNAPL due to precipitation infiltration or water table elevation changes dissolves soluble constituents resulting in a dissolved phase contaminant plume [6]. Even polycyclic aromatic hydrocarbons (PAHs) that are poorly soluble in water, can under certain conditions be leached out from the contaminated soil and pose a potential threat to groundwater [7]. It should be emphasized that as a result of the long-standing infiltration of LNAPL through the layer of cohesive soils it can come to the change of their original properties and to the worsening of their isolating ability [8]. Thus, in the case of oil pollution there is a necessity of the remediation and reclamation of both soil and groundwater with use of the properly attuned techniques [4]. Sites that need the remediation are characterized not only by morphologic changes but also by the disturbance of biological processes and the contamination with toxic substances [9]. It should be emphasized that reclamation of the degraded lands entails not only the land clearance, but also the need to the revitalization of their physical, chemical and biological properties [10]. If the LNAPL layer floats on the groundwater table the initial remediation step should be its recovery [11]. In order to a proper design of LNAPL recovery there is required an assessment of the mobile LNAPL volume [12] that can be determined on the base of the LNAPL thickness measured in the wells bored in several points of the contaminated area [13]. However, the LNAPL thickness measured in the observation well (the apparent thickness) is always different from the LNAPL layer thickness in the porous medium (the actual thickness) [14–17]. This difference depends on the properties of soil and the amount and properties of LNAPL [16]. Additional factors influencing the difference between apparent and actual thicknesses are the fluctuations of the water table level (hydraulic head fluctuations) and rate of these fluctuations [18–20].

When the hydraulic head changes the LNAPL lens moves up and down leading up to the increase of the volume of soil contaminated with LNAPL [18, 19]. During the descent of the water table level the LNAPL lens moves down leading up to the increase of the vertical extent of the interval of porous medium contaminated with LNAPL. Then, during the rising of the water table level a part of LNAPL lens moves up, but significant amount of it is trapped as a residual phase below the water table. This phenomenon has an impact on the decrease of the free product volume and the concurrent increase of the residual contamination zone [19]. Instead, during the descent of the water table level that follows the previous hydraulic head rise a part of LNAPL remains in the soil under the capillary fringe as adsorbed or residual phase, but a part of

LNAPL drops trapped previously below the water table is released. This results in the re-increase of the mobile LNAPL volume and thus the apparent thickness [19]. During the water table fluctuations changes the relationship between apparent and actual thicknesses and it can lead up to the imprecise estimation of the actual LNAPL thickness in the geological formation [18–20].

In the case of fluctuating water table very great influence on the relationship between apparent and actual thicknesses has the movement of free product and water between the well and geological formation. This is very often neglected at the estimation of the actual LNAPL thickness [18, 19]. If the hydraulic head in the geological formation rises, LNAPL drains from the well and flows into the adjacent formation. However the repletion of LNAPL recharges back into the well due to the pressure difference until the equilibrium is reached [18, 19]. However the apparent LNAPL thickness is in this case smaller than its equilibrium value. The faster the water table level rises, the smaller the apparent LNAPL thickness will be. If the hydraulic head in the aquifer falls, LNAPL flows into the monitoring well. Then the repletion of LNAPL drains back into the soil. In this case the apparent LNAPL thickness is larger than its equilibrium value. The faster the water table level falls, the larger the apparent LNAPL thickness will be [18, 19].

Water table fluctuations complicate the estimation of the actual LNAPL thickness and volume. When hydraulic head varies, the LNAPL volume estimates on the base of the apparent thickness may be significantly different from the actual LNAPL volume in the geological formation [18–20].

The focus of the experiments described in this article was to investigate the impact of hydraulic head fluctuations on the measured apparent and actual LNAPL thicknesses on the groundwater table in the case of sandy, homogeneous soil.

Materials and methods

The experiments were performed with use of sandy soil with effective grain size $d_{10} = 0.33$ mm, Hazen's uniformity coefficient $U = 2.03$ and hydraulic conductivity (at 10 ^{o}C) $k_{10} = 4.2 \cdot 10^{1}$ m $\cdot$ d^{-1}. As LNAPL was used the rapeseed oil with the density $\rho = 918$ kg $\cdot$ m^{-3} and the dynamical viscosity coefficient $\mu = 0.07$ kg $\cdot$ m^{-1} $\cdot$ s^{-1} (at the temperature of 20 ^{o}C).

The experiments were carried out in 10 cm diameter Plexiglas column with inbuilt filter-tube used as monitoring well (with diameter of 3.5 cm) (Fig. 1).

In the experimental column was also inbuilt the 3 cm diameter equalizing column with perforated bottom used to the changing of the water table elevations during the experiments. The column was packed with the soil sample. The perforated tube for injection of LNAPL was located into the soil (see Fig. 1). Then the experimental column was filled with water until the water table reached the assumed elevation (below the outfall of perforated tube). After 3 days about 600 cm^{3} of LNAPL was injected to the column directly above the capillary fringe zone. LNAPL was stained with the dye – Sudan III for better visibility of the liquid in the column. After next 4 days, the apparent and actual LNAPL thicknesses were measured in the well and in the soil. The actual

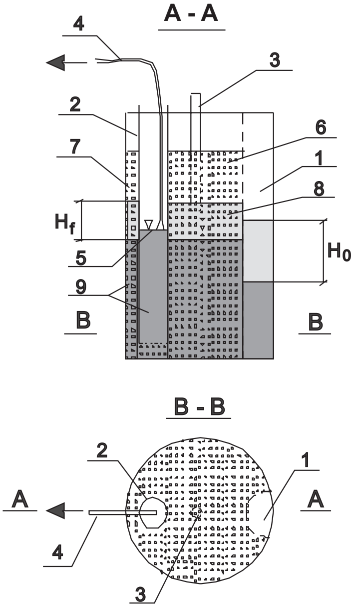

Fig. 1. The experimental set-up: 1 – observation well, 2 – equalizing column, 3 – perforating tube for LNAPL injection, 4 – tube for pump of water from the equalizing column, 5 – water table, 6 – soil, 7 – porous air, 8 – LNAPL, 9 – water; H_0 – apparent LNAPL thickness, H_f – actual LNAPL thickness

thickness was in this case the distance between the top and the bottom of LNAPL layer in the soil – the interval of porous medium containing LNAPL. Then the hydraulic head was lowered a few centimeters and after next 3–4 days the apparent and actual LNAPL thicknesses were measured. These procedures were repeated several times, until the LNAPL-water interface in the well has reached the well bottom. Then the hydraulic head was raised a few centimeters and after 3–4 days the apparent and actual thicknesses were measured. These procedures were repeated several times, until the air-LNAPL interface in the soil reached the top of soil sample. The top of the experimental column was protected against LNAPL evaporation by the cover. Experiments were performed at the temperature of about 20 °C.

Results and discussion

Fig. 2 shows the changes of the vertical placements of the LNAPL layers in the observation well and in the soil brought about the groundwater table fluctuations.

Results show that the actual LNAPL thickness diminishes for the falling water table level and increases when the hydraulic head rises. When the hydraulic head has the lowest elevation (measurement 9) the actual thickness diminishes to the zero value. In the case of measurements 6–10 the elevation of LNAPL layer in the observation well is so low that the LNAPL layers in the well and in the soil lost their connectedness.

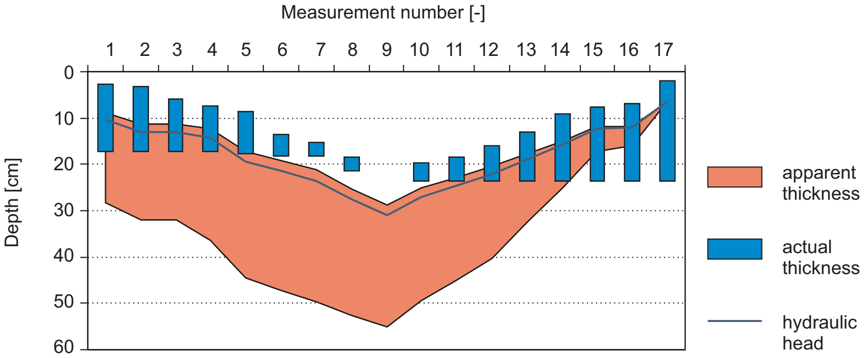

Fig. 2. The changes of the vertical placements of the LNAPL layers in the observation well and in the soil

Fig. 3 shows the impact of the water table fluctuations on the changes of the actual and apparent thickness values.

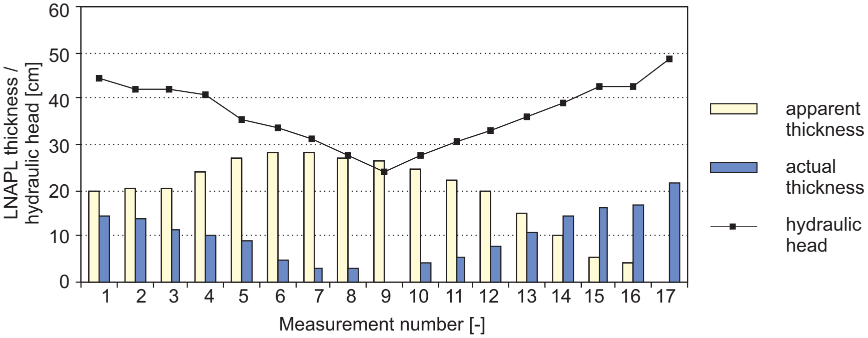

Fig. 3. Values of the apparent and actual thicknesses at varying hydraulic heads

The obtained results show that when the hydraulic head falls the apparent thickness of LNAPL rises and the actual thickness noticeably diminishes. At some point (measurement 9) the actual thickness has diminished to the zero value. Note that the maximal value of apparent thickness was obtained in the case of 7th measurement, ergo prior to the minimal values of the hydraulic head and of the actual thickness. During further abatement of the water table level (measurements 8 and 9) it was noticed very inconsiderable decrease of the apparent thickness and the considerable decrease of the actual thickness, until the disappearance of the LNAPL layer in the soil (measurement 9).

Instead when the hydraulic head rises the apparent thickness diminishes and the actual thickness increases. At some point (measurement 17) the apparent thickness has diminished to zero value. In this case whole volume of LNAPL has drained from the well into the adjacent formation.

The obtained results show noticeably that during the experiment was observed the increase of the vertical extent of the residual contamination zone.

Fig. 4 shows the relationships between the apparent and actual thicknesses during the groundwater table fluctuations.

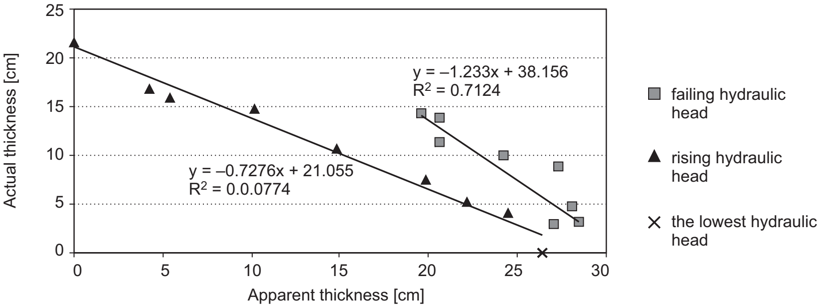

Fig. 4. Relationships between apparent and actual thicknesses at varying hydraulic heads

The results show that the graphs for falling and rising groundwater table level aren't coincident. It suggests the existence of the hysteresis phenomenon in the geologic formation LNAPL-, water- and air saturation during decrease and increase of the groundwater table.

The obtained results show that the water table fluctuations significantly complicate the estimation of the actual LNAPL thickness on the groundwater table on the base of the measured apparent LNAPL thickness. Results confirm that most available methods for estimation of the actual LNAPL thickness should be used only at the hydraulic equilibrium conditions. In the case of significant abatement of the hydraulic head the use of the apparent thickness for the estimation of the actual thickness may give the punitive results. Then in the case of the considerable hydraulic head rise the actual thickness estimated on the base of the apparent thickness can be largely understated. In an extreme case it may come to the disappearance of the LNAPL layer in the well, although LNAPL will be present in the soil.

The results confirm that it is very important or the hydraulic head change is the effect of the fall or rise of the water table level. The proper estimation of the actual LNAPL thickness in the geologic formation requires the study of the history of the hydraulic head changes in the contaminated site.

The diameters of the experimental column and the observation well have an effect on the experiment results. Very important was the relationship of the area of the soil cross section to the well cross section area. In the field conditions the well diameter cannot play so important role.

Conclusions

1. The results show that the water table fluctuations complicate significantly the estimation of the actual LNAPL thickness and volume on the base of the apparent thickness measured in the observation well.

2. When the hydraulic head falls the apparent thickness of LNAPL rises and the actual thickness diminishes.

3. When the hydraulic head rises the apparent thickness diminishes and the actual thickness increases. In the case of the rising water table level there can occur the situation in that the free product don't be present in the observation well.

4. The graphs showing the relationships between apparent and actual thicknesses for falling and rising groundwater table level aren't coincident. It can suggest on the hysteresis phenomenon.

5. During the experiment was observed an increase of the vertical extent of the residual contamination zone.

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WPŁYW WAHAŃ POZIOMU ZWIERCIADŁA WODY PODZIEMNEJ NA MIĄŻSZOŚĆ POZORNĄ LEKKICH CIECZY ORGANICZNYCH

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Politechnika Częstochowska

Streszczenie: Lekkie ciecze organiczne (LNAPL) infiltrujące do środowiska gruntowo-wodnego z uszkodzonych zbiorników magazynujących paliwa, rurociągów, cystern itp., a także z nieuszczelnionych składowisk odpadów komunalnych stanowią bardzo poważne zagrożenie dla środowiska wodno-gruntowego. W przypadku pojawienia się LNAPL na zwierciadle wody podziemnej pierwszy etap remediacji powinno stanowić jej sczerpanie. W celu odpowiedniego zaprojektowania operacji sczerpywania niezbędna jest znajomość objętości mobilnej LNAPL, którą ustala się na podstawie miąższości zanieczyszczonej strefy gruntu (miąższości rzeczywistej) lub na podstawie tzw. objętości jednostkowej LNAPL w ośrodku porowatym. Miąższość rzeczywistą i objętość jednostkową wolnego produktu oblicza się na podstawie miąższości LNAPL zmierzonej w studni obserwacyjnej (tzw. miąższości pozornej). Jednak miąższość pozorna zawsze różni się od miąższości rzeczywistej, a różnica między nimi zależy od właściwości gruntu, jak również od właściwości i ilości LNAPL. Dodatkowym czynnikiem, który wpływa na różnicę między miąższością pozorną i rzeczywistą, są wahania poziomu zwierciadła wody podziemnej (zmiany wysokości hydraulicznej).

Celem badań prowadzonych w ramach niniejszej pracy jest ustalenie wpływu zmian wysokości hydraulicznej na zmierzone wartości miąższości pozornej i rzeczywistej LNAPL na zwierciadle wody podziemnej. Otrzymane wyniki wskazują, że w przypadku obniżania się wysokości hydraulicznej miąższość pozorna wzrasta, natomiast miąższość rzeczywista maleje. Z kolei, w przypadku ponownego podwyższania poziomu zwierciadła wody podziemnej miąższość pozorna maleje, podczas gdy miąższość rzeczywista wzrasta. Przy znacznym podwyższeniu poziomu zwierciadła wody podziemnej może dojść do sytuacji, w której następuje całkowity zanik warstwy LNAPL w studni obserwacyjnej, mimo obecności wolnego produktu w ośrodku wodno-gruntowym. Uzyskane wyniki potwierdzają, że wahania wysokości hydraulicznej w znacznym stopniu komplikują ustalanie rzeczywistej miąższości oraz objętości mobilnej LNAPL w ośrodku porowatym na podstawie miąższości pozornej zmierzonej w studni obserwacyjnej.

Słowa kluczowe: LNAPL, miąższość rzeczywista, miąższość pozorna, wysokość hydrauliczna, wahania zwierciadła wody podziemnej

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ESTIMATING THE LEVEL OF WATER EUTROPHICATION IN PORAJ DAM RESERVOIR BASED ON SELECTED METHODS

OSZACOWANIE POZIOMU ZEUTROFIZOWANIA WODY ZBIORNIKA ZAPOROWEGO PORAJ NA PODSTAWIE WYBRANYCH METOD

Abstract: In this publication the chosen methods of estimation of the eutrophication were presented. These methods were used for the evaluation of the trophic status of the Poraj Reservoir water. In this research the Integral trophic state index was used. In addition the loading reservoir with total phosphorus was estimated, as well as the permissible and the critical loads of phosphorus according to Vollenweider were also defined. Furthermore, the trophic classification of reservoir water was achieved on the grounds of: the concentrations of biogenic substances in reservoir water, the concentrations of the chlorophyll *a*, the biomass of phytoplankton and the species composition of phytoplankton. The every chosen methods indicated on the advanced eutrophication processes occurring in this reservoir.

Keywords: eutrophication, dam reservoir, biogenic substances, chlorophyll *a*, biomass of phytoplankton

Introduction

Eutrophication is an increase of the water fertility level. The term was coined at the beginning of the 20th century by Thienemann & Naumann. This term meant precisely "water rich in nutrients" [1, 2].

The continuous addition of biogenic substances to the body of water results in the intensified eutrophication process. The problem touches especially dam reservoirs, since their catchments, as compared to *eg* lakes, occupy much larger areas [3]. Strongly eutrophic ecosystems, where the ecological balance have been seriously disturbed, are called hypertrophic ecosystems.

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The most notable result of water eutrophication is an excessive growth of phytoplankton [2, 4–8]. The increased algal biomass, often resulting in so-called water blooms, causes water to become feculent as a result of accumulated organic matter and colloidal substances, which are mainly released from dead photoautotrophic cells. The smell and taste of water change accordingly. Another negative effect of excessive growth of algae, in particular Cyanobacteria (Cyanophyta), is the appearance of toxic metabolites, as a result of dead or aging cells decomposing in water. Some authors claim that these toxins may not only be secondary metabolites but may play a crucial role in primary metabolism of some toxigenic species [9].

The aim of this research was to determine the level of eutrophication of Poraj Reservoir based on various research methods.

Materials and methods

The research of Poraj Reservoir water was conducted in years 2004–2006. This reservoir is located in northern area of Silesian Voivodeship, on the area of Poraj and Kozieglowy communes. The territories around the reservoir are considerably industrialised, there is an also developed farming well.

The River Warta forms the water quality of the reservoir. The water of river is polluted with sewage, which is derived from the Zawiercie and Myszkow areas, and they expose the reservoir of the delivery of a lot of pollutants. From majority of rural areas, deprived of the sewage system, the domestic wastewater is introduced directly into surface waters, which are located in the catchment area of the reservoir. This reservoir has recreation character, therefore multiple a resort centers are located in its neighborhood. They constitute a points sources of pollutants, which the considerably part get directly into the reservoir [10].

The reservoir of Poraj is typical lowland and shallow (the average depth is 4 m), about large surface (573,21 ha) (Table 1). The some morphometric-hydrologic parameters of reservoir, also the localization of Poraj dam Reservoir are presented appropriately in Table 1 and Fig. 1. The contour of reservoir and localization places for taking samples are presented on Fig. 2.

Table 1

Morphometric-hydrologic parameters of reservoir

The parameter	The data
Surface of reservoir bowl [km^2][a]	5.73
Total capacity [$\times 10^6$ m^3][a]	25.1
Depth average [m][a]	4
Retention time of water [in days][b]	124.8
Catchment area to section of dam [km^2][c]	389
Functions of reservoir[a]	fishing, flood protection, recreation, energy and guaranteeing of the permanent inviolable flow in the River Warta below the dam

[a] The Regional Management of Water Economy in Poznan; [b] it was calculated: total capacity / average daily inflow of the River Warta (which is the main inflow); [c] [10].

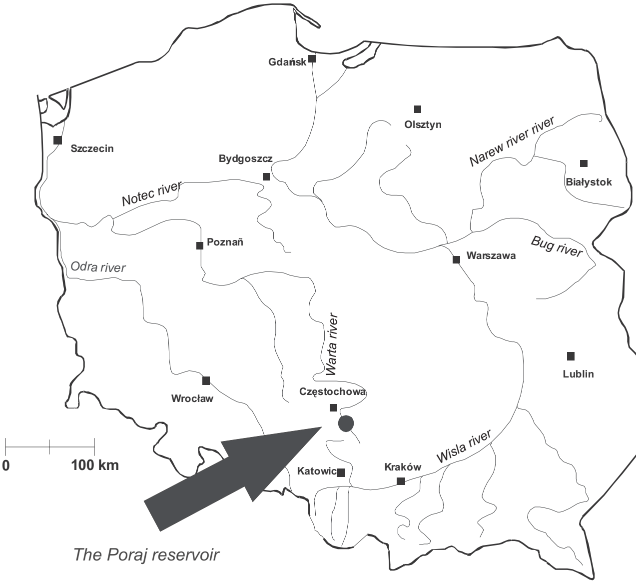

Fig. 1. Localization of Poraj dam Reservoir

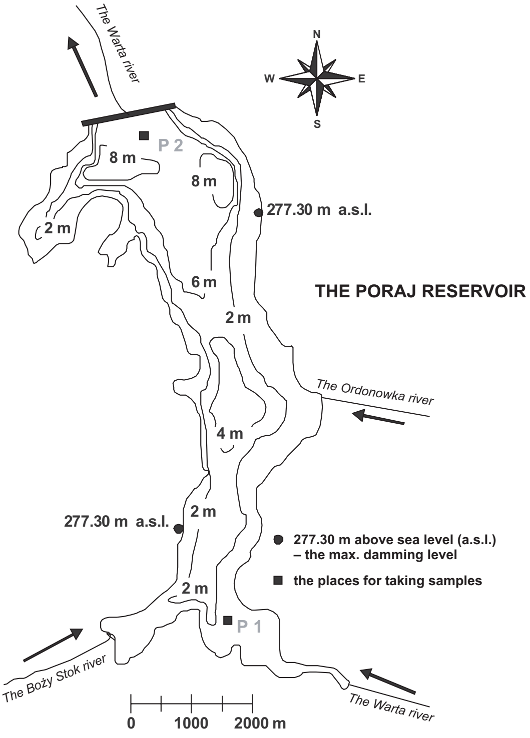

Fig. 2. Contour of reservoir and localization places for taking samples

The first of the diagnostic criteria used to establish the level of water eutrophication in the Poraj Reservoir was ITS (*Integral trophic state index*). It is calculated based on the degree to which the natural balance between production and decomposition of organic matter in the surface waters has been disturbed. If the production is greater than decomposition, the level of carbon dioxide (CO_2) decreases, while the level of oxygen (O_2) increases. In aquatic environments, the level of carbon dioxide can be determined based on the water pH, while the level of oxygen – based on oxygen water saturation [11, 12].

Oxygen water saturation was calculated by measuring the amount of dissolved oxygen and temperature according to Elbanowska et al [13].

For calculating *Integral trophic state index* the following formula was used [11, 12]:

$$\mathrm{ITS} = \frac{\sum \mathrm{pH}_i}{n} + a\left(100 - \frac{\sum [\mathrm{O}_2\,\%]}{n}\right) \tag{1}$$

where: pH_i – the measurement of the pH values during the t,
$[\mathrm{O}_2\ \%]$ – the concentration of oxygen in water in the moment of the measurement pH [%],
a – the empirical coefficient,
n – the number of measurements during the t.

The values of ITS indicator corresponding the different trophic states of water were presented in Table 2.

Table 2

The values of ITS indicator for water about different trophic status [11, 12]

The trophic status	The ITS
Ultraoligotrophy	6.3 ± 0.3
Oligotrophy	7.0 ± 0.3
Mesotrophy	7.7 ± 0.3
Eutrophy	> 8.3 ± 0.3

The trophy of reservoir water was also defined according to the criteria OECD, which are given by Dojlido [14]. The criteria OECD give a boundary values for average annual concentrations of total phosphorus ($\mathrm{mg} \cdot \mathrm{dm}^{-3}$) and average annual concentrations of chlorophyll *a* ($\mu\mathrm{g} \cdot \mathrm{dm}^{-3}$). The trophy of reservoir water was also estimated according to the Gizinski & Falkowska [15], who give the ranges of concentrations of total nitrogen ($\mathrm{mg} \cdot \mathrm{dm}^{-3}$) most often appearing in water of lakes (Table 3).

For more exact illustrating of eutrophication degree of reservoir water the loading reservoir of total phosphorus was estimated, the permissible and the critical loads of phosphorus according to Vollenweider [16] were also defined. The estimated value the loading of total phosphorus was compared to the values of permissible and critical loads.

Table 3

The ranges of concentrations and the boundary values of highly mentioned parameters, which are characteristic for defined trophic types of water [14, 15]

The trophic type of lake	The boundary values of total phosphorus concentrations [mgP · dm^{-3}]	The boundary values of chlorophyll *a* concentrations [μg · dm^{-3}]	The ranges of total nitrogen concentrations [mgN · dm^{-3}]
The oligotrophic lakes	< 0.01	< 2.5	0.5–1.0
The mesotrophic lakes	0.01–0.035	2.5–8	0.6–1.5
The eutrophic lakes	0.035–0.1	8–25	0.7–4.2
The hipertrophic lakes	> 0.1	> 25	1.2–6.0

The average volume of the flow [m^3 · s^{-1}] (which is essential for estimate of total phosphorus loading of reservoir) was computed using a data including flow [m^3 · s^{-1}] in days, in which the samples were collected from Warta River, on the inflow into the reservoir. For computing the volume of water flow in the area of influx Warta River into the reservoir was applied the analogy method, because water gauge station is located in Kreciwilk locality. The data, which concerning of the flows, was obtained from Institute of Meteorology and Water Management (IMGW), department in Katowice.

The chemical and biological research of reservoir water was conducted in years 2004–2006. The analyses of chemical parameters were realized according to Polish Standards by Silesian Voivodeship Inspectorate of the Environmental Protection (WIOS), department in Bielsko-Biala. The biological analyses were conducted using the light microscope Nikon Eclipse 200 in the laboratory of University in Bielsko-Biala. For identification of phytoplankton species the following keys were used: Sieminska [17], Starmach [18], Hindak [19]. The phytoplankton biomass was calculated for biovolume by comparing the shape of algae to their geometrical figures [20]. For evaluation the phytoplankton biomass the following converter was used [20]:

$$1\ \mu m^3 = 1/1 \cdot 10^9\ mm^3 = 1/1 \cdot 10^9\ mg \qquad (2)$$

Additionally, for the assessment of trophic state the classification, which including biomass of phytoplankton (mg · dm^{-3}) was accepted. This classification was suggested by Heinonen [21] (Table 4).

Table 4

The ranges of average values of phytoplankton total biomass, which are characteristic for definite trophic types of water [21]

The trophic type of lake	The ranges of average values of phytoplankton biomass [mg · dm^{-3}]
The oligotrophic lakes	0.14–0.68
The mesotrophic lakes	1.21–1.98
The eutrophic lakes	3.45–6.93
The hipertrophic lakes	17.5

The each identified species of phytoplankton was consulted with specialists.

Results

The conducted correlation analysis proved the occurrence of statistically highly significant correlation between the pH values and percentages of oxygen water saturation values, because statistically highly significant value of coefficient correlation *r* (r = 0.81***) was ascertained (Fig. 3).

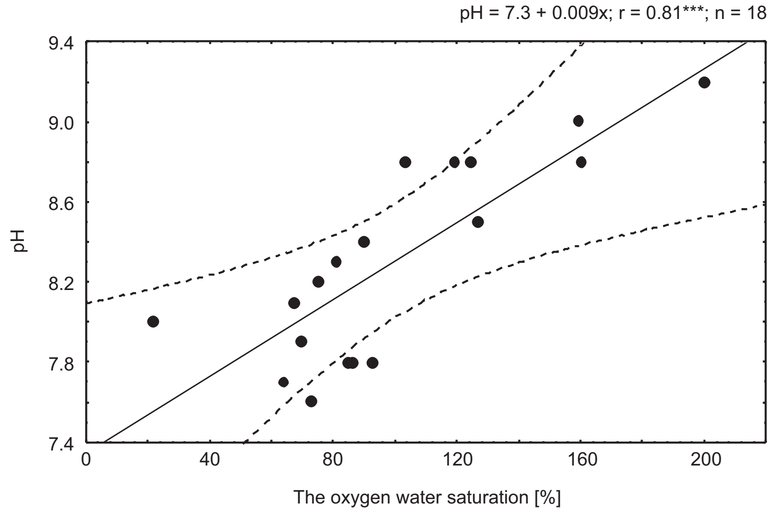

Fig. 3. The correlational relationship behind the pH values and percentages of oxygen water saturation values in the Poraj dam Reservoir

The preliminary classification of water was done as shown in Table 2. As a result of the above described research, the calculated value of ITS indicator (8.30) indicates the eutrophic character of the reservoir.

A more detailed research then followed, including the accumulation of biogenic substances such as total nitrogen and total phosphorus. The analysis concluded occurring the very highly of average concentrations of both these compounds in reservoir water. The exact amounts were respectively: 2.42 mg N $\cdot$ dm^{-3} i 0.21 mg P $\cdot$ dm^{-3}. The amount of nitrogen was slightly below the upper limit denoting eutrophic water, while the amount of phosphorus was significantly above this level, thereby concentrations of phosphorus qualified water of reservoir in the hypertrophy range (Table 5).

The results concerning of total phosphorus loading of reservoir, as well as the results including of the permissible and critical loads indicated for emphatic speedup the eutrophication in the reservoir, because the load of total phosphorus, flowing with Warta River into the reservoir, exceeded over fivefold the value of phosphorus critical load (Table 6). The minimum value of load flowing into the reservoir was jotted down in 2006 year, instead the maximum value was jotted down in 2004 year. In the course of years of research it is possible so to state a downward trend in the inflow of loads of this biogenic compound into the reservoir.

Table 5

The average concentrations of total nitrogen and total phosphorus of reservoir water

The parameter	The boundary values for eutrophy	The accounted values			
		2004	2005	2006	The average value for period 2004–2006
The total nitrogen [mgN · dm^{-3}][a]	0.7–4.2	2.57	2.51	2.19	2.42
The total phosphorus [mgP · dm^{-3}][a]	0.035–0.1	0.28	0.26	0.1	0.21

[a] It was calculated on base of spring, summer and autumn data.

Table 6

The loading of this reservoir with total phosphorus and the permissible and critical loads of phosphorus

The parameter	The accounted values			
	2004	2005	2006	The average value for period 2004–2006
The loading of total phosphorus [g · m^{-2} · year^{-1}]	6.808	3.592	2.594	4.331
The permissible load [gP · m^{-2} · year^{-1}][b]	0.417	0.387	0.411	0.405
The critical load [gP · m^{-2} · year^{-1}][b]	0.834	0.774	0.822	0.81

[a] It was calculated on the basis of average annual concentration of total phosphorus and total nitrogen (12-months data) and on the basis of average annual flow of water (years 2004–2006) – at the inflow of the Warta River into the reservoir; [b] It was calculated according to Vollenweider [16] criteria.

The final trophic classification of reservoir water was achieved on base of the species composition and biomass of phytoplankton, because a living organisms are excellent bioindicators and they constitute supplementing remaining research methods.

The high concentrations of chlorophyll *a* and biomass of phytoplankton in all years of research in water of reservoir were ascertained (Fig. 4). The values of these parameters exceeded much boundary values for eutrophy. The minimum concentrations of chlorophyll *a* and biomass of phytoplankton were jotted down in 2006 year (appropriately 39.51 μg · dm^{-3} and 10.84 mg · dm^{-3}), instead the maximum concentrations were jotted down in 2005 year (appropriately 109.66 μg · dm^{-3} and 31.18 mg · dm^{-3}).

The observed numerous species of phytoplankton, typical for eutrophic water, expressly indicated on the advanced eutrophication of water in this reservoir. These species of phytoplankton constitute bioindicators of eutrophy. The strong development of cyanophyta (*Microcystis aeruginosa* (Kutz.) Kutz., *M. viridis* (A. Br. in Rabenh.) Lemm), green algae (*Pediastrum* sp., *Coelastrum* sp., *Scenedesmus* sp.) and diatoms (*Aulacoseira granulata* (Ehr.) Ralfs (Ehr.) Simonsen), *Fragilaria crotonensis* Kitt., *Nitzschia palea* (Kutz.) W. Sm.) was ascertained in water of reservoir. The presence of

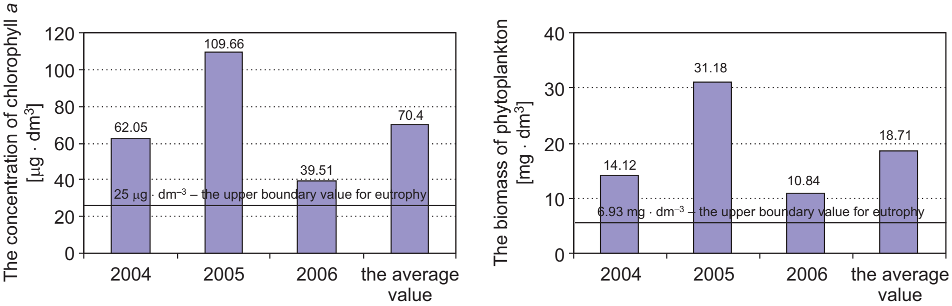

Fig. 4. The average concentration of chlorophyll *a* and the average biomass of phytoplankton on the background of boundary values for eutrophy in water of reservoir

typical species for hipertrophic water, *ia Planktothrix agardhii* (Gom.) Anagh. & Kom. was also jotted down.

Discussion

The Poraj Reservoir is located in the urban area, where the anthropogenic impact on the environment is acutely noticeable, which also includes water pollution.

The increasing eutrophication of the reservoir was confirmed not only by the ITS index but also by chemical analyses of water (raised levels of total nitrogen and total phosphorus), but mainly by the load of total phosphorus contributing with Warta River into the reservoir. A well-advanced eutrophication has also been confirmed by biological indicators (the total biomass of phytoplankton, determined by direct measuring and determined by indirect calculations based on concentrations of chlorophyll *a*) and the appearance of certain species of phytoplankton, which are typical for eutrophic water.

Whenever a significant eutrophication of water occurs, the carbon balance is seriously disturbed, resulting in the raised levels of oxygen as well as raised pH of water. This is due to the intensive photosynthesis process, during which a phytoplankton organisms absorb the carbon dioxide present in water and make for simultaneously to increase alkalinity of water. At the same time, the levels of oxygen water saturation go up. Raised pH of water, caused by water blooms during summer stagnation have been observed by researchers: Mazurkiewicz-Boron [22] wrote about it in the context of Dobczycki submountain Reservoir, Szelag-Wasielewska [23] in the Rusalka lowland Reservoir, while Neverova-Dziopak [11] and Kowalewski [12] in the strongly eutrophic Sulejowski Reservoir.

The concentrations of biogenic substances (total nitrogen and total phosphorus) in this reservoir were very high and in every case exceeded norms indicating water eutrophication (in case of total nitrogen, the amounts exceeded the lower boundary value, while in case of phosphorus also the upper boundary value for eutrophy). Phosphorus, as one of the most eutrophogenic substances, is one of the main culprits of speedup eutrophication process. In case of the discussed reservoir this biogenic substance played the particular role, because the load of this element carried in by the River Warta exceeded the critical load of total phosphorus more than fivefold. Such high levels of phosphorus may possibly come from the sewage treatment plant in Myszkow, as well as from improperly led farming on the banks of the river. The fact that the Poraj Reservoir is located in the urban area is also an important factor. In addition, the catchment here has a agriculturally – industrial character, which prompts in a considerable surface water inflow and the presence of various industry-related pollutants. The catchment area of this reservoir is located nearby of urban agglomerations (Myszkow and Zawiercie), therefore the supply of the public and industrial wastewater undeniably has the serious participation in water pollution. According to Strutynski et al [24] the urban wastewater is one of important sources of nitrogen and phosphorus in water environment. Some additional pollution may occur in the summer months due to several tourist sites and camps located near the reservoir.

The water eutrophication has also been confirmed by the high levels of chlorophyll *a* and large amounts of phytoplankton biomass (the eutrophication boundary values were exceeded by them). As pointed out by Ryding and Rast – after Wilk-Wozniak [25] – eutrophic reservoirs are characterized by high levels of phytoplankton biomass. On the other hand, according to research by Dojlido [14], the concentrations of chlorophyll *a* exceeding 8 $\mu g \cdot dm^{-3}$ also indicate eutrophication, while the levels above 25 $\mu g \cdot dm^{-3}$ classify water reservoir as hypertrophic.

The appearance of various species of algae, characteristic for eutrophic water, also constitutes as proof of the progressive eutrophication and the water degradation in the reservoir. These are mainly: (cyanobacteria *Microcystis viridis* (A. Br. in Rabenh.) Lemm., *Microcystis aeruginosa* (Kutz.) Kutz., green algae *Coelastrum* sp. and *Scenedesmus* sp., diatoms *Fragilaria crotonensis* Kitt.) [26–28]. Similar species of phytoplankton have been observed in the above mentioned eutrophic Sulejowski reservoir by Rakowska et al [29] and Lepistö & Rosenström [26] in the context of eutrophic Finnish lakes. Moreover, the research conducted by Negro et al [27] confirms that *Aulacoseira granulata* (Ehr.) Ralfs (Ehr.) Simonsen), which has been detected in the reservoir, prefers eutrophic water. According to Reynolds [28] the cyanobacteria *Microcystis* sp. is characteristic for sunny and eutrophic waters, instead cyanobacteria *Planktothrix agardhii* (Gom.) Anagh. & Kom indicates hipertrophy.

The biological analyses of the Poraj Reservoir confirmed that throughout the years of research, planktonic algae and cyanobacteria have thrived in the water. The case is serious especially where cyanobacteria are concerned, because they produce toxic substances, which may be dangerous for health of people. The recreation functions of the reservoir obligate to its protection and the matter of water cleanness should be priority. Unfortunately the biogenic substances flowing into the reservoir favour the growth of cyanobacteria, as a result the toxins secreting by some of cyanobacteria constitute threat for health of resting people. These toxic substances often trigger not only skin allergies (mainly dermatotoxins), but also damage to deeper body tissues, *ia* liver cells (mainly hepatotoxins) [9].

The building some new sewage treatment plants and sewage systems in particular localities will certainly enhance sanitary conditions of this reservoir.

Conclusions

1. Every apply methods indicated on a deepening eutrophic processes in Poraj Reservoir.
2. The calculated value of the ITS indicator (8.30) showed eutrophic character water of reservoir.
3. The load of total phosphorus, flowing with Warta River into the reservoir, exceeded that over fivefold the value of phosphorus critical load. It indicates on a speedup the eutrophic processes in this reservoir.
4. The high concentrations of biogenic substances, as well as the high concentrations of chlorophyll *a* were ascertained in water of reservoir (the average values of these parameters appropriately amounted: 2.42 mg N $\cdot$ dm^{-3}, 0.21 mg P $\cdot$ dm^{-3} and 70.4 μg $\cdot$ dm^{-3}).
5. The high trophic status of reservoir water was confirmed by the high biomass of phytoplankton and the species of phytoplankton, typical for eutrophic water (i. a. *Microcystis viridis* (A. Br. in Rabenh.) Lemm., *Aulacoseira granulata* (Ehr.) Ralfs (Ehr.)).

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OSZACOWANIE POZIOMU ZEUTROFIZOWANIA WODY ZBIORNIKA ZAPOROWEGO PORAJ NA PODSTAWIE WYBRANYCH METOD

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Abstrakt: W artykule zostały zaprezentowane wybrane metody oszacowania poziomu eutrofizacji. Metody te użyto do oceny statusu troficznego wód zbiornika zaporowego Poraj. W badaniach został zastosowany Integralny wskaźnik stanu troficznego. Dodatkowo oszacowano obciążenie zbiornika ładunkiem fosforu ogólnego oraz obliczono ładunki dopuszczalne i niebezpieczne fosforu ogólnego według Vollenweidera.

Ponadto, klasyfikacji troficznej wód zbiornika dokonano na podstawie koncentracji związków biogennych oraz chlorofilu *a*, a także biomasy fitoplanktonu oraz składu gatunkowego fitoplanktonu. Wszystkie wybrane metody wskazały na postępujące procesy eutrofizacyjne w wodach tego zbiornika.

Słowa kluczowe: eutrofizacja, zbiornik zaporowy, związki biogenne, chlorofil *a*, biomasa fitoplanktonu

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CONTAMINATION OF SOIL AND PINE BARK BY HEAVY METALS IN THE SELECTED FORESTS

ZANIECZYSZCZENIE GLEBY I KORY SOSNY METALAMI CIĘŻKIMI W WYBRANYCH LASACH

Abstract: The soil accumulates many chemical pollutants including heavy metals. In order to evaluate pollution of the environment the soil samples were taken on four forest stands in the Mogilski forest, in the Niepołomicka primeval forest, in Skalki Twardowskiego Landscape Park and in the grove in Zawada. At these stations also collected necrotic bark of the common pine (*Pinus sylvestris* L.), which is a popular tree in Poland. The outer bark of common pine is extremely sensitive biomarkers of environmental pollution. The content of heavy metals in the forest soils at four positions, and the bark of pine forest growing on these soils. Chemical analyses of heavy metals were performed by determining the contents of general forms of lead, cadmium, copper, nickel and zinc using the AAS method. The range of metals content in soils amounted to properly: 0.47–2.32 ppm Cd; 9.98–73.7 ppm Pb; 72.5–224.9 ppm Zn; 6.39–12.17 ppm Ni and 7.69–10.85 ppm.

The results indicate that the concentrations of heavy metals do not exceed the standards set out in the Regulation of the Minister of the Environment on soil quality standards and earth quality standards (Polish Journal of Laws DzU 2002 No. 165, item 1359 of 4 October 2002). The content of lead, copper and nickel in the studied soils were higher than in the bark of trees growing in this stations. The concentration of cadmium was varied at different positions. The highest content of Cd was observed both in the soil (2.32 ppm) and in the necrotic pine bark (1.55 ppm) in Skalki Twardowskiego Landscape Park, that is a part of Bielansko Tyniecki Landscape Park.

Keywords: heavy metals, necrotic bark of pine, forest soil

The development of industry, transport and a significant increase in urbanisation have contributed to environmental pollution by chemical substances, including heavy metals. Heavy metals pose a serious threat to all ecosystems. An increased heavy metal content in soils can affect the biological properties of the soil, be a potential source of danger to plants and to groundwater, and consequently contaminate the food chain [1–6]. It is therefore necessary to monitor their concentration in the individual elements of ecosystems [7]. The distribution and concentration of trace elements in soils depends

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primarily on the content and quality of the humus, their grain size composition, and the acidity of the soil [8–10]. The content of heavy metals in soil is largely related to the distance from roads, industrial plants, traffic density, terrain, and the way they are used [10–12].

Environmental contamination with heavy metals is a current global problem and therefore requires constant control and monitoring. For this purpose, different bioindicators are used. A good bioindicator for air pollution with acidifiers and heavy metals is the bark of trees, both deciduous and coniferous [10–20]. It is dead tissue and is no longer growing, in contrast to other bioaccumulators such as mosses, lichens, and the leaves of various plants. Pollutants accumulate on the outermost layer of the bark, which is exposed to prolonged activity from polluted air [21]. Chemical analyses of bark enable not only examination of the current layers of accumulation of elements and pollutants, but also an assessment of the course of this process in previous years. Both in Poland and other countries, the bark of pine trees (*Pinus sylvestris* L.), common in Poland, is used to assess the degree of pollution by atmospheric air [11, 15, 16, 21–23]. It is easy to remove the outer layer of bark from this tree. Clear changes occur in its physical and chemical properties due to air pollution. To evaluate the pollution level are applied, among others, such physicochemical properties of the bark as the pH of water extracts and the capability to absorb some chemical elements [16, 23]. The pH also depends on the species, age and the health of the trees, and on the soil they grow on, the storage tests, techniques, harvest time and the degree of porosity of the cortex [22, 23].

The aim of this study was the comparison of accumulation of Pb, Cd, Ni, Cu and Zn in soil and in necrotic pine bark at four forest positions.

Material and methods

In order to evaluate pollution of the environment the soil samples were taken with the use of soil sampler (25 cm^2) in four forest sites – I in forest 100 m from the street in Zawada (in the district Bochnia), II in Mogilski Forest about 3 km away from the Arcelor Mittal Poland in Krakow, III in Niepolomice Forest and IV in the Park Skalki Twardowskiego (Krakow).

On the selected sites soil was collected from a depth of 10cm during spring 2011– 2012. At each station also collected necrotic bark of the common pine (*Pinus sylvestris* L.). The environmental condition was analyzed owing to the detection of reaction of the soil, its humidity, content of heavy metals – Pb, Cd, Ni, Zn, Cu, pH and content of the metals in the pine bark obtained near the area where the soil samples were taken. In forest, from which soil was sampled, outer bark samples were taken from *ca* forty year old trees, having similar size of 40 to 50 cm breast height diameter, *ca* 2 to 4 m away from each other. The bark, was taken from the trunk at the height of 1.5 m above the ground. Chemical analyses of heavy metals were performed by determining the contents of general forms of lead, cadmium, copper, nickel and zinc using the AAS method.

In order to determine the pH value, the bark samples were dried at 65 °C for 3 hours, and next they were ground in mortar and pulverised using impact mill. From each sample, 2 g each of bark powder was weighed and 8 cm^3 distilled water added. After 48 hours, pH was measured using a WTW 330 pH-meter [24].

Chemical analyses of heavy metals were performed by determining the contents of general forms of lead, cadmium, nickiel, copper and zinc using the AAS method. Dried samples of bark and soil (2.5 g) were subjected to mineralisation process. For this purpose dry water was poured over 3 cm^3 of 65 % HNO_3 and heated for about 4 hours. The filtered liquid was poured into measure flasks and filled with distilled water to volume of 25 cm^3. In solutions of the soil prepared in that manner the content of heavy metals on the spectrometer of atomic absorption was marked (AAS firmy Cole-Parmer, BUCK 200A).

Also examined the correlation between the content of heavy metals in soil and bark at every position. The differences were statistically significant at $p < 0.05$. All the analyses were performed using STATISTICA 10 computer program.

Results

The soils in the test positions displayed pHs from the acidic to the slightly alkaline. However, pH of bark in all positions was acidic (Table 1).

Table 1

Selected parameters of the soil and pine bark in the studied sites

Parameters	I	II	III	IV	
pH of soil	5.33 (5.12–5.6)	5.63 (5.43–5.76)	4.23 (4.15–4.32)	7.65 (7.56–7.74)	
pH of bark	3.97 (3.62–4.31)	3.53 (3.13–3.70)	3.13 (2.92–3.81)	3.71 (3.31–3.97)	
Heavy metals in soil (mean in mg/kg d.m.)					
Pb	100*	12.53	9.9	73.7	59.63
Cd	4*	0.63	0.91	0.47	2.32
Ni	100*	12.17	8.91	6.39	15.79
Cu	150*	8.95	7.69	8.48	10.85
Zn	300*	93.98	224.97	91.63	72.47

* Limit values for the heavy metal content set out in the Minister of Environment Regulation on the soil quality standards and earth quality standards for group B (Polish Journal of Laws DzU 2002, No. 165, item 1359 of 4 October 2002).
I, II, III, IV– study sites.

The reaction of the bark at localities examined fluctuated from pH 3.13 to 3.97 (Table 1). The metal content in the soils was, respectively: 0.47–2.32 mg/kg Cd; 9.9–73.7 mg/kg Pb; 72.47–224.97 mg/kg Zn; 6.39–15.79 mg/kg Ni and 7.69–10.85 mg/kg Cu (Table 1). However, concentrations of heavy metals found do not exceed the limits stipulated in the Minister of Environment Regulation on the soil quality and farmland quality standards (Polish Journal of Laws DzU 2002, No. 165, item 1359 of 4 October 2002) [25]. Concentrations of the heavy metals tested – Pb, Cd, Ni, Cu and Zn – slightly exceed the limits for metal content in soils containing contaminants of anthropogenic origin established by Kabata-Pendias et al [8]. They amount to 70 mg for lead, 150 mg for zinc and 1 mg/kg for cadmium. The results obtained are characterised by these contents exceeding these limits at individual sites (Pb at III, Zn at II, and Cd at site IV). A high zinc content of 225 mg/kg d.m. was noted at site II (in Mogilski Forest), where the soil pH was 5.63 (Table 1).

A change in the soil pH greatly influences the concentration and mobility of zinc. The increasing pH of the soil is accompanied by a reduction in the zinc concentration, which may be caused by the increased binding of this element by the oxides of iron and aluminium, or their precipitation into less soluble forms [8].

Copper at all soil sites showed low concentrations (7.61–10.85 mg/kg), not exceeding the limit values. The highest concentration of cadmium was recorded in the soil at site IV (Table 1). Cd is one of the trace elements that is especially dangerous to man and the environment as a whole. Of all the elements, it is the most prone to bioaccumulation by plants from soil and water, and thus entry into the food chain [6, 8].

The concentration of heavy metals in the pine bark presented in Table 2.

Table 2

Content of heavy metals in the pine bark [mg/kg]

Sites	Pb	Zn	Cu	Cd	Ni
I	5.61	92.98	5.13	1.29	3.87
II	5.93	83.37	5.81	0.43	5.06
III	34.38	143.3	5.42	1.05	7.34
IV	41.85	32.37	10.23	1.55	8.72

The statistical analysis showed no significant correlation between the concentration of lead, zinc, copper and nickel in the soil and in the bark at any tested positions ($p \geq 0.09$).

The highest concentration of zinc amounting to 143 mg/kg d.m. was recorded in the bark at site III (Table 2).

Accumulation of lead, copper and nickel in the pine bark was significantly lower than in the soil on which grow tested trees (Fig. 1–3).

According to Kabata-Pendias [8], a concentration of this element in plants above 100 mg/kg d.m. indicates contamination of the environment. Plants are good accumulators of zinc and have a high resistance to an increase in its content. Klos et al [26] emphasise that the accumulation of selected metals in lichen thalli may be due to different sources of origin. The analytes contained in atmospheric aerosol can be locally lifted from the soil in the form of dust, or may be derived from distant emissions. The increased zinc content at the test site may be derived from alluvial sources, in this case, dust from the Sendzimir Steelworks in Nowa Huta (Fig. 4).

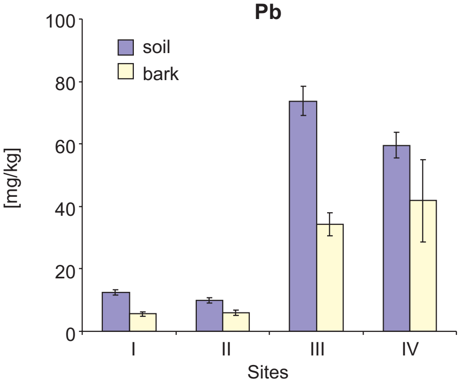

Fig. 1. Content of Pb in the soil and bark

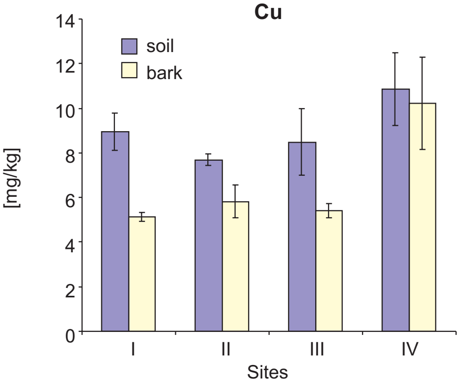

Fig. 2. Content of Cu in the soil and bark

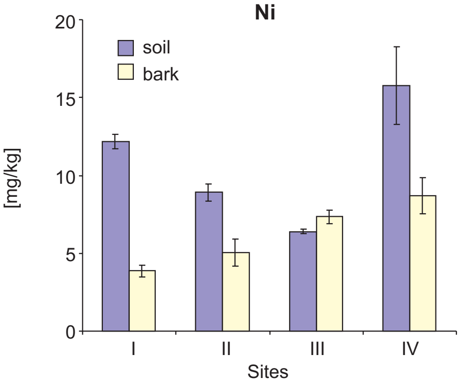

Fig. 3. Content of Ni in the soil and bark

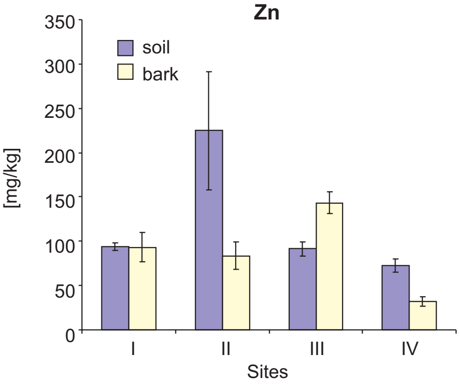

Fig. 4. Content of Zn in the soil and bark

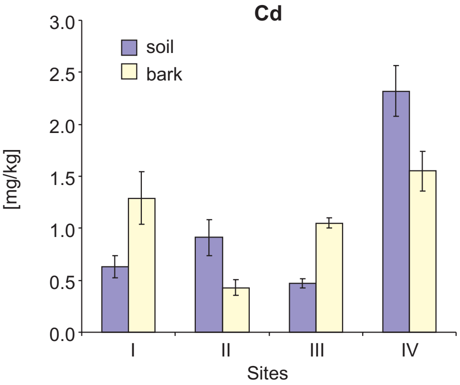

Fig. 5. Content of Cd in the soil and bark

The concentration of Cd in the bark at most sites was higher than in the soil and exceeded 1 mg/kg (Table 2). Only at site II (Mogilski Forest) was the concentration of this metal in the bark two times lower than in the soil (Fig. 5). The highest cadmium content in the outer bark of pine reported was similar to that in the soil, at site IV (2.32 mg/kg in soil and 1.55 mg/kg in pine bark) (Fig. 5). However, the metal concentration in that position was not significantly correlated with its content in the bark ($p = 0.881$).

Only in the Niepolomice forest (at site III) showed a significant correlation between concentrations of Cd in soil and pine bark ($p = 0.003$). At this site, the average concentration of cadmium in the soil was determined at 0.47 mg/kg d.m., and in the bark, concentration was 1.05 mg/kg (Fig. 5). Such a correlation has also been demonstrated in similar soil and bark studies, *inter alia* in China [17]. A higher cadmium content in bark than in soil may indicate that this metal is most likely accumulated directly from atmospheric (industrial, transport) pollution.

Conclusions

1. The results indicate that the concentrations of heavy metals – lead, cadmium, copper, zinc and nickel in the soil do not exceed the standards set out in the Regulation of the Minister of the Environment on soil quality standards and earth quality standards for group B (Polish Journal of Laws DzU 2002, No. 165, item 1359 of 4 October 2002).
2. Generally accumulation of heavy metals in pine bark was lower than the concentration of metals in the soil.
3. Higher content of cadmium in the bark than in the soil may indicate that this metal is probably the cumulative from air pollution (such as industrial pollution, communication).
4. There were no significant correlations between the studied metals in the soil and in the bark.

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ZANIECZYSZCZENIE GLEBY I KORY SOSNY METALAMI CIĘŻKIMI W WYBRANYCH LASACH

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Abstrakt: Gleba kumuluje wiele zanieczyszczeń chemicznych w tym również metale ciężkie. Dla oceny zanieczyszczenia środowiska pobierano próbki glebowe na czterech stanowiskach leśnych – w Lesie Mogilskim, w Puszczy Niepołomickiej, w ok. 40-letnim zagajniku sosnowym w Parku Skalki Twardowskiego i w zagajniku w Zawadzie. Na tych stanowiskach pobierano również korę martwicową sosny zwyczajnej (*Pinus sylvestris* L.), która jest popularnym drzewem w Polsce. Kora martwicowa sosny zwyczajnej jest wyjątkowo czułym biowskaźnikiem zanieczyszczenia środowiska. Badano zawartość metali ciężkich w glebie na czterech stanowiskach leśnych i w korze sosen rosnących na tych glebach. Analizy chemiczne dotyczące metali ciężkich przeprowadzono, wyznaczając zawartość ogólnych form ołowiu, kadmu, miedzi, cynku i niklu metodą AAS. Zawartości metali ciężkich w badanych glebach wynosiły odpowiednio: 0,47–2,32 mg/kg Cd; 9,98–73,7 mg/kg Pb; 72,5–224,9 mg/kg Zn; 6,39–15,79 mg/kg Ni i 7,69–10,85 mg/kg. Wyniki badań wskazują, że stężenia metali ciężkich nie przekraczają norm określonych w Rozporządzeniu Ministra Środowiska w sprawie standardów jakości gleby oraz standardów jakości ziemi (DzU 2002, nr 165 poz. 1359 z dn. 4.10.2002). Zawartość ołowiu, miedzi i niklu w glebie na wszystkich stanowiskach była wyższa niż w korze rosnących na tym obszarze drzew. Stężenie kadmu było zróżnicowane na poszczególnych stanowiskach. Najwyższą zawartość Cd odnotowano zarówno w glebie (2,32 mg/kg), jak i w korze martwicowej sosny (1,55 mg/kg) na stanowisku usytuowanym w Parku Skalki Twardowskiego należącym do Bielańsko-Tynieckiego Parku Krajobrazowego w Krakowie.

Słowa kluczowe: metale ciężkie, kora sosny, gleby leśne

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NITROGEN ACCUMULATION IN SCOTS PINE (*Pinus sylvestris* L.) NEEDLES NEAR NITROGEN PLANTS "PUŁAWY"

AKUMULACJA AZOTU W IGŁACH SOSNY ZWYCZAJNEJ (*Pinus sylvestris* L.) W POBLIŻU ZAKŁADÓW AZOTOWYCH „PUŁAWY"

Abstract: The paper concerns nitrogen content in the needles of Scots pine (*Pinus sylvestris* L.) depending on the concentration of this element in the soil and on the distance from the Nitrogen Plant "Pulawy" S.A., which emits air nitrogen pollutions. The studies have shown a significant relationship between the concentrations of nitrate and total nitrogen in the organs of assimilation of scots pine and ammonium and nitrate nitrogen content in soil and the distance from the emission source – Nitrogen Plants "Pulawy".

Keywords: pine needles, nitrogen accumulation, nitrogen plant emissions

Nitrogen Plants "Pulawy" are located inside a forest about 4 km away to the north from the center city of Pulawy. In the first period of activity in the years 1966–1985 there has been intense environmental degradation and death of a lot of pine trees [1–4]. The main reason for such rapid disappearance of forests and expand the borders of individual degradation zones was saturation of the atmosphere and soil with nitrogen compounds [2, 5, 6]. The cause of secondary, but equally important was the poor quality of the soil environment and the resulting nutritional pine trees [7–9].

In January 1990, Nitrogen Plants, "Pulawy" were located by the Minister of Environmental Protection, Natural Resources and Forestry on the list of 80-plus companies in the country – onerous environmental degradation. Ecological friendly actions initiated in 1985 resulted in a significant reduction of pollutants emitted by Nitrogen Plants, "Pulawy", an average of about 65 %, while increasing production by about 50 %.

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The largest reduction was achieved with: ammonia 88 % and 85 % of the fertilizer dust, ash particles 80 %, 62 % nitrogen oxides, sulfur dioxide and 27 % [1, 3, 4]. As a result, there has been a significant reduction in emissions and the negative impact of the factory on the environment.

The aim of this study was to assess the nitrogen content in the needles of Scots pine (*Pinus sylvestris* L.) depending on the content of this component in the soil and on the distance from the Nitrogen Plant "Pulawy", which emits air pollution.

Methods

The study was conducted in the years 2007–2009, in an area located in the zone of influence of the Nitrogen Plant "Pulawy" S.A. Material for the study was taken from various distances from the source of pollution, the transect from the plant, according to the main direction of migration of contaminated air in this area: Control – the south-west (SW) at distance of 11.0 km and northeast (NE) at a distance of 160, 260, 400, 800, 1500, 1900, 2500 m from Nitrogen Plants. Soil and plant material (pine needles) for investigations were collected at three different times: at the beginning of the growing season – first decade of April, in the middle of growing season – first decade of July and the end of the vegetation season – first decade of October.

In the soil were determined: total nitrogen by the Kjeldahl method and mineral nitrogen – $N_{min.}$ ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) extracted from soil with 1 % solution of K_2SO_4. The concentration of mineral nitrogen in the extract was determined by the colorimetric method: Ammonium $N\text{-}NH_4^+$ with Nessler reagent and nitrate nitrogen $N\text{-}NO_3^-$ with sodium salicylate.

Pine (*Pinus sylvestris* L.) was selected as an indicator plant to study due to needle existing on the plants for 3–4 years and their actively involving in the uptake of the pollutants from the atmosphere. In the laboratory of Regional Agricultural Chemical Station in Lublin in pine needles were determined: total nitrogen by distillation (KQ/PB-70) and $N\text{-}NO_3^-$ by flow colorimetric method. For the statistical evaluation of results used analysis of variance with Tukey confidence intervals and coefficients of linear correlation (Pearson). Correlation coefficients were calculated between the contents of nitrogen in the soil and the plant. Calculations have been made with program Statgraphics Centurion XV.

Results and discussion

Uptake of nitrogen by plant roots from the soil solution concerns mainly mineral fractions of the element ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) known as active forms present in the soil solution, and to a lesser extent, organic-soluble fraction of nitrogen [5, 7, 10–12]. While ammonium is adsorbed by soil colloids, nitrates are present only in the soil solution and are more readily taken up by plants, but also easily leached into groundwater [13–15].

Average ammonium nitrogen content (Table 1) in the soil profile was significantly differentiated depending on the distance from the emission source. The highest concentration of this form of nitrogen were stated in objects located close to Nitrogen Plants "Pulawy" (160 m) and (260 m).

Table 1

The content of N-NH_4^+ [mg · kg^{-1} d.m.] in the soil profile depending on the depth of soil profile and the distance from source of emission

Distance [m] (A)	Depth [cm] (B)						Means (A)
	0–5	5–20	20–40	40–60	60–80	80–100	
Control	1.1	0.6	0.2	0.2	0.08	1.3	0.6
160	7.0	1.5	1.0	0.4	0.6	1.5	2.0
260	6.1	1.3	0.7	0.5	0.8	0.5	1.7
400	2.2	1.0	1.3	0.5	0.6	0.7	1.1
800	2.1	1.2	1.1	0.6	0.3	0.5	1.0
1500	4.0	1.0	0.7	0.8	0.3	0.8	1.3
1900	2.6	1.2	0.6	0.2	0.4	0.8	1.0
2500	1.8	0.9	0.3	0.6	1.4	0.1	0.9
Means (B)	3.4	1.1	0.8	0.5	0.6	0.8	

$LSD_{0.05}$ for: A = 0.4; B = 0.3; A × B = 0.9.

Ammonium nitrogen content in soil profiles decreased with increasing distance, and the lowest average content was found in control. In the soil profile located close to the emitter average ammonia nitrogen content for all layers was 3.4 times higher than in the control and 2.3 times than the furthest point away from the factory.

The content of N-NO_3^- in soils (Table 2) was characterized by high volatility and was respectively higher than ammonium content. Mean values for the nitrogen in the form of nitrate are varied in a broad range from 0.6 mg · kg^{-1} and 40.8 mg · kg^{-1}.

Table 2

The content [mg · kg^{-1} d.m.] of N-NO_3^- in the soil profile depending on the depth of soil profile and distance from source of emission

Distance [m]	Depth [cm]						Means (A)
	0–5	5–20	20–40	40–60	60–80	80–100	
Control	3.4	1.0	0.9	0.6	1.4	1.3	1.4
160	26.8	13.9	13.2	8.9	8.1	2.3	12.2
260	40.8	17.2	13.0	5.4	6.7	7.1	15.1
400	9.2	1.6	3.5	1.1	2.3	2.7	3.4
800	19.0	4.5	3.2	3.1	1.4	1.6	5.5
1500	21.9	3.1	1.9	1.9	1.7	2.5	5.5
1900	24.3	5.0	3.8	2.7	3.0	3.2	7.0
2500	15.2	8.5	5.6	5.9	3.9	2.6	7.0
Means (B)	20.1	6.8	5.6	3.7	3.6	2.9	

$LSD_{0.05}$ for A = 2.4; B = 2.1; A × B = 5.9.

The study showed significant differences between the concentration of nitrate nitrogen, and the distance from the emission source. The highest content of N-NO_3^- in the soil was found in places closest to the source of emission (160 m and 260 m), and the lowest in control. Also concentration of this form of nitrogen was higher in soil located at the furthest from the emitter than in the control.

Compared to other research objects relatively low levels of nitrates in the soil was found 400 m away from emitter (reclaimed sand dune), which must be combined with favorable conditions for leaching of nitrates and mobile wind erosion associated with terrain, which resulted in the loss of this component outside of the soil environment [3, 4, 16–18].

An important and very useful indicator of changes in the environment is the chemical composition of assimilatory organs of plants, which are more sensitive to environmental and atmospheric pollution in comparison with other components of the ecosystems [5–7, 19, 20]. The availability of different forms of nitrogen in soil and nitrogen metabolism play a key role in ionic balance of plants, which largely controls the uptake and distribution of the cations in the plant [10, 11, 13, 14]. The chemical composition of the plants is dependent on many factors, not only the content of different nutrients in the environment, but also on the mutual balance between them [10, 11, 14–16].

Scots pine (*Pinus sylvestris* L.) has been selected as an indicator of response on air pollution due to existing of the needles on the plants for 3–4 years and are actively involved in the uptake of the pollutants from soils and the atmosphere [5, 6, 19, 20].

Studies have shown significant differences in the nitrogen content of pine needles depending on the distance from the Nitrogen Plants "Pulawy" (Fig. 1). The content of this element in the organs of assimilation of scots pine decreased with increasing distance from emitter, excluding object of 400 m (reclaimed sand dune), which should be associated with poorer habitat. In addition, the soil of the test area was characterized by a lower content of nitrogen in the form of both ammonium and nitrate compared with other objects in the soil. The accumulation of nitrogen in plants was dependent on the concentration of mineral nitrogen in the soil. This confirms the positive correlation coefficient, which in the surface layer of soil (0–5 cm) was significant only for the NH_4^+ (R = 0.52), while in the deeper one (5–20 cm) for both forms of nitrogen: NH_4^+

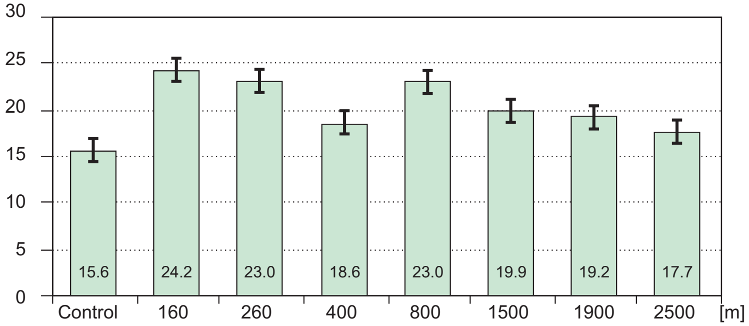

Fig. 1. The average content of total nitrogen [g · kg^{-1} d.m.] in pine needles (y axis), depending on the distance from the emitter (x axis)

(R = 0.40) and NO_3^- (R = 0.26). Many authors [1, 7, 9, 21] indicates that the pine tree in the wild in acidic forest soils is adapted for the use of ammonium ions. The uptake of nitrogen by scots pine is also beneficially affected due to the content of the nitrates in the soil and the balance between NH_4^+ and NO_3^- ions.

Pine trees located in the vicinity of the pollutants emitter characterized by a higher content of nitrogen in the needles, compared with control plants. The highest average content of total nitrogen in pine needles were found in the experimental area closest to Nitrogen Plants "Pulawy" of 160 m (24.2 g · kg^{-1} d.m.), and the lowest at the farthest distance from the emitter 2500 m (17.7 g · kg^{-1} d.m.). Comparative optimum content of nitrogen in the pine needles usually ranges, from 12.0 to 18.0 g · kg^{-1} d.m. [1, 8, 13]. Taking these values as a reference points it must be noted that the content of total nitrogen in the needles of trees from the area of impact of the Nitrogen Plant was higher than optimal [7] and varied in a particular objects from 24.2 to 17.7 g · kg^{-1} d.m.

Only research material from an object far away from the emission source was located in the upper limit of the optimum. Needles of control object corresponded to the optimal content for both ranges and average nitrogen content in the bioindicator research plant material was 15.6 g · kg^{-1} d.m. The nitrogen content in the pine needles in almost all objects in area of nitrogen fertilizers manufactory impact exceeded plant physiological maximum (18.0 g · kg^{-1} d.m.) and could be treated as evidence of negative impact of air pollution on trees.

Statistical analysis revealed significant differences of the nitrogen content in needles depending on the time vegetation season and material download years in the study (Fig. 2). The highest concentrations of this element in the organs of pine assimilation were stated in 2008 year, and the lowest in 2009. High nitrogen accumulation in plant tissues probably was related to the high amounts of rainfall this year and good soil moisture was beneficial for N uptake by plants. In the spring there were significant differences between the content of the element in all years, while in summer and autumn only between the years 2007 and 2008, and the year 2009. According to Parzych [21], small differences in the content of nitrogen and phosphorus in pine needles in the seasons between the years due to the variable availability of nutrients and

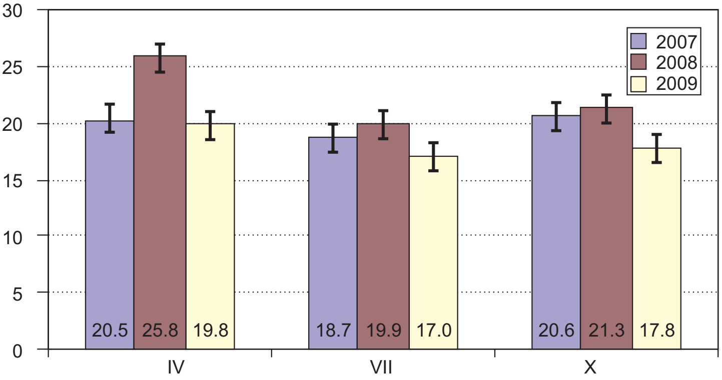

Fig. 2. The total nitrogen content in the needles of pine (y axis) [g · kg^{-1} d.m.] depending on the date and year (x axis)

water in the soil. The decrease of uptake both cations and anions by plants may also be affected the temperature drop. In general, the maximum concentration of nitrogen in the needles were in April (19.8–25.8 g · kg^{-1} d.m.), and the minimum in July (17.0–19.9 g · kg^{-1} d.m.). Low nitrogen content in the needles in July was probably associated with fast growth of biomass weight, and so-called "Dilution effect" and a small water capacity of the soils and drying effect in the summer. The content of N in the needles increased again in October can be associated with the extension of the growing season.

The nitrate content of pine needles (Table 3) showed significant differences depending on the distance from the Nitrogen Plant "Pulawy" and timing of sampling for analysis. The content of nitrate in assimilation organs of trees decreased with increasing distance from the emission source. In April the content of $N\text{-}NO_3^-$ was significantly higher in needles of pine located closest to Nitrogen Plant, than in the control and plants from further away. In all the research objects the highest concentration of nitrates in pine needles was recorded in early stages, in the 1st decade of April. During the growing season – 1st decade of July and the end of the vegetation time in October nitrate concentration in pine needles decreased. Nitrate content in the organs of assimilation largely depends on the precipitation, which can wash out the NO_3^- anions from plant tissue [21].

Table 3

The content of nitrate [mg · kg^{-1} d.m.] in the pine needles, depending on the distance from the Nitrogen Plant "Pulawy" and time

Time (A)	Distance [m] (B)								Means (A)
	Control	160	260	400	800	1500	1900	2500	
IV	27.7	157.3	182.1	58.8	36.0	35.9	36.2	15.6	68.7
VII	5.5	133.7	29.6	24.9	14.2	11.5	10.6	4.7	29.3
X	4.8	114.9	14.6	6.4	12.6	14.5	18.8	23.0	26.8
Means (B)	12.7	135.3	75.4	30.0	20.9	20.6	21.9	14.4	

$LSD_{0.05}$: A – 12.4; B – 20.5; A × B – 48.1.

The high content of nitrates in the pine organs of assimilation from the places nearest to the source of emissions, is likely to be related to deposition of nitrogen impurities on needles surface and the possibility of NO_3^- intake through the stomata or outside stomata [1, 5, 20].

Conclusions

The study of the nitrogen content in the needles of Scots pine (*Pinus sylvestris* L.) depending on the content of this component in the soil and the distance from the Nitrogen Plant "Pulawy", which emits air pollution containing nitrogen compounds allowed to draw the following conclusions:

1. Average content of ammonium and nitrate nitrogen in the soil profile were significantly differentiated depending on the distance from the emission source. The

highest concentration of this forms of nitrogen were stated in objects located close to Nitrogen Plants "Pulawy".

2. Scots pine (*Pinus sylvestris* L.) has been given as a bioindicator of response on air pollution due to existing of the needles on the plants for 3–4 years and are actively involved in the uptake of the pollutants from soils and the atmosphere.

3. The concentrations of nitrate and total nitrogen in the organs of assimilation of scots pine decreased with increasing distance from emitter of gaseous and particulate pollutants.

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AKUMULACJA AZOTU W IGŁACH SOSNY ZWYCZAJNEJ (*Pinus sylvestris* L.) W POBLIŻU ZAKŁADÓW AZOTOWYCH „PUŁAWY"

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Abstrakt: Praca dotyczy zawartości azotu w szpilkach sosny (*Pinus sylvestris* L.) w zależności od zawartości tego składnika w glebie i odległości od Zakładów Azotowych „Puławy", które emitują do atmosfery gazowe i pyłowe zanieczyszczenia azotowe. Badania wykazały istotne zależności pomiędzy zawartością azotu azotanowego(V) i ogólnego w organach asymilacyjnych sosny oraz zawartością azotu amonowego i azotanowego w glebie i odległością od źródła emisji z Zakładów Azotowych „Puławy".

Słowa kluczowe: igły sosny, akumulacja azotu, emisje zakładów azotowych

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and Monika MIERZWA[1]

FERTILIZATION OF SPRING WHEAT (*Triticum aestivum* L.) WITH COMPOST OF BIODEGRADABLE WASTE *VERSUS* CADMIUM, LEAD, NICKEL AND NITRATES CONTENTS IN GRAIN

NAWOŻENIE PSZENICY JAREJ (*Triticum aestivum* L.) KOMPOSTEM Z ODPADÓW BIODEGRADOWALNYCH A ZAWARTOŚĆ KADMU, OŁOWIU I NIKLU ORAZ AZOTANÓW W ZIARNIE

Abstract: Research hypothesis adopted in the conducted experiments assumed that composted biodegradable waste used for fertilization should have a positive influence not only on spring wheat grain yield but also on its biological value. The investigations aimed at an assessment of the effect of fertilization with compost produced of biodegradable waste on the content of lead, cadmium, nickel and nitrates(V) in spring wheat grain. Spring wheat grain yield receiving mineral fertilizers was larger in the first year of research in comparison with the yield harvested on the fertilizer treatments where manure and compost were used. Residual effect of fertilization with manure and compost on spring wheat grain yield became apparent only in the third year of the research. Content of lead and nickel was higher in the wheat grain fertilized with manure and compost, whereas cadmium content was higher in grain from the object where exclusively mineral fertilizers were applied. Assessed content of the studied elements do not exclude the analysed grain from its use for animal feed. A lower content of nitrate nitrogen was determined in wheat grain (irrespectively of the year of the experiment) from the objects where manure and compost were applied in comparison with the exclusively mineral treatment.

Keywords: compost, spring wheat, cadmium, lead, nickel, nitrates nitrogen

Introduction

Composting of biodegradable waste in large agglomerations is an alternative for their management [1]. It is only a part solution of the existing problem because the quality of produced composts not always complies the requirements. Generally, soil fertilization

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with compost brings positive results for its physical, chemical and biological properties [2, 3]. However, there is a risk of an unfavourable impact of compost, particularly its bigger doses on seed germination, limiting the availability or excessive release of nitrogen mineral forms and soil pollution with heavy metals [4]. These hazards cause that the use of composts based on municipal waste as fertilizers is relatively small.

The quality of obtained biomass is a crucial element determining its destiny. Content of undesirable substances, including heavy metals and nitrates limits the use of produced agricultural products, which in result may affect economic condition of farms [5].

Deepening deficit of organic matter in soil is not an adequate argument for an unlimited application of processed waste as fertilizers [6, 7]. Ill-considered use of processed waste substance, incompatible with the principles of good practice may cause irreversible environmental effects. Therefore, the diagnostics of quality parameters and the environmental risk assessment, including plant quality, are crucial elements of management of the processed organic waste material.

The research hypothesis in the presented investigations assumed that while using composted biodegradable waste one may expect their beneficial effect not only on spring wheat yield but also on its biological value. The aim of the research was an assessment of the effect of fertilization with compost based on biodegradable waste on lead, cadmium, nickel and nitrates content in spring wheat grain.

Material and methods

The research was conducted as a field experiment located 10 km west of Krakow (49.9963 N; 19.7011 E). The data obtained from meteorological station were presented in Tables 1 and 2.

The soil from the researched area was classified to Stagnic Gleysol. Selected properties of the soil prior to the experiment outset were shown in Table 3.

The experiment was set up with randomised block method. The plot area was 30 m^2. The experimental design comprised 4 objects in four replications: soil without fertilization (0), soil receiving mineral fertilizers (MF): 110.0 kg $N \cdot ha^{-1}$, 58.6 kg $P \cdot ha^{-1}$ and 120.0 kg $K \cdot ha^{-1}$, soil fertilized with pig manure (PM) dosed 3.23 $t \cdot ha^{-1}$ of d.m., soil fertilized with compost of biodegradable waste (C) dosed 2.83 $Mg \cdot ha^{-1}$ of d.m.

Selected properties of the manure and composts were compiled in Table 4. The field was limed before the experiment outset in compliance with 1/2 value of hydrolytic acidity (962.0 kg $CaO \cdot ha^{-1}$). The following spring, following the application of basic cultivation measures, pig manure and compost were distributed evenly on the plot surfaces and ploughed. Two weeks later a supplementary mineral treatment was applied and mixed with the soil using soil cultivating aggregate. Nitrogen dose supplied with organic fertilizers was 110.0 kg $N \cdot ha^{-1}$. Phosphorus and potassium were supplemented with mineral fertilizers to the level equal on all treatments (except the control): phosphorus to 58.6 kg $P \cdot ha^{-1}$ as a single superphosphate and potassium to 120.0 kg $K \cdot ha^{-1}$ as 60 % potassium salt. In the second and third year of the experiment, identical

Table 1

Monthly and periodic precipitation totals in study years [mm]

Year	Month												Σ Mar. III –Aug. VIII
	I Jan.	II Feb.	III Mar.	IV Apr.	V May	VI Jun.	VII Jul.	VIII Aug.	IX Sep.	X Oct.	XI Nov.	XII Dec.	
2005	66.4	32.6	20.7	49.1	61.3	40.6	113.4	102.6	26.5	7.7	29.9	46.7	387.7
2006	57.5	48.5	60.1	56.5	51.9	89.1	14.1	104.1	17.2	31.9	20.9	16.1	375.8
2007	100.6	42.2	61.1	15.4	51.7	72.1	71.0	76.4	179.8	48.3	90.4	21.4	347.7
1961–1999	34.0	32.0	34.0	48.0	83.0	97.0	85.0	87.0	54.0	46.0	45.0	41.0	434.0

Table 2

Mean daily air temperature in study years [^{o}C]

Year	Month												Mean Mar. III – Aug. VIII
	I Jan.	II Feb.	III Mar.	IV Apr.	V May	VI Jun.	VII Jul.	VIII Aug.	IX Sep.	X Oct.	XI Nov.	XII Dec.	
2005	–1.2	–4.3	–0.2	6.8	11.4	14.4	17.6	15.4	12.5	7.1	3.9	–0.7	10.9
2006	–2.4	–3.0	0.2	5.6	10.9	15.0	18.6	15.6	13.4	9.1	6.3	0.9	11.0
2007	3.2	1.2	6.0	8.5	15.2	18.4	19.4	19.0	12.4	7.7	0.8	–1,1	14.4
1961–1999	–3.3	–1.6	2.4	7.9	13.1	16.2	17.5	16.9	13.1	8.3	3.2	–1.0	12.3

as in the first year component doses were applied, exclusively as mineral fertilizers, in order to supplement nitrogen, phosphorus and potassium taken up with wheat yield.

Table 3

Physical and chemical properties of the soil before experiment (0–20 cm layer)

Parameter		Value	Parameter		Value
pH (KCl)	—	5.6	Ni_{total}	[mg · kg^{-1} d.m.]	33
Organic matter	[g · kg^{-1} d.m.]	26	Bulk density	[g · cm^{-3}]	1.52
N_{total}		1.59	Solid particle density		2.58
$P_{available}$	[mg · kg^{-1} d.m.]	712	Total porosity	[cm^3 · cm^{-3}]	0.41
$K_{available}$		298	Sand	[g · kg^{-1} d.m.]	280
$Mg_{available}$		368	Silt		580
Pb_{total}		36	Clay		140
Cd_{total}		0.96	Texture	—	Silty loam

Table 4

Physical and chemical properties of the organic materials

Determination	Unit	Pig manure (PM)	Compost (C)
N_{total}	[g · kg^{-1} d.m.]^b	34	39
P_{total}		13	5.80
K_{total}		22	30
S_{total}		4.76	3.61
Pb	[mg · kg^{-1} d.m.]^b	1.20	9.3
Cd		0.80	1.42
Ni		10	10
EC^a	[mS cm^{-1}]	2.89	2.62
pH (H_2O)		8.23	7.31
Organic matter	[g · kg^{-1} d.m.]^b	831	531
Ash		169	469
Water content	[g · kg^{-1} f.m.]^c	774	563

[a] EC = electrical conductivity; [b] data are based on 105 °C dry matter weight; [c] f.m. = fresh matter.

The test plant in the presented investigations was spring wheat, ‘Jagna’ c.v. Assumed plant density per 1 m^2 area was 485. Chemical measures were applied during vegetation to protect the plantation against weeds and fungal diseases.

The length of wheat vegetation period in the successive years depended on the weather conditions. Wheat was harvested at full grain maturity: on 13 August, 2005 in the first year, on 3 August, 2006 in the second and on 31 July, 2007 in the third. In order to determine wheat grain yield in field conditions, the plants were gathered from the area of 4 m^2, separately from each plot.

The manure applied in the experiment came from pigs and prior to the application had been stored on manure plate for 6 months. The compost used for the experiment (further referred to as compost) was produced from municipal plant waste and other biodegradable waste using Mut-Kyberferm technology in the following proportions: 25 % grass, 20 % wood pellets, 20 % leaves and 10 % organic waste from marketplaces, 5 % tobacco dust and 20 % coffee manufacturing waste. The compost originated from a composting plant located in Krakow.

In fresh samples of the manure and compost dry mass content was assessed (at 105 ^{o}C for 12 h), pH by potentiometer, electrolytic conductivity by conductometer, total nitrogen content after sample mineralization in a concentrated sulphuric acid by Kjeldahl's method, following N-NO_3 reduction with Devarda's alloy. In dried and ground material assessed were: organic matter and mineral content with thermal method after sample mineralization in a chamber furnace (at 450 ^{o}C for 5 h) and dissolving of ash in diluted nitric acid (1:2, *v/v*). Phosphorus concentration was determined by colorimetry on Backman DU640 spectrophotometer and potassium using flame photometry on Philips PU 9100X apparatus. Contents of lead, cadmium and nickel were assessed using ICP-AES method on JY 238 Ultrace apparatus. Sulphur content was determined with ICP-AES method after material mineralization in a concentrated nitric acid. The analyses were conducted according to the methodology described by Baran and Turski [8] and Krzywy [9], and the results were presented in Table 4.

Lead, cadmium and nickel concentrations were assessed in dried and ground wheat grain after sample mineralization in a chamber furnace (at 450 ^{o}C, 5 h) and dissolving the remains in diluted nitric acid (1:2, *v/v*) [10]. The content of analysed elements in the prepared solutions was assessed using ICP-AES method on JY 238 Ultrace apparatus. Total nitrogen content in grain was determined by Kjeldahl's method and nitrate nitrogen with phenol disulfonic acid by colorimetry.

Analysis of plant material was conducted in four replications. The precision of the conducted Pb, Ni and Cd assessments was determined using reference material NCS DC733448 (China National Analysis Center for Iron and Steel). The data concerning the precision and accuracy of the assessments were presented in Table 5.

Table 5

Amounts (mean ± SD) of metals released for material NCS DC733448, as well as data for analytical precision and accuracy

Metal	The value obtained in current study [$mg \cdot kg^{-1}$ d.m.]	Recommended value [$mg \cdot kg^{-1}$ d.m.]	Precision	Accuracy
Pb	6.8 ± 0.1	7.1 ± 1.1	1.4	–4.2
Cd	0.18 ± 0.01	0.14 ± 0.06	5.5	28.5
Ni	2.0 ± 0.1	1.7 ± 0.4	5.0	17.6

A two-way ANOVA (factors: fertilization x years of cultivation) in totally randomised design using f-Fisher test was conducted for spring wheat grain yields and the content of analysed trace elements and nitrate nitrogen. The significance of differences between arithmetic means was verified on the basis of homogenous groups determined by Tukey's test at the significance level α ≤ 0.05. All statistical computations were made using Statistica PL packet [11].

Results

The yield of spring wheat fertilized with mineral fertilizers (M) in the first year was larger in comparison with the yield harvested on the plots where spring wheat was fertilized with pig manure (PM) and compost (C) (Table 6), however the differences were not statistically significant. Also in the second year of the experiment grain yield was the largest on the object where wheat was fertilized only with mineral fertilizers (M). The residual effect of fertilization with pig manure (PM) and compost (C) on spring wheat grain showed itself only in the third year of the research. Increase in grain yield was 25 % and 26 %, respectively, in comparison with the grain yield harvested in the object where wheat received mineral fertilizers (M), and was statistically significant.

Table 6

Grain yield and thousand grain weight

Treatments		Grain yield [Mg d.m. · ha^{-1} ± SD]	Thousand grain weight [g d.m. ± SD]
Fertilizer	Year		
No fertilization (0)	1.	2.53^{ab} ± 0.13	32.7^{ab} ± 1.61
	2.	1.93^{a} ± 0.25	27.1^{a} ± 1.24
	3.	2.55^{ab} ± 0.10	30.8^{ab} ± 1.24
	mean	2.33^{a} ± 0.16	30.2^{a} ± 1.36
Mineral fertilization (M)	1.	4.13^{cde} ± 0.47	34.0^{ab} ± 6.47
	2.	4.81^{de} ± 0.45	33.3^{ab} ± 1.44
	3.	4.30^{cde} ± 0.30	32.9^{ab} ± 3.54
	mean	4.42^{b} ± 0.41	33.4^{ab} ± 33.8
Pig manure (PM)	1.	3.36^{bc} ± 0.16	36.8^{b} ± 1.27
	2.	4.01^{cd} ± 1.01	33.8^{ab} ± 1.80
	3.	5.39^{de} ± 0.31	30.7^{ab} ± 1.95
	mean	4.25^{b} ± 0.49	33.8^{ab} ± 1.67
Compost (C)	1.	3.07^{abc} ± 0.62	34.8^{b} ± 1.73
	2.	4.31^{cde} ± 0.90	34.4^{b} ± 1.12
	3.	5.42^{e} ± 0.11	31.4^{ab} ± 1.13
	mean	4.27^{b} ± 0.54	33.6^{ab} ± 1.33

Means marked by the same letters did not differ significantly at α ≤ 0.05 according to the Tuckey test; factors fertilization × year ± SD (standard deviation), n = 4.

In the first year of the investigations, the thousand grain weight indicating grain filling ranged from 34.0 g to 36.8 g (Table 6), irrespectively of the applied treatment. In the subsequent years, a lower thousand grain weight was registered, no matter which fertilization was used. Independently of applied fertilization and year of research, obtained differences were not significant.

Lead content in spring wheat grain was not significantly diversified either due to applied fertilization or the year of research (Table 7).

Table 7

Lead, cadmium and nickel content in grain of spring wheat

Treatments		Pb	Cd	Ni
Fertilizer	Year	[mg · kg^{-1} d.m. ± SD]		
No fertilization (0)	1.	$0.24^{a} \pm 0.08$	$0.16^{abc} \pm 0.03$	$0.99^{b} \pm 0.20$
	2.	$0.24^{a} \pm 0.11$	$0.17^{abc} \pm 0.03$	$0.69^{ab} \pm 0.20$
	3.	$0.24^{a} \pm 0.07$	$0.17^{abc} \pm 0.02$	$0.39^{a} \pm 0.11$
	mean	$0.24^{a} \pm 0.09$	$0.17^{a} \pm 0.03$	$0.69^{a} \pm 0.17$
Mineral fertilization (M)	1.	$0.13^{a} \pm 0.02$	$0.30^{c} \pm 0.12$	$0.76^{ab} \pm 0.05$
	2.	$0.28^{a} \pm 0.15$	$0.28^{bc} \pm 0.01$	$0.53^{ab} \pm 0.10$
	3.	$0.22^{a} \pm 0.02$	$0.20^{abc} \pm 0.02$	$0.40^{a} \pm 0.11$
	mean	$0.21^{a} \pm 0.06$	$0.26^{b} \pm 0.05$	$0.56^{a} \pm 0.09$
Pig manure (PM)	1.	$0.13^{a} \pm 0.06$	$0.13^{a} \pm 0.03$	$0.69^{ab} \pm 0.09$
	2.	$0.57^{a} \pm 0.15$	$0.26^{abc} \pm 0.10$	$0.52^{ab} \pm 0.13$
	3.	$0.28^{a} \pm 0.07$	$0.15^{ab} \pm 0.02$	$0.47^{ab} \pm 0.12$
	mean	$0.32^{a} \pm 0.09$	$0.18^{a} \pm 0.05$	$0.56^{a} \pm 0.11$
Compost (C)	1.	$0.29^{a} \pm 0.10$	$0.12^{a} \pm 0.04$	$0.89^{ab} \pm 0.19$
	2.	$0.42^{a} \pm 0.29$	$0.22^{abc} \pm 0.02$	$0.90^{ab} \pm 0.28$
	3.	$0.23^{a} \pm 0.03$	$0.17^{abc} \pm 0.03$	$0.63^{ab} \pm 0.24$
	mean	$0.31^{a} \pm 0.14$	$0.17^{a} \pm 0.03$	$0.81^{a} \pm 0.24$

Means marked by the same letters did not differ significantly at $\alpha \leq 0.05$ according to the Tuckey test; factors fertilization × year ± SD (standard deviation), n = 4.

Lead contents in wheat grain from the objects receiving pig manure (PM) and compost (C) were higher in the second and third year of the experiment than the values noted on the object where only mineral fertilizers (M) were applied. Mean lead content for three years in wheat grain fertilized with pig manure (PM) and compost (C) was higher than mean Pb content in wheat grain fertilized with mineral fertilizers (M), respectively by 33 % and 47 %.

An opposite relationship was registered for cadmium content (Table 7). Irrespectively of the year of research, the highest amounts of this element were assessed in wheat grain fertilized only with mineral fertilizers (M). Analysis of mean Cd content in wheat grain over three years shows significantly largest quantities of this element in wheat grain treated with mineral fertilizers (M).

Nickel content in wheat grain, independently of applied fertilization or year of research, did not exceed 1 mg · kg^{-1} d.m. (Table 7). The biggest quantities of nickel, independently of the year of research, were registered in wheat grain fertilized with compost. Mean for years content of this element in wheat grain fertilized with compost (C) was over 40 % higher than the average Ni content assessed in wheat grain from objects receiving mineral fertilizers (M) and pig manure (PM).

Total nitrogen content in wheat grain from the objects where pig manure (PM) and compost (C) were used in the first year was lower than the content noted in the wheat grain from mineral treatment (M) (Table 8). In the subsequent years N content in grain from these objects was increasing and in the third year of the experiment reached higher value that in grain treated exclusively with mineral fertilizers (M).

The highest values for nitrate nitrogen, irrespectively of the year of research, were assessed in wheat grain receiving mineral fertilizers (M) (Table 8). The content of this element in wheat grain from pig manure (PM) or compost (C) treatment was on average from 1 % to 6 % lower. An average share of N-NO$_3$ in total N content did not exceed 0.3 %.

Table 8

Content of total nitrogen and N-NO$_3$ in grain of spring wheat

Treatments		Total N [g · kg^{-1} d.m. ± SD]	N-NO$_3$ [mg · kg^{-1} d.m. ± SD]
Fertilizer	Year		
No fertilization (0)	1.	20.1^{a} ± 0.88	28.0^{a} ± 9.46
	2.	22.0^{abc} ± 0.72	40.1^{abc} ± 6.51
	3.	26.1d^{ef} ± 0.28	34.1^{ab} ± 3.98
	mean	22.8^{a} ± 0.62	34.1^{a} ± 6.65
Mineral fertilization (M)	1.	24.6^{cd} ± 0.76	56.6^{c} ± 8.12
	2.	23.7^{bcd} ± 1.38	49.0^{bc} ± 4.51
	3.	27.7^{ef} ± 0.58	52.8^{bc} ± 5.30
	mean	25.3^{a} ± 0.91	52.8^{b} ± 5.98
Pig manure (PM)	1.	21.5^{ab} ± 1.82	28.6^{a} ± 9.14
	2.	25.3^{def} ± 1.13	25.0^{a} ± 9.24
	3.	28.2^{f} ± 1.59	26.8^{a} ± 8.24
	mean	25.0^{a} ± 1.51	26.8^{a} ± 8.87
Compost (C)	1.	19.0^{a} ± 1.03	36.5^{ab} ± 2.02
	2.	25.0^{cde} ± 1.02	21.2^{a} ± 6.27
	3.	27.9^{ef} ± 0.88	28.8^{a} ± 3.07
	mean	23.9^{a} ± 0.98	28.9^{a} ± 3.79

Means marked by the same letters did not differ significantly at $\alpha \le 0.05$ according to the Tuckey test; factors fertilization × year ± SD (standard deviation), n = 4.

Discussion

Beside climatic factors (temperature and rainfall) also soil factors determine the amount and quality of obtained biomass. Fertilization with natural and organic materials usually less affects growth and development of plants and so called lack of residual effect is also often observed, which results from using up a considerable amount of nutrients from these fertilizers already in the first year of their application. Conducted research confirmed a lesser effectiveness of fertilization with manure and compost based on biodegradable waste applied in the first and second year of the experiment in comparison with mineral treatment. However, in the second year of the research the disproportions between grain yields from the individual fertilizer treatments were much smaller. Such high regression in wheat grain yield in the first year of the experiment observed on manure and compost treatments resulted from difficult plant access mainly to nitrogen. The causes of this situation should be sought in a relatively short period of time during which applied organic materials were undergoing mineralization process in the soil. Spring application of natural or organic fertilizers, particularly under unfavourable weather conditions causes a slowdown of transformation processes leading to a release of fertilizer components [12]. Despite a relatively weaker fertilizer effect, in comparison with mineral fertilization, spring application of natural and organic fertilizers may limit losses of fertilizer components, mainly nitrogen, which released from organic bonds and not absorbed by plants is dispersed in the environment. A lack of significant residual effect of the applied manure and compost fertilization in the second year of the research has not been corroborated by the results in the paper by Skowronska et al [13] or Woloszyk [14]. However, it should be emphasized that the authors quoted above applied markedly higher doses of organic materials (composts, sewage sludge) in conversion to the amount of supplied nitrogen. In presented own investigations a significantly better yield forming effect in comparison with mineral fertilization was obtained only in the third year of research. According to Sienkiewicz [12] manure fertilizer effect on wheat grain yield was significant, although the author emphasizes that the fertilization was not equally effective over the three year period of investigations. Moreover, Sienkiewicz [12] points up that a lower effectiveness of manure should be attributed to unfavourable weather conditions – too high rainfall.

The uptake but also translocation of trace elements in plant is determined, beside soil factors, also by the plant species. Research conducted by MacNicol and Beckett [15] confirm that roots are the first barrier inhibiting trace element translocation to the shoots. The barriers on the way of trace element transport from the roots to the shoots are generally efficient in all plants for lead and copper. In case of cadmium they apparently depend on the plant species. According to Chu and Wong [16] and Galler [17] such barriers also exist on the way of metal transport within the shoots. In the Authors' own investigations, lead and nickel content was higher in wheat grain fertilized with manure and compost, whereas cadmium content was higher on the object where only mineral fertilizers were applied. However, it should be emphasized that the contents of analysed elements fell within the range which did not exclude the analysed grain from use for fodder [18, 19]. Analysis of the obtained results considering trace metal content registered by other authors shows that, irrespectively of applied fertilization, assessed contents of lead, cadmium and nickel were higher than noted by Bednarek et al [12] in winter forms of wheat from Lublin region. On the other hand, Scigalska et al [21] assessed comparable to presented lead contents, higher contents of nickel but lower of cadmium in winter and spring forms of triticale. However, the authors quoted above emphasize higher content of the analysed elements in spring forms in comparison with winter ones.

Despite a relatively stable level of total mineral contents in plant biomass, occurring biochemical processes may considerably modify the relationships between individual components in a plant. From the perspective of fodder quality, nitrate nitrogen content is an important indicator. On the basis of conducted experiment (independently on the year) a lower content of nitrate nitrogen was assessed in wheat grain from the objects where manure and compost were applied in comparison with the treatment where wheat was fertilized only with mineral materials. Application of big doses of nitrogen may be a good method to obtain large grain yield, still its quality may worsen [22, 23]. Presented investigations have demonstrated that applied mineral fertilization favoured accumulation of nitrogen in wheat grain in a non-protein form. This situation may result among others from inadequate plant nutrition with sulphur. It impairs plant ability for nitrate nitrogen reduction [24]. This hypothesis may be confirmed by supplying sulphur with manure and compost, which affected nitrogen metabolism in plant. Also Jasiewicz et al [25] revealed that fertilization with organic materials (compost, sewage sludge or manure) increased the proportion of protein nitrogen forms in this element total content, whereas bigger doses of mineral fertilizers obviously decrease its share.

Conclusion

1. In the first year of the experiment the yield of spring wheat grain receiving mineral fertilizers was higher in comparison with the yield harvested from the objects where wheat was fertilized with manure and compost. The residual effect of manure and compost treatment on wheat grain yield became apparent only in the third year of the experiment.
2. Lead and nickel content was higher in wheat grain fertilized with manure and compost, whereas cadmium content in grain from the object where exclusively mineral fertilizers were used. Determined contents of the analysed elements do not exclude the use of the analysed grain for fodder.
3. A lower content of nitrate nitrogen was assessed in wheat grain (irrespectively of the year of research) from the treatments where manure and compost were applied in comparison with the object where wheat was fertilized only with mineral materials

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NAWOŻENIE PSZENICY JAREJ (*Triticum aestivum* L.) KOMPOSTEM Z ODPADÓW BIODEGRADOWALNYCH A ZAWARTOŚĆ KADMU, OŁOWIU I NIKLU ORAZ AZOTANÓW W ZIARNIE

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Abstrakt: W przeprowadzonych badaniach hipoteza badawcza zakładała, że stosując do nawożenia przekompostowane odpady biodegradowalne można oczekiwać ich korzystnego wpływu nie tylko na plon ziarna pszenicy jarej, ale także na jego wartość biologiczną. Celem przeprowadzonych badań była ocena wpływu nawożenia kompostem z odpadów biodegradowalnych na zawartość ołowiu, kadmu i niklu oraz azotanów(V) w ziarnie pszenicy jarej. Plon ziarna pszenicy jarej nawożonej nawozami mineralnymi w pierwszym roku badań był większy w porównaniu do plonu zebranego w obiektach, w których pszenicę nawożono obornikiem i kompostem. Następczy wpływ nawożenia obornikiem i kompostem na plon ziarna pszenicy jarej ujawnił się dopiero w trzecim roku badań. Zawartość ołowiu i niklu była większa w ziarnie pszenicy nawożonej obornikiem i kompostem, natomiast kadmu w ziarnie z obiektu, w którym zastosowano wyłącznie nawozy mineralne. Oznaczone zawartości badanych pierwiastków nie wykluczają użycia analizowanego ziarna do celów paszowych. Stwierdzono mniejszą zawartość azotu azotanowego w ziarnie pszenicy (niezależnie od roku badań) z obiektów, w których zastosowano obornik i kompost w porównaniu do obiektu, w którym pszenice nawożono wyłącznie mineralnie.

Słowa kluczowe: kompost, pszenica jara, ołów, kadm, nikiel, azot azotanowy

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ECOLOGICAL EFFECTS OF UREA PHOSPHATE ON SOIL DIAZOTROPHS IN SPRING. PART II[*]

EKOLOGICZNE SKUTKI DZIAŁANIA FOSFORANU MOCZNIKA NA DIAZOTROFY GLEBOWE W OKRESIE WIOSENNYM. CZ. II[*]

Abstract: The main of the present study was to evaluation of the influence urea phosphate (UP) on the number and intensity of nitrogen fixing by diazotrophs. Soil samples were taken from the experiment established on brown soil. The examination focused on the following treatment of soils: I – soil, II – soil + urea phosphate, III – soil + manure, IV – soil + manure + urea phosphate. The study results evidenced that urea phosphate limited the total number of diazotrophs in the soil only after 30 days compared to soil and soil with manure. Moreover, theirs number in the presence of urea phosphate was higher in soil with manure than in unmanured soil. Potential ability of nitrogen fixing was stimulated by urea phosphate after 30 and 90 days in both manured and unmanured soil. It shows the lack of correlation between the number of diazotrophs and their N-fixing activity. Biochemical examinations proved that application of urea phosphate increased of mineral nitrogen concentration (especially of N-NH_4) in the soil solution.

Keywords: urea phosphate, soil, diazotrophs, N-fixing activity, spring

Atmosphere is one of the biggest sources of nitrogen which contain about 78 % of it. However, this element in gas form is not available to most living organisms. Various diazotrophic Proteobacteria are known for their nitrogen fixing capabilities. Among the aerobic diazotrophs *Azotobacter* sp*.*, *Azotococcus* sp**.**, *Azotomonas* sp., *Biejernickia* sp. are the major ones. Some facultative anaerobes such as *Pseudomonas* sp*.*, *Bacillus* sp., *Klebsiella* sp.; microaerophilic *Azospirillum* sp. and some genus of *Clostridium* sp. posses the ability to reduce atmospheric dinitrogen into ammonia under anaerobic conditions and use it directly for its growth [1–5].

In all these cases nitrogen fixation and the conversion of molecular nitrogen into ammonium is catalyzed by a multimeric enzyme complex-nitrogenase [1, 6–8]. More recent studies have shown that certain environmental factors, such as pH, dissolved oxygen, ammonium concentration, the availability of organic carbon compounds, the

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lack or low content of nitrogen assimilation form, the presence of a sufficient amount of iron, molybdenum, copper and phosphorus, affecting their growth, survival and biochemical activities. [4, 8–12]. Due to the fact that diazotrophs use of soil organic matter, application of natural or organic fertilizer significantly influences their existence and nitrogen fixation process. Particular importance is the large ratio of C/N and the content of mineral forms of nitrogen [13–16]. The aim of this study was to evaluate the effect of urea phosphate (UP) used as a disinfectant of manure on the total number and the nitrogen fixing activity of free-living diazotrophs.

Materials and methods

The research material was brown soil pH_{KCl} 5.5 sampled from the layer of soil from 0–25 cm in the spring period. The experiment was conducted under laboratory conditions in three replications with the following treatments:

– I soil (S),

– II soil + manure (40 $Mg \cdot ha^{-1}$) (S + M),

– III soil + manure (40 $Mg \cdot ha^{-1}$) + urea phosphate (4.8 %) (S + M + UP),

– IV soil + urea phosphate (4.8 %) (S + UP).

The soil samples were incubated at 22 ^{o}C, kept in constant humidity on the level 60 % of total water capacity and were taken after 7, 30 and 90 days. Microbiological analysis included a determination [17]: the total number of diazotrophs by incubating the cultures on Fenglerowa’s medium at 28 ^{o}C for 7 days and potential activity of the fixing nitrogen in the soil by modified Pochon methods (Dn). This method uses the phenomenon of the emergence of ammonia as the first product formed during the process of fixation of nitrogen from the atmosphere. Determination of the amount of diazotrophs was carried out in four replication, and the result are given as average values of replicates per 1 g d.m. soil. Chemical analysis of samples were taken from the average determinations related to pH by potentiometric method and the content of mineral forms of nitrogen ($N\text{-}NH_4$ and $N\text{-}NO_3$) in the soil solution by Grewelinga-Peech method [18]. Statistical calculations were done by the variance method. Significant differences between average were measured according to the Duncan’s multiple range test. Data indexed by the same letter are statistically not significantly different ($p < 0.05$).

Results and discussion

The results of microbiological tests showed that urea phosphate significantly limited the number of diazotrophs in the soil only after 30 day of study (3.80 log cfu/g d.m. soil) compared with the soil (5.18 log cfu/g d.m. soil) and soil with manure (5.2 log cfu/g d.m. soil) (Fig. 1).

Furthermore, their population in the soil with manure and urea phosphate was 10-fold higher than in soil with urea phosphate, but these differences were not significant. However, after the application of manure, a significant increase in the number of nitrogen-fixing bacteria was observed only after 7 days compared to the other objects. Taking into consideration the duration of the experiment a gradual increase in population of diazotrophs in all treatments was observed, wherein the most frequently occurred in the 30 day of study (Fig. 1).

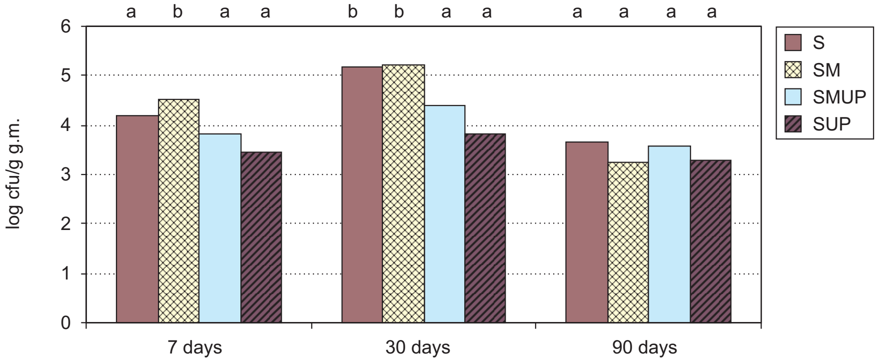

Fig. 1. The number of diazotrophs in the soil depending on the of fertilization [cfu/1 g d.m]; small letters significant differences ($p < 0,05$): S – soil without fertilization; SM – soil + manure; SMUP – soil + manure + urea phosphate; SUP – soil + urea phosphate

Assessment of the intensity of atmospheric nitrogen fixation by diazotrophs showed that in soils with urea phosphate process is the least ran only on 7 day test (significant differences), while the most intense occurred after 30 and 90 days (Fig. 2).

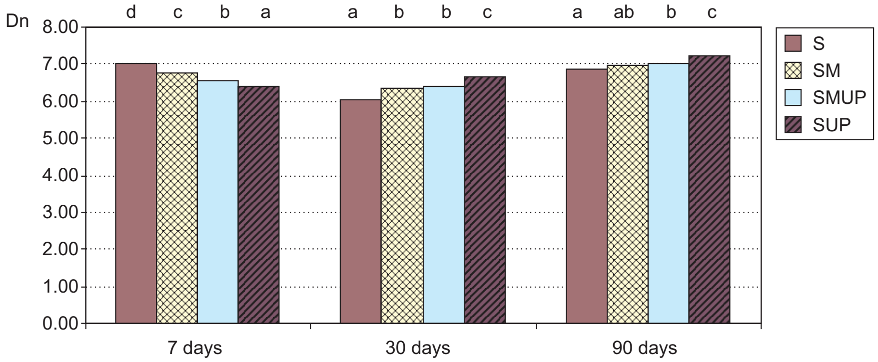

Fig. 2. The potential activity of the fixing nitrogen in the soil depending on the fertilization; explanations are given in Fig. 1

Analyzing the duration of the experiment, it was found that in all examined treatments, this process proceeded the most intense in 90 days, even though the smallest number of diazotrophs at this time was observed. Increase in activity of diazotrophs in fertilized soil along with over time is explained by the gradual decomposition of organic matter in soil. For example, the addition of cellulose, ammonium sulphate increases the production of nitrogenase enzyme. Furthermore, the addition of different carbon sources (sucrose, starch, mannitol) may promote growth of free-living nitrogen-fixing bacteria [11]. Additional source of nitrogen results in faster development of cellulolytic microorganisms, and consequently causes the stronger growth of microorganisms fixing atmospheric nitrogen [14].

Therefore, taking into account both the number and activity of diazotrophs observed lack of relationship between these parameters. The number of nitrogen fixing bacteria and their activities are not always correlated. This is probably due to the fact that in the process of nitrogen fixation the diazotrophs are involved, that differ in terms of morphological and physiological. Therefore, they can exhibit different yield atmospheric nitrogen fixation process [14, 19].

The results contained in Fig. 3 indicate that the use of urea phosphate resulted in a slight decrease of soil reaction after 7 and 30 days of study, while after 90 days the pH value remained at similar levels in all treatments from 5.21 (SUP) to 5.39 (S).

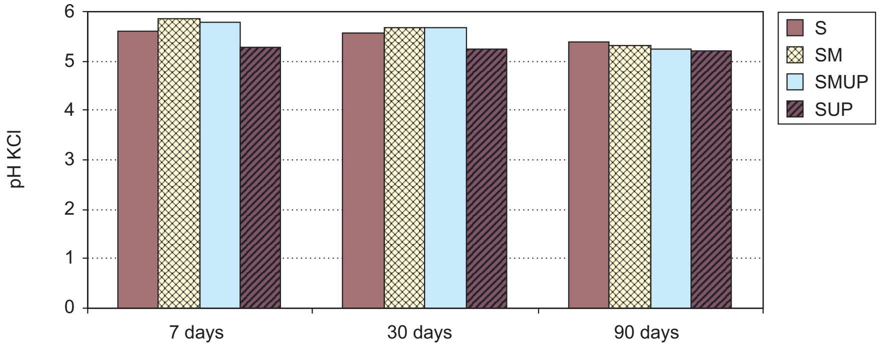

Fig. 3. The pH of the soil depending on the fertilization; explanations are given in Fig. 1

Biochemical examination on the content of mineral forms of nitrogen showed that the use of urea phosphate increased their content in the soil solution to a considerable degree than farmyard manure. In unfertilized soil samples and fertilized with manure the ammonium nitrogen content did not exceed the value of 7.44 mg/kg d.m soli. However, after application of the urea phosphate, content of this form of nitrogen significantly increased after 7 days, especially in the treatment of soil with urea phosphate (144.77 mg/kg d.m soil). The growing tendency was continued until the end of the study (Fig. 4).

In the soil fertilized only with farmyard manure the content of N-NO_3 was lower throughout the study period compared to the soil unfertilized. Whereas, it was higher in soil with urea phosphate after 30 and 90 days, while in soil with farmyard manure and urea phosphate after 90 days (Fig. 5).

Increasing the content of ammonium ions in soil with urea phosphate can be caused not only by diazotrophs activity, but also, due for instance to an intensive growth of other microorganisms that are active in the transformations of nitrogen (*eg* ammonificators, nitrificators), or the amount of urea phosphate introduced into the soil [20–23]. Especially important forms of nitrogen for microorganisms and plants are

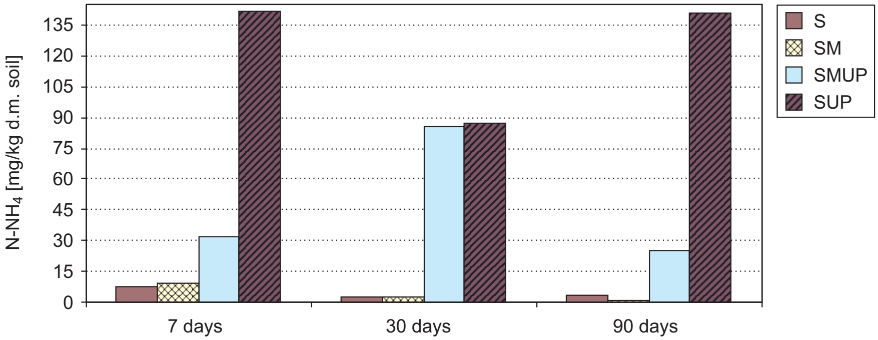

Fig. 4. The content of ammonium nitrogen (N-NH_4) in the soil depending on the fertilization [mg/kg d.m. soil]; explanations are given in Fig. 1

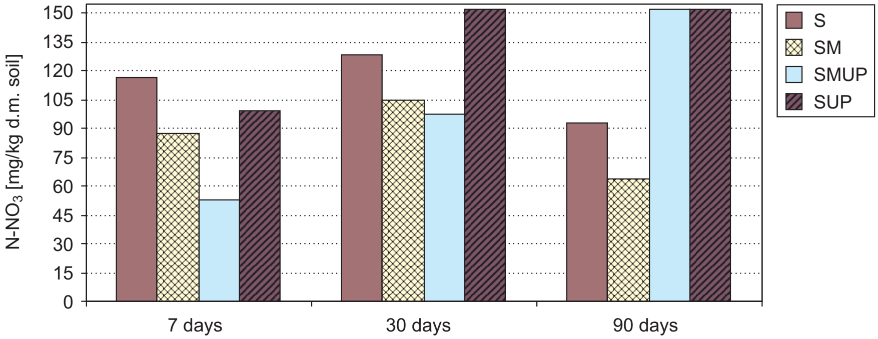

Fig. 5. The content of nitrate nitrogen (N-NO_3) in the soil depending on the fertilization [mg/kg d.m. soil]; explanations are given in Fig. 1

NH_4^+ and NO_3^- ions, which can be assimilated in them, contribute to their growth [24]. The nitrogen fixing bacteria preferentially take advantage of the ammonium ion. However, it was found that higher concentrations of nitrogen forms acts by inhibiting both the nitrogenase enzyme activity and its synthesis (by inhibition of enzyme glutamine synthetase formation), or just by inhibiting the synthesis of new nitrogenase or else decreasing the number of diazotrophs [4, 8, 25].

Nevertheless, the response of diazotrophs to the presence of these ions is not the same. Some of them such as *Azospirillum* sp*.*, *Azotobacter* sp. immediately respond to inhibition of synthesis of the nitrogenase, while in other representatives this reaction occurs gradually [10, 26]. Thus this process remains in the environment so long as the ammonium ions are present [1, 13, 25]. Moreover, nitrates or amino acids can inhibit the nitrogen fixation, although their influence on diazotrophs is less than that found for the ammonia [10]. In the case of nitrates, it is probably an indirect effect of reducing NO_3^- to NH_4^+. More often, the ammonia can exert inhibitory effects on the enzyme nitrogenase, but not nitrates [26]. However, the effect of nitrogen ions may be weaker in an environment that is the soil. The presence of antagonistic microflora capable of assimilation the bounded forms of nitrogen, can promote the survival of the diazotrophs population [8]. In addition, fertilization of soil with fertilizer rich in carbohydrates (high ratio of C / N) causes both the number and intensity the process of the nitrogen increases [6].

Conclusions

1. Application of urea phosphate as a manure disinfectant resulted in a slight reduction in the number of diazotrophs only after 30 days, whereas the increase in the intensity of nitrogen fixation in the soil.
2. It had been found a gradual increase the intensity of the nitrogen fixation in all tested treatments, with the highest values were recorded after 90 days study.
3. Urea phosphate influenced to the increase of the content of mineral nitrogen, especially ammonium nitrogen in the soil solution

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EKOLOGICZNE SKUTKI DZIAŁANIA FOSFORANU MOCZNIKA NA DIAZOTROFY GLEBOWE W OKRESIE WIOSENNYM. CZ. II

Samodzielna Katedra Biotechnologii i Biologii Molekularnej
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Abstrakt: Celem prezentowanych badań była ocena wpływu fosforanu mocznika (FM) na liczebność i aktywność wiązania azotu atmosferycznego przez diazotrofy w okresie wiosennym. Przedmiotem badań była gleba brunatna właściwa. W badaniach zastosowano następujące kombinacje: I – gleba, II gleba + obornik, III – gleba + obornik + fosforan mocznika, IV gleba + fosforan mocznika. Wyniki badań wykazały, iż fosforan mocznika ograniczał liczebność diazotrofów w glebie tylko po 1 miesiącu badań w porównaniu do gleby i gleby z obornikiem. Ponadto ich liczebność w obecności FM była większa w glebie z obornikiem niż w samej glebie. Potencjalna aktywność wiązania wolnego azotu była stymulowana przez fosforan mocznika po 30 i 90 dniach, zarówno w glebie nienawożonej, jak i glebie z obornikiem. Wskazuje to na brak zależności między ich liczebnością a intensywnością wiązania tego pierwiastka. Badania biochemiczne na zawartość mineralnych form azotu wykazały, iż zastosowanie FM zwiększyło ich zawartość, zwłaszcza N-NH_4 w roztworze glebowym.

Słowa kluczowe: fosforan mocznika, gleba, diazotrofy, intensywność wiązania azotu

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STUDY ON WASTE GARDEN ROCKWOOL WITH REGARD TO CONTENT OF BASIC NUTRIENTS

BADANIE ODPADOWEJ WEŁNY MINERALNEJ POD KĄTEM ZAWARTOŚCI PODSTAWOWYCH SKŁADNIKÓW ODŻYWCZYCH

Abstract: Variety of researches on garden rockwool used for carnations, cucumbers and tomatoes crops were presented. Research objective was to determine residual content of phosphorus, iron, magnesium, calcium and nitrogen after a completed crop cycle. Primal focus was set on following the rules of Sustainable Development in agriculture. Environmental protection, strictly dependant of relevant waste management, such as post-process rockwool management, was one main aims of these studies. Economic progress connected with recycling most important residual nutrients from waste rockwool was also of a great importance. Phosphorus, iron, calcium, magnesium and nitrogen contents were analyzed in specimens assembled from a one-year vegetation process with application of typical fertilizers used in carnations, cucumbers and tomatoes crops. Every sample was dried at a temperature of 30 °C. Afterwards samples underwent a grinding process on 0.40 mm sieve in order to reduce size of fibers and homogenize sample 0.40 mm. Total iron residue was examined in mineralized samples with a spectrophotometric method usage. Total phosphorus was determined in samples that underwent wet mineralization, using a spectrophotometric method based on a vanadate-molybdate complex. Total calcium and magnesium were examined, after their preparation by using the same mineralization process as samples prepared for iron residue, by titration of accordingly arranged samples with adequate solution content. Total nitrogen was examined by Kjeldahl's method. Humidity and pH were measured in order to evaluate any possibility of reusing the post-process rockwool. Acquired results indicate a large-scaled chance of processed waste rockwool being used successfully in gardening and fertilizing, as well as an insight of prospect of economical profits from recycled residual nutrients.

Keywords: gardening mineral wool, calcium, magnesium, iron, phosphorus, nitrogen, fertilizer, pH, moisture

Introduction

Fast developing population enforces much faster development of technology in almost every strand of human activity. The one of highest priority is aspect of

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nourishment. Higher need of food entails need of advanced technology of fertilization, cultivation methods, usage of components in fertilizers and crop protection chemicals. However, there is high danger of devastating environment during exploiting soil with such an intensive development without caution and it makes high risk of pollution by remains of some types of cultivation [1, 2]. In connection with above threats it is necessary to follow rules of Sustainable Development in designing new technologies which would be applied in agriculture [3–5].

Current trends in gardening encompass in majority greenhouses. This type of cultivation requires concerning such aspects as air humidity, sun exposure, watering and fertigation method, type of base. Those aspects are very important in context of quality and economical issue [6, 7]. Method of fertigation and watering are collocated with base. There are aeroponic systems, where water and minerals are given by sparging not grounded roots or system using various types of base [8]. The most commonly used bases are based on peat, rockwool, bark, coconut fibers, sawdust, polyurethane foam and phenolic foam [9–12]. However, the most popular is rockwool used with different components [13].

Rockwool is made from basalt rock. Ready to use rockwool proceed by stages of melting rock, stretching and rolling to get low-diameter fibers. This intermediate product is subsequently blown to achieve fibers of μm diameter. Properties of rockwool, like low density and thermal conductivity, it's possibility of processing with a low costs made it perfect material for thermal insulation in construction [14–16]. In gardening rockwool was being used at the end of XX century, when methods of its adaptation to usage in cultivation was laid down. After amelioration rockwool has lot of advantages, inter alia, good control of pH, temperature or moisture, what is interrelated with possibility of controlling parameters of fertigation. With comparison to organic base, rockwool is more resistant to expansion of pathogens [17].

Total outlay for production of construction and garden rockwool in 2011 was estimated on 25 466 mln USD, whereas in 2006 it was 21 969 mln USD. In Poland in 2006 it was 169.83 mln USD and in 2011 – 200.31 mln USD, what comprise 3.15 % of total outlay in the Europe and 0.79 % in the world [18].

Rockwool used in gardening is formed into blocks, slabs or cubes. Ways of cultivation are different dependable on types of plants, and they varies in types of fertilizers and their dose, moisture and porosity of rockwool [6]. Rockwool is often reused two or three times before wasting, what could benefit in quality of plants [7].

However, there are difficulties with rockwool after finished crop cycles when it is worked out. There is no complex method of total utilization. Current methods are based on melting waste rockwool, but it leads to pollution of rockwool with leftovers. There are possibility of steaming rockwool for cleanse, but its properties are not as good as postprocess rockwool [19].

Materials and methods

Aim of this study was assessment of waste garden rockwool prior to possibility of reusing leftovers to produce fertilizers. Rockwool after crop cycle contain some mineral substances which haven't been exploited by plants and which have crucial role in fertilizers industry. Quantification of these minerals is important due to design of extraction process. According to type of cultivation waste rockwool contain different amount of total minerals. Assessment was made on samples after tomatoes, cucumbers and carnations crop. Each sample was studied for contain of total phosphorus, phosphorus soluble in water and 2 % solution of citric acid, total iron, calcium, magnesium and nitrogen. Additional there were made studies concerning basic properties of rockwool such as pH and moisture.

Samples contained roots leftovers, depended on type of cultivation. After cucumbers crop there were lots of roots, root system was strongly expanded. After tomatoes crop amount of roots was noticeable, but root system was not developed. In sample after carnation crop there were few roots, almost unnoticeable.

Samples was prepared by drying in temperature 300 ^{o}C and mechanically grounded on 0.40 mm sieve. As a result was obtained fine-grained, dusty fibres.

Assay of total phosphorus and soluble in water and 2 % solution of citric acid was made by using spectrophotometric method based on a vanadate-molybdate complex according to PN-88/C-87015. Preparing sample for analysis of total phosphorus was about mineralization of rockwool in 3 : 2 (by volume) mixture of sulphuric acid and aquafortis. Samples for assay of phosphorus soluble in water and 2 % solution of citric acid was prepared by agitation of rockwool with water or citric acid solution and percolation of mixture. Quantification was made on spectrophotometer Jasco V-630 with wavelength 430 nm.

Assay of total nitrogen was made using Kjeldahl's method according to PN-EN 13654-1:2002, by reduction of nitrates to ammoniacal nitrogen with chromium powder, mineralization with concentrated sulphuric acid, distillation and titration of ammonia.

Assay of calcium and magnesium was made by titration of sample with EDTA for calcium and magnesium in pH 10 and Eriochrome Black T as an indicator, and only for magnesium in pH 13 and calconcarboxylic acid as an indicator.

Assay of iron was made by using spectrophotometric method based on a complex of 2,2'-bipyridyl with iron. Quantification was made on spectrophotometer Jasco V-630 with wavelength 520 nm.

Assay of pH was made using 1 g of rockwool in 20 cm^3 of water. Measurement was performed one hour after preparation.

Assay of moisture was performed by subtraction mass of sample after drying in 105 ^{o}C from mass before drying in relation to mass before drying.

Results and discussion

Table 1 consist results of study concerning basic properties of rockwool, performed according to PN.

Result of study of pH shows that only waste rockwool after cultivation of cucumbers has acidic reaction. Rockwool after tomatoes and carnations crop have neutral reaction.

Moisture was dependable on storage time and type of crop. For rockwool after cultivation of tomatoes and cucumbers was highly moist, whereas after cultivation of carnations sample was practically dry.

Table 1

Results of moisture and pH analysis of garden rockwool before and after completed crop cycle

Sample		Moisture [% (m/m)]	pH
Waste garden rockwool	After cultivation of tomatoes	77.4	6.56
	After cultivation of carnations	1.0	6.76
	After cultivation of cucumbers	66.7	5.25

Table 2 consist results of study concerning content of three different forms of phosphorus in waste garden rockwool, performed according to PN.

Table 2

Content of total phosphorus, phosphorus soluble in water and 2 % solution of citric acid after completed crop cycle

Waste garden rockwool	Total P_2O_5 [% (m/m)]	P_2O_5 soluble in water [% (m/m)]	P_2O_5 soluble in 2 % solution of citric acid [% (m/m)]
After cultivation of tomatoes	3.53	0.14	2.92
After cultivation of carnations	4.59	0.09	0.83
After cultivation of cucumbers	0.70	0.03	0.13

The highest content of total phosphorus was in cultivation of carnation and after cucumbers was the lowest. Phosphorus soluble in water compromise only insubstantial part of total phosphorus for every type of rockwool, and only for rockwool after carnations crop phosphorus soluble in 2 % solution of citric acid is significant.

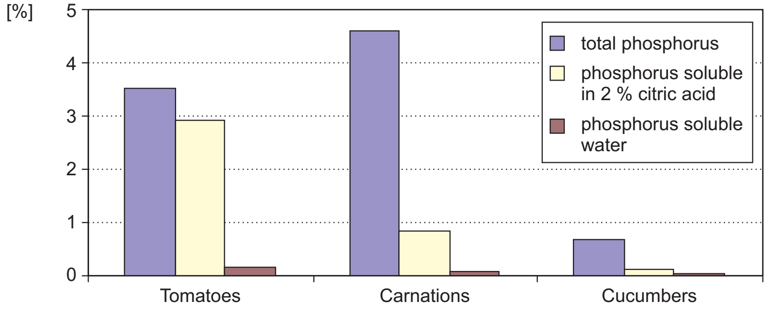

Fig. 1. Comparison of content of three form of phosphorus in waste garden rockwool

In every type of waste rockwool phosphorus soluble in water comprises a small content of total phosphorus. However, amount of phosphorus soluble in 2 % citric acid is very similar to amount of total phosphorus in rockwool after cultivation of tomatoes, but this is also minor form for carnations and cucumbers.

Table 3 consists results of study concerning total content of basic minerals in waste rockwool, performed according to PN.

Table 3

Content of total nitrogen, calcium, magnesium and iron after completed crop cycle

Waste garden rockwool	Total calcium [% (m/m)]	Total magnesium [% (m/m)]	Total iron [% (m/m)]	Total nitrogen [% (m/m)]
After cultivation of tomatoes	0.34	0.24	0.14	0.60
After cultivation of carnations	0.31	0.26	0.13	0.34
After cultivation of cucumbers	0.35	0.26	0.10	0.57

Content of magnesium and calcium is similar for each of type of rockwool. Also content of iron is comparable. But content of nitrogen is highest for rockwool after cultivation of cucumbers and tomatoes. In rockwool after cultivation of carnations content of nitrogen is almost a half smaller.

Conclusions

Content of mineral elements in inertial, waste garden rockwool vary dependable on type of plant cultivated in each base. Differences are engendered by constitution of fertilizers, requirement of plant for mineral elements, moisture and pH. Discrepancy between content of total phosphorus and phosphorus soluble in water and 2 % solution of citric acid may depend on amount of roots leftovers in garden rockwool. Amount of magnesium, calcium and iron is comparable for each type of cultivation. The highest content of nitrogen is observed in rockwool after crop cycle of cucumbers and tomatoes.

Introductory studies concerning mineral leftovers in waste garden rockwool allows to continue studies on possibilities of its reusing in technology of fertilizers, concerning ability of extraction of minerals by using different types of extractant and parameters.

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BADANIE ODPADOWEJ WEŁNY MINERALNEJ POD KĄTEM ZAWARTOŚCI PODSTAWOWYCH SKŁADNIKÓW ODŻYWCZYCH

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Abstrakt: Przedstawiono wyniki różnych badań przeprowadzonych na ogrodowej wełnie mineralnej zastosowanej w uprawie goździków, ogórków oraz pomidorów. Celem analizy było ustalenie pozostałości fosforu, żelaza, magnezu, wapnia i azotu po ukończonym cyklu upraw. Głównie skupiano się na stosowaniu zasad zrównoważonego rozwoju w rolnictwie. Jednym z głównych powodów analizy była zależna od odpowiedniego zarządzania odpadami, takimi jak odpadowa wełna ogrodnicza i ochrona środowiska. Bardzo istotny był również rozwój ekonomiczny związany z odzyskiwaniem najważniejszych pozostałych pierwiastków.

Fosfor, żelazo, wapń, magnez oraz azot były analizowane w próbkach pobranych z jednorocznych upraw, z zastosowaniem odpowiednich nawozów, goździków, pomidorów oraz ogórków. Każda próbka była suszona w temperaturze 300 °C. Następnie próbki zostały starte na sicie o średnicy oczek 0,40 mm w celu zmniejszenia wymiarów włókien i homogenizacji próbki.

Całkowita zawartość żelaza została zbadana w zmineralizowanych próbkach za pomocą metody spektrofotometrycznej. Całkowitą zawartość fosforu określono w próbkach, które przeszły mokrą mineralizację, przy użyciu metody spektrofotometrycznej wykorzystującej kompleks wanadowo-molibdenowy. Całkowita zawartość wapnia i magnezu została zbadana po odpowiednim przygotowaniu, przy użyciu tej samej metody mineralizacji, którą przeszły próbki do badać żelaza, poprzez miareczkowanie przyrządzonych roztworów odpowiednimi odczynnikami. Całkowity azot został zmierzony przy użyciu metody Kjeldahla.

Słowa kluczowe: ogrodnicza wełna mineralna, wapń, magnez, żelazo, fosfor, azot, nawozy, pH, wilgotność

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PESTICIDE RESIDUES IN FRUITS AND VEGETABLES PRODUCED IN ORGANIC, INTEGRATED AND CONVENTIONAL SYSTEMS

POZOSTAŁOŚCI PESTYCYDÓW W OWOCACH I WARZYWACH WYPRODUKOWANYCH W SYSTEMACH EKOLOGICZNYM, INTEGROWANYM I KONWENCJONALNYM

Abstract: Presented research concerns the evaluation of residues of synthetic pesticides in crops, depending on the system of production: conventional, integrated and organic. The study was conducted in 2005–2009 on 1134 samples of plant material derived from the four provinces: Lubelskie, Podlaskie, Warminsko-Mazurskie and Mazowieckie. The 123 active substances of pesticides were searched in the plant material.

This research has been shown that in the samples of fruit produced in the integrated system percentage of samples with residues below the maximum residue levels (MRL) is larger (55 %) than in the samples from conventional production (28 %), and less with above MRL (3 %). The pesticide residues were also detected in 8 % in samples of fruits and vegetables from organic production.

Keywords: pesticide residues, fruits, vegetables, conventional, integrated, organic production

Pesticide residues in crops are primarily the result of application of synthetic pesticides for the purpose of protection of crops against undesirable pests or the result of persistence in the environment. These residues must be present in an amount that is as small as possible and are usually detected in very low concentrations ($mg \cdot kg^{-1}$, $\mu g \cdot kg^{-1}$). A maximum residue level (MRL) is defined for every pesticide used in the production of food [1, 2].

Crops in Poland are mainly produced in the *conventional system* (CC), and also in *integrated system* (IC) and *organic system* (EC). The nature of each of these systems is defined by the scope of applied pesticides, the degree of interference in the natural environment, as well as by expectations concerning the quality and quantity of obtained

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crop yield. The goal of integrated production is to achieve high crop yield of good quality, free from substances that are unfavorable for human health, as well as protection of the natural environment. Chemical protection of plants against diseases, pests or weeds should be replaced by alternative methods whenever possible, such as: biological, physical, and agrotechnical methods. Conventional farming is a farming method directed towards maximization of profit, which is achieved thanks to high crop yield obtained in specialized farms that apply production technology based on high consumption of pesticides and very low costs of labor, which also create a series of negative consequences. The philosophy of organic farming is based on the prohibition of using chemical agents, including mineral fertilizers and synthetic pesticides [3]. The goal of this method of production is to obtain products of a high biological value that are free from chemical residues.

The goal of the study was to evaluate the presence of pesticide residues in crops from northeastern Poland depending on the system of their production.

Materials and methods

Crops samples (1134) were obtained within the framework of an official inspection of pesticide residues conducted in the years 2005–2009 on the order of the Ministry of Agriculture and Rural Development, realized in cooperation with the State Plant Health and Seed Inspection Service (PIORiN). Crops originated from three farming systems: integrated (265 samples), conventional (774), and organic (95). 123 active substances of pesticides were sought for in the plant material. A procedure developed by the authors, based on sample dispersion in a solid phase MSPD (*Matrix Solid Phase Dispersion*) [4, 5], was used to determine these substances. Extraction with a simultaneous purification step was carried out with the application of adsorption column chromatography. Instrumental determinations where carried out using gas chromatographs with simultaneous EC and NP detection. The applied method fulfills the validation criteria required for analysis of pesticide residues [6] and is accredited by the Polish Centre for Accreditation (AB 839), and the laboratory regularly participates in proficiency tests to confirm its competencies. Evaluation of pesticide residues was carried out based on *maximum residue levels* (MRL) according to applicable legal provisions.

Results

The distribution of pesticide residues in crops originating from organic, integrated, and conventional production is presented in Fig. 1.

The most numerous group of tested samples contained crops produced in the conventional system (68.25 % of all samples), among which 69 % did not contain pesticide residues and 3 % were stated to have violated MRLs. In the group of crops originating from integrated production, which constituted 23.37 % of all samples, no residues were stated in 42 % of samples, and MRL violations were noted in 3 % of samples. The least numerous group was represented by plant material originating from

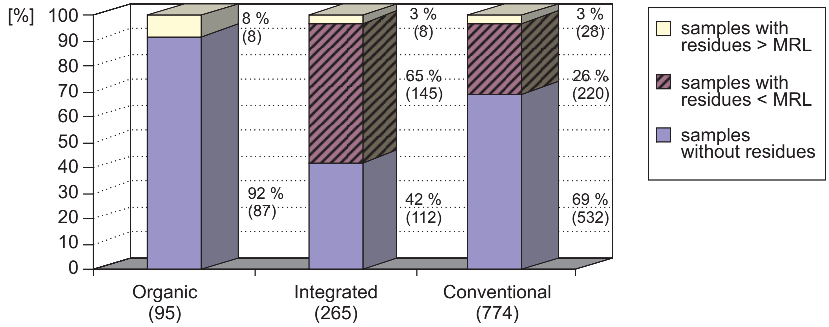

Fig. 1. Distribution of pesticide residues in plant material originating from organic, integrated, and conventional production

organic farms, comprising 8.38 % of all plant material. Pesticide residues were noted in 8 % of samples, which is considered to be an MRL violation in organic products.

The distribution of pesticide residues in fruits and vegetables originating from organic, integrated, and conventional production is presented in Fig. 2.

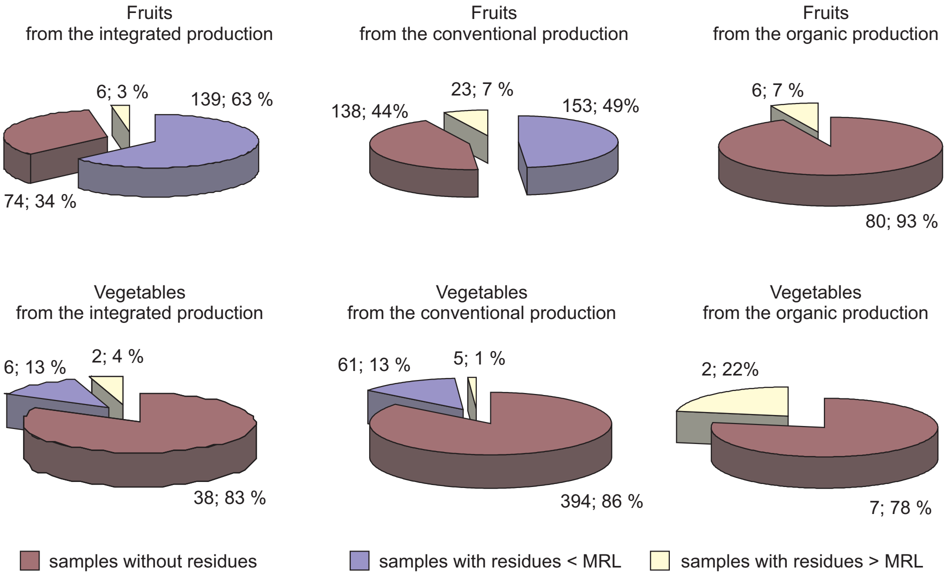

Fig. 2. Distribution of pesticide residues in fruits and vegetables depending on the system of production

Vegetable samples from organic, integrated, and conventional production were laden with pesticide residues in a lesser degree (22 %, 17 %, 14 %, respectively) than fruits (7 %, 66 %, 56 %, respectively). The smallest share of samples without residues was noted for organic fruits (93 %), and the highest percentage of MRL violations was noted for vegetables from this system of production (22 %). The highest percentage of samples with pesticide residues and MRL violations was noted for fruits (66 %) originating from integrated production and vegetables (22 %) originating from organic production.

Discussion

Integrated farming in Poland is mainly oriented towards growing fruits and vegetables. Pesticides were intensively used in this production, which led to a larger amount of samples in which residues were determined. The results of these studies, which were conducted in the years 2005–2009, indicate that among crop samples originating from integrated production pesticide residues were stated below acceptable limits in 55 % of samples, and MRL violations were noted in 3 % of samples.

According to Lozowicka [7], 48 % of Polish samples originating from integrated production in 2006 contained residues. MRL violations were stated in 2.6 % of these samples.

In the presented studies, 28 % of the crop samples from the conventional system contained residues below MRLs. MRL violations were noted in 3 % of these samples. In 2006, the presence of pesticides was stated in 21 % of such samples, and a level of these substances that was higher than the acceptable level was noted in 3.2 % of samples.

It should be noted that the share of samples from the conventional system of production in which pesticides were detected (31 %) is smaller than for the integrated system (58 %). In samples originating from IC, pesticide residues were detected in over half of the analyzed material, and the number of cases in which MRLs were violated is only smaller by 0.2 %. It is commonly recognized that conventional production should be distinguished by greater pesticide consumption, and thus, by a greater amount of residues in tested products. Both the economic aspects of applying pesticides and the structure of cropland in individual types of production, including the type of plant grown and its qualities (susceptibility to pests, and thus the necessity of applying chemical plant protection) could have influenced this different state of affairs. The lack of strict and systematic inspection combined with incorrect treatment techniques and failure to observe guidelines for use of preparations could have contributed to the detection of pesticide residues in crops from both of these systems.

The contents of pesticide residues in crops from northeastern Poland shown in this study are similar to the distribution for the whole of Poland [8–11]. Date from the literature shows that integrated farming produces food that is visibly less contaminated in comparison to that from conventional farming [12]. For example, Tsakiris [13] indicates that peaches produced in the conventional system in Greek contained pesticides in concentrations that were three times greater than in peaches from the integrated system. This study prove that in Greece, IC offers fruits of the highest quality, is safe for the environment, and integrated farming methods have been implemented successfully. The situation in northeastern Poland is different than in the entire country [14]. As a result of conducted studies, it can be concluded that, in fruit samples from integrated production, a greater percentage of samples contains residues below MRLs in comparison with conventional production, and a smaller percentage of samples violate MRLs. However, a smaller percentage with residues above MRLs was demonstrated for vegetables from conventional production.

According to data from the literature, the number of samples from organic production with pesticide residues, *eg* in the Austria, is equal to 8.2 % [15], in the Czech Republic 14 % [16], and in Belgium 8 % [16]. In years 2007–2010, the presence of pesticides was noted in 4.4 % of Polish crop samples [18, 19]. In northeastern Poland, these agents were present in 8 % of samples, of which 22 % were vegetable samples and 7 % were fruit samples.

The presented data attests to the fact that synthetic pesticide residues are generally detected in organic products from around the world, which may indicate their use, and this is inconsistent with the assumptions of organic farming [20, 21]. The presented results indicate a need for continuing inspections of pesticide residues.

Conclusions

1. In the studied region, tested conventional products are characterized by a lower content of pesticide residues in comparison with integrated production. Both the economic aspects of pesticide application and the crop structure in individual types of production, as well as the type of tested samples have an influence on this state of affairs.
2. The conducted studies show that a greater percentage of fruit samples from integrated production contain residues below MRLs as compared with conventional samples, and a smaller percentage of samples violate MRLs. The situation is reversed in the case of vegetables.
3. The unfavorable trends of pesticide detectability in crops produced in the organic system shown in this work require rectification through the application of economical mechanisms and modernization of techniques and programs of plant protection, as well as through the education of farmers and fruit-growers.
4. Systematic testing of pesticide residues made it possible to demonstrate tendencies of changes of food of plant origin contamination with these residues at the stage of primary production in northeastern Poland, depending the method of production.
5. The burdening of crops with pesticides in the region of northeastern Poland is comparable with tendencies for the whole of Poland, but it is still present in a degree that is clearly lesser than in highly developed countries. This fact should be used to promote these crops both on the domestic market and in exporting. A necessary condition for such promotion is the continuation of systematic and representative laboratory tests according to internationally recognized standards of analytical procedures, as have been presented in this paper.

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POZOSTAŁOŚCI PESTYCYDÓW W OWOCACH I WARZYWACH WYPRODUKOWANYCH W SYSTEMACH EKOLOGICZNYM, INTEGROWANYM I KONWENCJONALNYM

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Abstrakt: Przedstawione badania dotyczą oceny pozostałości środków ochrony roślin w płodach rolnych wyprodukowanych w systemie konwencjonalnym, integrowanym i ekologicznym. W latach 2005–2009 przeprowadzono analizy 1134 próbek materiału roślinnego, pochodzących z czterech województw: lubelskiego, podlaskiego, warmińsko-mazurskiego i mazowieckiego. W materiale roślinnym poszukiwano 123 substancji aktywnych pestycydów. W badaniach wykazano, że w próbkach owoców produkowanych w systemie integrowanym próbki z pozostałościami poniżej najwyższego dopuszczalnego poziomu mają większy udział (55 %) niż w próbkach z produkcji konwencjonalnej (28 %), a nieznacznie mniejszy z przekroczeniami NDP (3 %). Pozostałości pestycydów wykryto również w 8 % próbek owoców i warzyw z produkcji ekologicznej.

Słowa kluczowe: pozostałości pestycydów, owoce, warzywa, produkcja konwencjonalna, integrowana, ekologiczna

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INFLUENCE OF FERTILIZATION WITH MICROELEMENTS TIMOTHY GRASS (*Phleum pratense* L.) ON THE DEVELOPMENT OF SPAD INDEX

WPŁYW NAWOŻENIA MIKROELEMENTOWEGO TYMOTKI ŁĄKOWEJ (*Phleum pratense* L.) NA KSZTAŁTOWANIE SIĘ WSKAŹNIKA SPAD

Abstract: The paper presents results from a 4-year field study into the usefulness of SPAD test for nourishment evaluation in timothy-grass of Skald cultivar grown for seed. The readings of SPAD values were taken with the aid of an optical device Minolta-502. Foliar feeding in this study included copper, manganese and zinc. The values of SPAD readings varied with different measurement time and different micronutrient for foliar feeding. Relative chlorophyll concentration index was increasing with consecutive stages of plant growth up to anthesis and as the result of any micronutrient applied. For practical use this will mean the necessity of supplementing the macronutrients from primary inorganic soil fertilisation with some foliar micronutrient feeding.

Keywords: timothy-grass, chlorophyll, chlorophyll meter, SPAD readings, micronutrient feeding

Timothy grass (*Phleum pratense* L.), known under common names as herd's grass or cat's tail, is one of the best fodder grass species from nutritional viewpoint. Its capacity to accumulate nitrates is low, therefore this species can be safe in animal nutrition. Another advantage will be its high digestibility. Owing to high monosaccharide concentration timothy is eaten willingly by animals [1, 2]. It proved itself to be an excellent component in silages. It is winter hardy and resistant to lodging. Its production grows in response to increasing doses of nitrogen fertilization. The species is allogamous and anemophilous, so timothy grass can be easily cross-pollinated by its different cultivars or another grass. Hence in timothy-grain cultivation one should take into account the placement of a field in order to assure its appropriate spatial isolation as

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required by the rules of field qualification [3]. Not only financial feasibility assessment is indispensable prior to making choice of seed cultivation, but also an earlier analysis and calculation, which have involved the requirements of production process [4]. A prerequisite here is adequate knowledge of present technology for such production. In order to obtain high yielding one should, above all, diagnose properly the nutritional status of plants [5, 6].

Chemical analysis of a plant is a widely accepted method of determination mineral deficiencies and preventing them [7]. The paper is a review focused on a method of chlorophyll content determination serving as an indirect measure of plant nutritional status. Similarly to cereals, grass plants cultivated for seed are especially vulnerable to copper, manganese and zinc deficiency [8]:

– copper influences tissue formation and anatomy, participates in nitrogen transformations as well as in the synthesis of protein and vitamin C,

– manganese improves intensity of respiration, carbon dioxide assimilation and carbohydrate synthesis; the lack of manganese causes chlorosis,

– zinc is involved in the synthesis of chlorophyll and vitamins; its deficiency affects plant growth and development.

The aim of this study was to determine SPAD values for Skald cultivar of timothy-grass during four developmental phases, *eg* stalk formation, head emergence, anthesis and milky ripeness, in different micronutrient fertilization conditions.

Materials and methods

A field experiment was set up on the area of the Plant Breeding Station in Skrzeszowice, which belongs to Malopolska Plant Breeding Company – HBP, Krakow. Skrzeszowice lies within the area of Gmina Kocmyrzow-Luborzyca in the Krakow County. The station is situated in the northern part of Lesser Poland Voivodeship, 25 km northeast of Krakow, and lies within Proszowice Plateau on Lesser Poland Upland, which is between Vistula Valley, Nida Valley, Miechow Upland and Wodzislaw Hills.

The investigation into diversity of SPAD values (chlorophyll content) in timothy grass, Skald cultivar, was conducted in years from 2006 to 2009.

The experiment was established according to randomized block design with four replications on degraded black earth formed from loess; the soil falls to class I, or wheat group of very good quality, by the agricultural soil classification system. Its chemical properties were as follows: pH_{KCl} – 7.32, available nutrients per 1 kg of soil – 190 mgP_2O_5, 155 mgK_2O and 69 mgMg. The soil was moderately rich in available manganese and zinc, while the level of available copper forms was low.

For the growing seasons (April to September) of the years 2006, 2007, 2008 and 2009 total precipitation amounted to 396.0, 553.0, 439.6, and 503.8 mm respectively, while the respective mean air temperatures equalled 11.9, 10.0, 10.2, and 13.7 ^{o}C (Table 1). The grains were sown in rows 40 cm wide at seeding rate of 4 $kg \cdot ha^{-1}$ without protective plants.

Table 1

Hydrologic and thermal conditions yearly from 2006 to 2009 against multi-year averages (1991–2004) for Plant Breeding Station in Skrzeszowice

Month	Year				
	2006	2007	2008	2009	1991–2004
Total rainfall [mm]					
IV	41.5	27.0	47.0	0.4	51.8
V	65.0	67.0	32.0	94.5	68.0
VI	64.0	84.0	53.5	123.1	74.9
VII	31.0	60.0	144.5	69.9	92.4
VIII	101.5	64.0	39.0	49.0	65.2
IX	22.0	163.0	61.7	22.7	56.3
X	14.0	54.0	46.4	65.0	49.6
XI	57.0	34.0	15.5	79.2	30.4
Total	396.0	553.0	439.6	503.8	488.6
Average air temperature [^{o}C]					
IV	7.8	5.4	4.6	11.4	8.6
V	12.5	11.5	9.8	14.2	14.3
VI	15.4	16.6	15.2	16.8	17.2
VII	19.0	16.6	15.5	20.4	19.1
VIII	15.9	15.5	14.8	18.8	18.6
IX	13.0	9.4	10.0	15.1	13.2
X	6.4	4.7	8.1	7.9	8.6
XI	5.1	0.5	3.9	5.2	3.3
Mean	11.9	10.0	10.2	13.7	12.9

Soil mineral fertilization was applied as follows. In the year of planting pre-seed fertilizers were spread including 50 kgN $\cdot$ ha^{-1} as ammonium nitrate, 60 kgP$_2$O$_5$ $\cdot$ ha^{-1} as triple superphosphate, and 80 kgK$_2$O $\cdot$ ha^{-1} as 57 % potassium chloride. Additionally in autumn phosphorus and potassium fertilizers were top-dressed at the same rates as used before. For each year of plant usage a phosphorus-and-potassium dose in spring was identical (60 kgP$_2$O$_5$ $\cdot$ ha^{-1} as triple superphosphate and 80 kgK$_2$O $\cdot$ ha^{-1} as 57 % potassium chloride), while the nitrogen one was 100 kgN $\cdot$ ha^{-1}. Foliar feeding was proceeded with the use of single chelated micronutrients including zinc, manganese and copper. Types and doses of the micronutrients were as follows:

Chelated zinc, 14 % Zn (EDTA+DTPA ligands) at a dose of 100 gZn $\cdot$ ha^{-1} during stalk elongation and head emergence phases.

Chelated manganese, 14 % Mn (EDTA+DTPA ligands) at a dose of 100 gMn $\cdot$ ha^{-1} during stalk elongation and head emergence phases.

Chelated copper, 12 % Cu (EDTA+DTPA ligands) at a dose of 60 gCu $\cdot$ ha^{-1} during stalk elongation and head emergence phases.

In the consecutive years of this study the influence of foliar fertilization with micronutrients on chlorophyll content was analyzed. Leaf greenness (SPAD) indices were measured with a Minolta SPAD 502 DL chlorophyll meter using upper leaves, in four stages of development including stalk elongation, head emergence, anthesis and milky ripeness. Measurements were done using thirty fully developed leaves from each plot.

The fundamental part of the instrument is a clip provided with a light source at one end, and a light detector on the opposite end. When the measuring clip is open, one can insert an analysed leaf inside. When the instrument is closed, the light source present at an arm of the clip will illuminate the leaf. The amount of light passing through the leaf, is sensed by the light detector placed at the other arm. The light detector identifying radiation of 650 nm wavelength, is able to detect an amount of light absorbed by chlorophyll. Additionally light of 940 nm wavelength is measured, which is absorbed by other parts of leaf structure. This enables correction to a result calculated by microprocessor and relative units are shown at a display. After two seconds one can begin next measurement.

In order to eliminate expansion of dicotyledonous weed species a spray was performed in the tillering phase of grass development. For massive weed growth Chwastox Extra 330 SL, Aminopielik D, Starane 250 SL and Gold 450 EC were employed at doses of 1.5, 1.5, 0.6, and 1.5 $dm^3 \cdot ha^{-1}$ respectively. Individual stubborn weeds were eradicated by spot application of Roundup herbicide. Then monocotyledonous weeds were destroyed, at the beginning of September and in spring as soon as the growth had started, with the aid of Stomp 330 EC, where 5 $dm^3 \cdot ha^{-1}$ dose of preparation was dissolved in 300–400 dm^3 of water. The experimental field was also sprayed preventively against pests with Owadofos 50 solution (1 $dm^3 \cdot ha^{-1}$). The first spray was performed in early springtime, once the plants had started growth, and the next took place after head emergence, but before flowering. Timothy-grass harvest fell between the end of July and the beginning days of August. Seed crop was gathered with a Wintersteiger plot combine harvester. Having collected the grain experimental plots were cleared of waste straw and after-harvest remnants.

Obtained data underwent statistical analysis with the aid of the ANOVA procedure. The values of one-sided confidence limits were found by means of Tukey’s test at confidence level $\alpha = 0.05$.

Results and discussion

Chlorophyll pigments in a plant are among its most important compounds that influence the intensity of photosynthesis, biomass production or seed yielding. Measured chlorophyll content in leaves showed significant differentiation with regard to time of reading and type of foliar micronutrient fertilization (Table 2). Mean values of SPAD readings for the years of the study in zinc-fertilized objects were higher than in these receiving foliar sprays of manganese or copper. An increase in SPAD reading scores with regard to reading time was also observed. The values of leaf greenness index (or SPAD) in experimental plants varied with the time of reading and fell between 38.75 and 44.97.

Table 2

Leaf greenness (SPAD) index in timothy-grass of Skald cultivar over some developmental stages and with different micronutrient feeding (four-year averages for whole study)

Item	Developmental Stage			
	Stalk formation	Head emergence	Anthesis	Milky Ripeness
Control	38.75	39.64	41.45	39.03
Cu	39.00	39.99	43.93	39.56
Mn	38.89	39.30	42.74	39.15
Zn	39.44	40.74	44.97	40.59
Standard deviation	0.30	0.62	1.52	0.71
Coefficient of variation [%]	0.76	1.55	3.51	1.79
$LSD_{\alpha = 0.05}$	0.26	0.60	1.65	0.54

After reaching a maximum at the anthesis stage the scores were diminishing steadily, so that at the milky ripeness phase they were below the level for head emergence. Mean values for specific points of time were slightly higher than similar ones obtained in the same species by Olszewska et al [9]. This difference can be attributed to the fact that in her work the plants grew on more deficient soil, namely fine and light loamy sand. Our findings correspond with experimental results by different authors which showed higher chlorophyll concentration in plants growing in adequate nutritional conditions [10–12].

Machul [13] in his studies demonstrated that with increasing level of nitrogen fertilization SPAD readings in maize leaves were growing. The role of micronutrients in an improvement of SPAD readings, then, is ascribed to the fact that zinc is an element involved in nitrogen metabolism; it engages in synthesis of chlorophyll and vitamins C, B and D, influencing plant growth and development, enhancing drought resistance and affecting tolerance to frost, which was supported by some investigations [8, 14].

Next, manganese is an element indispensable in photosynthetic process, protein synthesis, uptake and assimilation of many nutrients; it regulates the levels of plant hormones (growth) and iron uptake, which has an effect on chlorophyll synthesis and enhances winter resistance.

Finally, copper is involved in the synthesis of nitrogen compounds and vital for optimum uptake of mineral nitrogen (N and Cu synergism), improving winter hardiness, enhancing resistance to fungal and bacterial diseases; it affects plant respiration, chlorophyll synthesis and stabilisation, activates many enzymes, lignin synthesis and photosynthetic process [15].

Chlorophyll content depends on nutritional status and supplies of nutrients, primarily nitrogen and sulphur, then magnesium and potassium [7].

Samborski and Rozbicki [6] reported that the absorbance of light not only depends on amounts of the pigment, but also on diffraction of light inside mesophyll, its reflection off a leaf surface and spatial distribution of chlorophyll within a leaf.

Podlesny and Podlesna [16] showed for narrow-leaved lupine and barley that in drought stress conditions the SPAD values of the leaves were lower. Although Olszewska et al [9] found high chlorophyll content in the leaves of grass grown in stress conditions, which is explained as an effect of anti-stress defence by plants.

Published results confirmed that up to now a chlorophyll meter SPAD-502 has found uses in monitoring the status of nitrogen nutrition in many plant species including maize, rice, spring and winter cereals, soya, sorghum, cotton, potato, tomato, tobacco, apple tree, grape vine, peanut and pepper [6, 10, 11, 17–19]. Moreover, the author's own research has demonstrated that a quite important issue would be not only to determine the nitrogen nutrition status of plants, but also to supply their feeding with micronutrients. Based on scores from a chlorophyll meter SPAD-502 the plants receiving foliar sprays of micronutrients were shown to have higher chlorophyll concentrations (or SPAD readings).

Conclusions

1. Foliar application of micronutrients had a significant influence on leaf greenness (SPAD) index. In all developmental phases the highest scores of SPAD reading were observed in plants from the zinc-fed object, while the lowest were noted for the one receiving manganese application.
2. The index of relative chlorophyll content was increasing with the plant growth. It obtained the highest value during the anthesis phase, whereas in the milky ripeness phase it diminished to the level as it had prior to head emergence.

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WPŁYW NAWOŻENIA MIKROELEMENTOWEGO TYMOTKI ŁĄKOWEJ (*Phleum pratense* L.) NA KSZTAŁTOWANIE SIĘ WSKAŹNIKA SPAD

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Abstrakt: W latach 2006–2009 przeprowadzono badania nad zróżnicowaniem wartości SPAD (zawartości chlorofilu) u tymotki łąkowej odmiany Skald na plantacji nasiennej nawożonej dolistnie miedzią, manganem i cynkiem. Doświadczenie polowe z tymotką łąkową przeprowadzono w Stacji Hodowli Roślin w Skrzeszowicach należącej do Małopolskiej Hodowli Roślin – HBP Kraków. Założono je metodą losowanych bloków w czterech powtórzeniach na czarnoziemie zdegradowany wytworzonym z lessu, I klasy bonitacyjnej, kompleksu przydatności rolniczej pszennego bardzo dobrego. Do nawożenia dolistnego zastosowano nawozy w formie chelatów. W okresie wegetacji mierzono indeks zieloności liści – chlorofilometrem SPAD-502 firmy Minolta. Pomiary wykonano na każdym poletku, na trzydziestu w pełni rozwiniętych liściach, w czterech fazach rozwojowych: strzelaniu w źdźbło, kłoszeniu, kwitnieniu i dojrzałości mlecznej.

Wskaźnik względnej zawartości chlorofilu wzrastał od fazy strzelania w źdźbło do fazy kwitnienia. Wszystkie testowane mikroelementy przyczyniły się do zwiększenia wartości indeksu zazielenienia liści. Największe wartości odczytów we wszystkich fazach rozwojowych stwierdzono u roślin z obiektu nawożonego cynkiem, a najmniejsze wartości odnotowano w obiekcie z aplikacją manganu. Dostarczone mikroelementy drogą dolistną na plantację nasienną tymotki łąkowej miały istotny wpływ na odnotowane wartości omawianego wskaźnika.

Słowa kluczowe: tymotka łąkowa, chlorofil, chlorofilometr, odczyty SPAD, nawozenie mikroelementami

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EFFECT OF SOIL CONTAMINATION WITH PETROLEUM PRODUCTS ON ESTERASE ACTIVITY IN EARTHWORM *Lumbricus terrestris*

WPŁYW ROPOPOCHODNYCH ZANIECZYSZCZEŃ GLEBOWYCH NA AKTYWNOŚĆ ESTERAZOWĄ DŻDŻOWNIC *Lumbricus terrestris*

Abstract: Among biomarkers of exposure the suitability of esterases especially carboxylesterases (CarE), metabolizing wide array of xenobiotics being important for organismal functioning, was very often underlined. Moreover, neurotoxic effects of constituents of petroleum products may be exert by inhibition of cholinesterase (ChE) isozymes in animals. Here, we examined esterase activity toward acetylthiocholine iodide and *p*-nitrophenyl acetate in earthworms *Lumbricus terrestris* reared through four weeks on loamy sand soil contaminated with petroleum products (unleaded petrol, used engine oil), in two weeks intervals. Such contamination was simulated in one m^3 volume soil containers, at a dose of 6 g $\cdot$ kg^{-1} of dry soil (experimental field in Mydlniki, Krakow suburb, Poland; 50.0815°N, 19.84730°E). We compared the effects of these contaminants action in soil collected in the year of treatment and one year later. We evaluated also effects of animals rearing on soil unremediated and remediated with "cocktail" of microorganism, and also checked the enzymatic activities response in two weeks intervals. We found lower ChE activity in animals reared on petroleum derivatives contaminated soil when compared with control animals, but only in the case of four weeks rearing on soil collected in the year of treatment. Similar response of ChE in animals reared on soil collected a year after the treatment, but estimated in earlier period of time – after two weeks exposure to petrol unleaded contamination was observed. CarE activity characterized higher variability with time than ChE activity. Generally, the activity of CarE lowered from the beginning to the end of the rearing (in all experimental groups). Animals reared on remediated soil, in most cases, had higher ChE activity than those reared on unremediated soil (control and diesel oil exposed animals).

Keywords: esterase, petroleum contamination, earthworm *Lumbricus terrestris*

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Contamination of agroecosystems by petroleum derivatives may be a threat for their invertebrate inhabitants [1, 2]. Among soil inhabitants, earthworms seems to be important in assessment of negative effect of soil contamination. Earthworms as a common standard organisms have a notable place in soil toxicology. The earthworms are an important factor in mixing the soil, increase the contact of microbials with toxins. They are presented as a model organism in respect to its role in removal of polycyclic aromatic hydrocarbons (PAHs) from soil. They may reduce time of remediation process in petroleum derivatives contaminated sites [3, 4].

There are plenty of petroleum contamination sources, like leakages from: tanks, pipes during distribution, petrol stations, different types of transport, industry [5]. In industrial soil, the concentrations of PAHs may go up even to 6 g $\cdot$ kg^{-1} of soil [6]. Serious soil pollution by PAHs in Poland was reported by Wcislo [7]. Petroleum products are usually mixtures of several substances having complicated fate in soil surrounding. Their compositions depend on source, refinery process and product specificity [8]. Petroleum contaminants, among them PAHs, modify properties of soil, evaporating to its air spaces. Most hydrocarbons are quickly removed from soil as volatile compounds or degraded readily by microorganisms, but some of them are more resistant and may accumulate in fat tissues of animals [9, 10]. The effects of soil petroleum contaminants might be typical for local sources of contamination. Schaefer and Juliane [4] measuring effects of different representatives of earthworms, observed their high mortality (above 58 %) when exposed for 28 days of rearing to soil contaminated with total petroleum hydrocarbons, with concentration higher than 4 g $\cdot$ kg^{-1} of soil.

Remediation of contaminated soil may make it less destructive. According to Salanitro et al [11] after contamination with total petroleum hydrocarbons (4–27 g $\cdot$ kg^{-1} of soil), alkanes, alkenes, aromatic and polar constituents of petrol derivatives were degraded continuously during remediation process, being less toxic for earthworm after 5 to 12 months after contamination (it took longer with lower organic contents in soils).

Natural remediation process, especially due to absence of suitable endogenous bacteria, may be enhanced by inoculation with PAH specific bacteria. Additional input of non indigenous bacteria not always lead to significant biomagnifications of the remediation process. Their compatibility to the site application is very important, however some of them are capable to metabolize wide spectrum of substances [9, 12–13].

Esterases of various organisms inhabited contaminated areas have a promising role as biomarkers. Wheelock et al [14] emphasised, next to cholinesterases, the usefulness of carboxyloesterases in that matter, especially, when recording toward *p*-nitrophenyl acetate as a substrate. Vejares et al [15] depicted the suitability measuring the effects of organophosphate pesticides on both enzymes: CarE – important as enzymatic barrier against organophosphate uptake during ingestion of contaminated soil, ChE activity – connected with proper neuronal transmission.

ChE activity was measured in earthworms toward different substrates, but dominant isozymes, as was revealed, had an affinity to acetylthiocholine iodide [16].

The present study examined the effects of different petroleum contaminants: unleaded petrol and diesel oil on cholinesterases and carboxylesterases activity in earthworms *Lumbricus terrestris.* The animals were reared on soil well-mixed with these products during four weeks period, firstly using soil collected two month after its contamination from containers located in the field and secondly, using soil collected a year after. We compared the effects using soil not only contaminated but also being mixed with cocktail of microorganisms. The main question was whether these esterases were universal and suitable biomarkers of such contamination.

Material and methods

Earthworms *Lumbricus terrestris* Linnaeus 1758 were purchased from a commercial sportfishing supply and were reared thought four weeks on loamy-sand soil contaminated with unleaded petrol or used engine oil. The used mature clitellate animals weight, at the beginning of the experiment, was 3–6 g. Twenty individuals were reared in each containers of 3L volume, filled with soil contaminated with unleaded petrol or used engine oil. Soil was collected on the surface layer (0–10 cm), dried on air and sieved (sieve with 1 mm diameter) and after 78–80 % rH moisturing was used for earthworms rearing (soil hygrometer TFH-100-E, Ebro GmbH). Every week, wet soil was mixed with 4 g dried powder of horse manure.

The contaminated soil used in the experiment, was originated from cubic containers of one m^3 volume with attest to use petrol substances. The containers, equipped with drainage and evaporation system, were kept in the field (Mydlniki, Krakow, Poland; 50.0815^{o}N, 19.84730^{o}E). They were placed into the ground and the soil inside them was filled up to the same level as that outside. Their walls in the upper part were perforated to allow the penetration by the field organisms. For better soil remediation process, the soil was aerated in gravity system using perforated tubes, digged to a depth of *ca* 70 cm. Before remediation mixture was applied to the top soil layer in the containers, multi-component fertilizer (Azofoska; 13.6 % N, 2.8 % P and 15.8 % K) in a dose of 100 g $· container^{-1} was applied. Leachates from containers were pumped regularly not to allow their flooding. In the June of 2010, the soil was contaminated with petroleum products: unleaded petrol or used engine oil, in concentration of 6 g · kg^{-1} of dry soil weight. In the August of 2010 and next, in the August of 2011, the upper part of the soil (*ca* 10 cm layer) was collected and air dried prior experiment provided in laboratory conditions.

The experiment were provided twice, firstly with usage of soil collected two months after contamination. Secondly, with the usage of soil collected one year later (fourteen months after its contamination).

The experimental group names are connected with petroleum derivatives contamination of soil, used in the experiment, on which earthworms were reared.

– Group C – control group – earthworms reared on soil without petrol contaminations,

– Group P – earthworms reared on soil contaminated with petroleum unleaded,

– Group EO – earthworms reared on soil contaminated with used engine oil,

– Group C + R – earthworms reared on soil uncontaminated and bioremediated with “cocktail” of microorganisms,

– Group P + R – earthworms reared on soil contaminated with petroleum unleaded and bioremediated,

– Group EO + R –earthworms reared on soil contaminated with used engine oil and bioremediated.

The loamy-sand soil, which was used in this study was characterized by concentration of 75 % of sand particles (mainly fine sand), 21 % of silt particles (mainly coarse silt) and 4 % of clay particles. The measured soil pH was: pH (KCl) 8.5 and pH (H_2O) 7.1. The soil contained 1.04 % of $C_{organic}$. The soil was also characterized in the respect of nitrogen and carbon concentration with VARIO MAX CNS elemental analyser. Nitrogen concentration in the control soil was 1.134 g $\cdot$ kg^{-1} dry weight of soil, and comparable values were found in the soil from other experimental groups. Only in the cases of groups P + R and EO + R higher concentrations of this element were found. Carbon concentration in control soil samples was 10.035 g $\cdot$ kg^{-1} dry weight of soil, and in the case of EO + R group it was three times higher than in the controls. The EO + R group significantly differentiated higher proportion of carbon to nitrogen than other experimental groups (Kafel, unpublished).

Animals from each experimental group were reared in two containers. After two and four weeks of rearing the earthworms were anaesthetized on ice, freezed in nitrogen liquid and homogenized in 0.05 M buffer Tris-HCl, pH 7.4 with 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 mM phenythiourea and 20 % sucrose. Homogenates were centrifuged for 10 minutes at 1000 g at 4 ^{o}C, and the obtained supernatants were centrifuged for 15 minutes at 15,000 g at 4 ^{o}C. Next, the supernatants were centrifuged at 100,000 g for 1 h at 4 ^{o}C. After that centrifugation, the supernatants were used for esterase enzymes measurements.

Carboxylesterase (CarE) activity was measured in presence of *p*-nitrophenyl acetate as a substrate. The measurement was taken at 400 nm for 3 min. Results were corrected by subtracting blanks contained buffer instead of sample. To calculate CarE activity, the extinction coefficient of 9.25 $mM^{-1} \cdot cm^{-1}$ was used [17].

Acetylcholinesterase (AChE) activity was determined according to Ellman et al [18]. The reaction mixture contained buffer, sample and acetylthiocholine iodide (a substrate) and 5,5'-dithio-bis(2-nitrobenzoic acid). The linear changes in absorbance were measured at 412 nm through 5 min. The activity was presented as nmol mg $protein^{-1} \cdot min^{-1}$.

The protein concentrations were assessed as in Bradford [19], using bovine serum albumin as a standard.

The assumption of variance homogeneity and normal distribute were tested before statistical analysis. If necessary, the data were log transformed. ANOVA was performed with LSD post-hoc test. Furthermore, linear regression analysis were done to evaluate the putative association among biochemical variables but we did not found any relationships. The data in the tables are presented as mean values ± SD of six-seven replicates in each experimental groups (each replicate was composed from homogenate from one individual).

Results

The animals reared through four weeks on soil collected two months after its contamination with petroleum derivatives characterized significant lower ChE activity than in control animals. In the case of animals from P group it was *ca* 28 % lower and in the case of animals from group EO it was almost 50 % lower in comparison with control values (Table 1). ChE and CarE activity was examined in individuals from experimental groups, which were reared on soil collected fourteen months after its contamination. At the start of the experiment, animals had lower activity ChE than in the control groups but higher in the animals from the other groups, when consider effects after two weeks exposure. After next two weeks exposure, control animals had lower activity than in previous measurement (measured after two week exposure) and opposite trend was found for animals from the other experimental groups.

Table 1

ChE activity [nmol · min^{-1} · mg · protein^{-1}] in earthworms *L. terrestris* reared through four weeks on the soil collected two months after contamination with petroleum derivatives: petrol unleaded and used engine oil (first year of the experiment). Different letters denotes significant differences between experimental groups (LSD, $p < 0.05$)

Experimental groups	ChE (Mean ± SD)	
Group C	50.44 ± 15.97	a
Group P	36.48 ± 7.82	b
Group EO	26.58 ± 5.87	b

When compared effects of petroleum derivatives contamination with proper (measured after the same period of rearing) control, the differences were found only after two weeks rearing but not after four weeks rearing. The earthworms analysed after two weeks of exposure derived from P and EO groups had more than 5 times lower activity than earthworms from the proper control group.

Regarding CarE activity changes, we found the lowering with time tendency. The highest activity was measured in animals at the start of the experiment. And then, in animals reared through two weeks from almost all examined groups, the activity was higher than in animals reared through four weeks. The only exception was found in the case of animals derived from EO + R group. In animals from this group, similar activity of the enzyme was measured in animals after two and four weeks of rearing (13.16 *vs* 11.53 nmol/min/mg protein, respectively). When compared effects of petroleum derivatives contamination with proper (measured after the same period of rearing) control, in the case of CarE, we found lower activity in animals from P groups (those being after two weeks of exposure) and in the case of animals from EO group (those being after four weeks of exposure). Moreover, animals reared through two weeks on remediated soil and exposed to petrol unleaded or used engine oil differentiated lower CarE activity than in control (Table 2).

Table 2

ChE and CarE activity [nmol · min^{-1} · mg · protein^{-1}] in earthworms *L. terrestris* reared through two and four weeks on the soil collected fourteen months after its contamination with petroleum derivatives: petrol unleaded and used engine oil, and additionally after bioremediation process. Different letters denote significant differences among experimental groups after the same time of treatment, star denotes differences with control values gained at the beginning of the experiment (from C group), dash denotes differences between the same groups in different time of rearing: two and four weeks (LSD, $p < 0.05$).

Experimental groups	ChE (Mean ± SD)		CarE (Mean ± SD)	
At the beginning of the experiment				
Group C	20.97 ± 10.67		32.82 ± 17.56	
After two weeks of the experiment				
Group C	48.09 ± 20.35	a *#	26.57 ± 10.49	a #
Group P	8.75 ± 4.17	c *#	16.52 ± 2.58	b *#
Group EO	6.85 ± 2.28	c *#	17.95 ± 5.49	a #
Group C + R	17.14 ± 7.98	b #	16.64 ± 3.12	b #
Group P + R	8.10 ± 3.97	ab *#	13.68 ± 5.45	b *#
Group EO + R	6.67 ± 1.67	ab *#	13.16 ± 2.69	b *
After four weeks of the experiment				
Group C	23.83 ± 3.97	b #	9.74 ± 2.37	b *#
Group P	18.23 ± 6.17	b #	10.72 ± 3.18	ab *#
Group EO	25.62 ± 6.33	b #	13.47 ± 2.15	a *#
Group C + R	38.80 ± 5.33	a *#	10.85 ± 3.54	ab *#
Group P + R	34.23 ± 13.66	ab *#	7.46 ± 1.81	b *#
Group EO + R	24.40 ± 8.81	ab #	11.53 ± 0.41	b *

Discussion

Responses of the earthworms' examined enzymes to soil contaminants: after treatment with petrol unleaded or used engine oil, if noted, generally, were connected with a decrease of animals activity in comparison with proper controls. Such reaction was revealed in the case of ChE activity in earthworms reared through four weeks on soil collected two months after its treatment. In the case of ChE and CarE in the earthworms reared on soil collected fourteen months after its treatment with petrol unleaded (but just after two weeks of rearing) and CarE activity in those treated with soil contaminated with used engine oil (after four weeks of rearing), the lower activity (in comparison with control) of both enzymes was recorded (Tables 1 and 2). Both enzymes are presented as effective biomarkers of different kind of contamination: with heavy metals or pesticides (organophosphate and carbamate) and PAHs [20–23]. In highly stressful situation the mentioned enzymes were inhibited by different xenobiotics. Such situation was shown by Alpuche-Goal and Gold-Bouchot [23] in the case of *H. lumieri* fish, for which lowered ChE activity was measured in brain and liver under benzo[a]pyrene and chlorpyrifos exposure. But, sometimes, the enzymes could also be an ineffective as biomarkers, as it was in the case of acetylcholinesterase (predominant ChE) activity measured in brains *of Sparus aurata*. A lack of effects on the enzyme to exposure of different PAHs present in petroleum contaminated environments (phenanthrene, pyrene and fluorine) were shown [24]. The ways of petroleum constituents action may be uptaken mainly by alimentary tract of the earthworms and also by their skin [25]. Their detoxifying responses may be important, because they fulfil a role as a barrier, bio-filter and bio-remediator of petroleum derivatives contamination. Sinha et al [26] presented, on the example of the earthworm *Eisenia fetida,* high reduction (above 99 %) of highly toxic hydrocarbons (C10-C14, C15-C28 and C29-C36) in soil of vermifilter bed.

But, regarding suitability esterase as biomarkers, it should be also analysed: the duration of exposure, the level of petroleum constituents in the soil in the following years after contamination and remediation process. Impact of petroleum substances on organisms in relation to time and concentration of petroleum derivatives mixtures was presented by Baggi [27], Stroomberg et al [28] and Singh et al [29].

Generally, CarE activity lowered with time of rearing, and in groups reared on control and unleaded petrol contaminated (remediated and unremediated) soil, there were noted significant differences among records done in animals being exposed through two weeks and four weeks. The highest activity of CarE was measured in animals at the start of the experiment (Table 2). This might be connected with lowering of the level of contaminants in the soil (unpublished data). These ubiquitous enzymes, have still not well established physiological functions, with wide range of substrate, apart from their participation in cellular metabolism, are important in detoxifying or inactivating toxins process [30, 31]. Sanchez-Hernandez and Wheelock [25] shown high relevance between level of carboxylesterase in different parts of alimentary tract of *L. terrestris* and in located there ingested soil. Their activity may be important for hydrogen constituents limitation.

In turn, in the case of ChE activity changes it is difficult to describe a general tendency. The changes were specific in each experimental group. When reactions are related to changes with time, general unspecific response of CarE and specific ChE are seen. Some confounding results may be connected with variation of the enzymes activity in specific tissues [15].

We have done measurements of ChE, twice, in the first year of experiment and in the next year, accordingly to time of current soil collection. It was seen a lower enzyme activity after four weeks of rearing on contaminated soils in the first year of exposure in comparison to control (Table 1). In the second year such duration of exposure (four weeks) did not exert impact on the enzyme activity (Table 2). It could be connected with, mentioned earlier in the Introduction part, lowering amounts of hydrocarbons with time.

The remediation process may be effectively augmented by additional input of microorganism into the soil. It supposedly might come to lowering levels of petroleum contamination constituents. Hubalek et al [32] shown that bioremediation process limited negative impact on *Eisenia fetida* development examined after 3 months from application of hydrocarbons to the soil (around 6 g · kg^{-1}) in the field (such contamination was done with mineral oil, hydraulic fluids and grease). After that period of time, soil contained about 4.5 g hydrocarbons · kg^{-1} of dry weight of soil. But, it is difficult to find any tendency in esterase activity changes in our study which would be connected with such supposing. Moreover, the earthworms responses in control groups having contact with soil treated with remediation cocktail indicated that changes in soil, originated from microbial activity, might be connected with availability of nutrients, tended to changed activity in group C + R in comparison with control group. Simultaneously provided examinations of nitrogen and carbon contents, showed some decline of these elements in soil collected after second week of the earthworms rearing (Kafel, unpublished). Such situations might exert impact on animals condition and these enzymes levels, apart from petroleum contaminants action.

Conclusions

1. CarE activity in *L. terrestris* seems to be a more applicable biomarker of petroleum derivatives contamination soil.
2. Effect of soil contamination with petroleum derivatives (at a dose of 6 g · kg^{-1} of dry soil) on CarE and ChE activity in *L. terrestris* was more pronounced with time of rearing (differences between effects of two or four weeks exposure).
3. Differences in esterases responses were found between animals reared on remediated with "cocktail" of microorganism and unremediated soil.

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WPŁYW ROPOPOCHODNYCH ZANIECZYSZCZEŃ GLEBOWYCH NA AKTYWNOŚĆ ESTERAZOWĄ DŻDŻOWNIC *Lumbricus terrestris*

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Abstract: Spośród biomarkerów ekspozycji szczególnie użyteczne są esterazy, a zwłaszcza karboksyloesterazy (CarE) metabolizujące szeroki zakres ksenobiotyków. Podkreśla się często, że odgrywają one również ważną rolę w funkcjonowaniu organizmów. Co więcej, neurotoksyczne efekty składników substancji ropopochodnych mogą być wywierane również na izoenzymy cholinoesteraz (ChE) u zwierząt. W tym doświadczeniu, w dwutygodniowych interwałach, badaliśmy aktywność esteraz wobec jodku acetylotiocholiny i octanu para-nitrofenylu u dżdżownic *Lumbricus terrestris*, hodowanych przez cztery tygodnie w glebie gliniasto-piaszczystej zanieczyszczonej produktami ropopochodnymi (benzyną bezołowiową, zużytym olejem silnikowym). Takie zanieczyszczenie symulowano w glebie umieszczonej w kontenerach o pojemności jednego m^3 w stężeniu 6 g · kg^{-1} suchej masy gleby (eksperyment prowadzono w Mydlnikach, na przedmieściu Krakowa, 50.0815°N, 19.84730°E). Porównano efekty działania tych zanieczyszczeń w glebie zebranej w roku kontaminacji i rok później. Oceniono także wpływ gleby remediowanej z użyciem „koktajlu” mikroorganizmów i nieremediowanej na hodowane zwierzęta, i sprawdzano u nich zmiany aktywności enzymatycznej, również w dwutygodniowych interwałach. Stwierdzono niższą aktywność ChE u zwierząt hodowanych w glebie skażonej substancjami ropopochodnymi w porównaniu do zwierząt kontrolnych, lecz tylko w przypadku czterotygodniowej hodowli na glebie zebranej w roku kontaminacji. Podobną odpowiedź ChE ustalono u zwierząt hodowanych w glebie skażonej benzyną bezołowiową zebranej rok później, ale w krótszym okresie czasu, już po dwóch tygodniach. Aktywność CarE cechowała się dużą zmiennością w czasie, w porównaniu ze aktywnością AChE. Generalnie, aktywność CarE obniżała się od początku hodowli wraz z upływem czasu (we wszystkich grupach eksperymentalnych). Zwierzęta hodowane w glebie poddanej remediacji w większości przypadków wykazywały się wyższą aktywnością ChE niż te, które były hodowane w glebie nie poddanej remediacji (grupy: kontrolna i eksponowana na zużyty olej silnikowy).

Słowa kluczowe: esterazy, zanieczyszczenia ropopochodne, dżdżownice *Lumbricus terrestris*

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THE EFFECT OF OIL DERIVATIVES ON THE ABILITY OF ENTOMOPATHOGENIC NEMATODE *Steinernema feltiae* TO FIND HOST

WPŁYW ROPOPOCHODNYCH NA ZDOLNOŚĆ NICIENIA *Steinernema feltiae* DO ODNAJDYWANIA ŻYWICIELA

Abstract: The aim of the research was to evaluate the effect of oil derivatives on entomopatogenic nematode *Steinernema feltiae*. The effect of unleaded petrol, diesel oil and used engine oil on the ability of *S. feltiae* infective juveniles to locate test insect was investigated. The experiments were carried out in laboratory conditions in four replications. In the first experiment the effect of oil derivatives on behavior of infective juveniles on agar medium in the presence of test insects was investigated. Oil derivatives were added to the medium at the rate of : 2000, 4000, 6000 and 8000 $mm^3 \cdot dm^{-3}$. Infective juveniles did not dispersed towards test insects on agar medium contaminated with oil derivatives. The application of high doses of oil derivatives had also negative effect on nematodes activity and mortality. In the second experiment infective juveniles were applied into contaminated soil. Soil was polluted with following oil derivatives: unleaded petrol, diesel oil and used engine oil at the rate of : 2000, 4000, 6000 i 8000 $mg \cdot kg^{-1}$. In soil contaminated with oil derivatives test insect mortality was significantly lower than in uncontaminated control. It was also revealed that less extensive infestation of test insects with nematodes was observed in contaminated soil. Reaction of *S. feltiae* to particular oil derivatives was diverse. The most toxic effect on nematode ability to find host was observed in soil polluted with unleaded petrol. The applied oil derivatives had also an adverse effect on the female/male ratio of nematodes infesting host insect.

Keywords: *Steinernema feltiae*, oil derivatives

Pollution with oil derivatives is one of the most important threat to the environment. The most devastating disasters are usually in marine environment, but on land there are also many accidents *ie* car accidents, which causes environment pollution. Oil derivatives cause substantial changes in soil environment affecting all living organisms [1]. However there are not many studies concerning the effect of oil derivatives on soil fauna [2] and only a few concerning nematodes [3].

Nematodes are used in soil ecotoxicology both in field and laboratory studies [4]. Raymond et al [5] investigated presence of wild nematodes in soil in the USA during

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degradation process of various oil kinds. Similar study was carried out by Prihonen and Huhta [3] in forest soil in Finland. More studies were done on the effect of oil derivatives on aqueous invertebrate [6–10]. It was revealed that oil derivatives has severe and long-lasting toxicological effects, primarily aromatic hydrocarbons.

An important group of nematodes are entomopathogenic ones, which are widely used in biological plant protection [11]. They naturally occur in soil environment and are regarded as possible indicators of the environment contamination [12]. Entomopathogenic nematodes were used to evaluate the effect of soil pollution with heavy metals [13]. Pathogenicity of entomopathogenic nematodes was significantly affected by heavy metal ions [14].

The aim of the research was to evaluate the effect of oil derivatives on ability of entomopatogenic nematode *Steinernema feltiae* to locate and kill host insect.

Material and methods

In the first experiment the effect of oil derivatives on behavior of infective juveniles (IJs) on agar medium was investigated. Oil derivatives were added to the agar medium at the rate of: 2000, 4000, 6000 and 8000 $mm^3 \cdot dm^{-3}$. In the control unpolluted medium was applied on Petri dishes (90 mm diameter). At the edge of the dish a drop containing 100 IJs was applied. The excess of water was removed by filter paper to facilitate movement of the IJs. In the center of the dish a larvae of test insect (*Tenebrio molitor*) wrapped in perforated aluminum foil was put. The distance from the IJs application point to the test insect was 4 cm. The movement of IJs was observed under stereomicroscope. After 3 hours the position of IJs was marked. Number of migrating IJs from application point and the number of IJs which were able to reach test insect were counted. IJs which had characteristic straight posture were recognized as dead. Migrating IJs and those that did not left application point but were moving were recognized as active. The experiment was carried out in 22 ^{o}C in four replications.

Soil was polluted with following oil derivatives: unleaded petrol, diesel oil and used engine oil. Unpolluted soil was used as control. In the conducted experiments sensitivity to oil derivatives was tested for of *Steinernema feltiae* (Nemasys) infective juveniles (IJs). Experiment was carried out in 120 cm^3 containers. Each container held 50 g of soil dry mass. Doses of 2000, 4000, 6000 and 8000 mg of oil derivatives per 1 kg soil d.m. were applied. Distilled water was used in the control. Entomopathogenic nematodes were applied at the bottom of the container. After that the container was filed with soil. The depth of soil in the container was about 5 cm, that was the distance the IJs had to the surface, where 10 test insects (*Tenebrio molitor* larvae) were put after 24 hours. The larvae were covered with perforated foil to avoid direct contact of insect with polluted soil and enable nematodes penetration inside the host. The experiment was carried out in 22 ^{o}C. The larvae mortality was checked every 24 hours. Dead larvae were removed into sterile Petri dishes and stored at 25 ^{o}C for 48 hours. After two days since the test insect death they were dissected under a binocular magnifying glass to count the number of nematodes able to actively penetrate into their bodies. Rate of male and female of nematodes entering insect body was also calculated.

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

Results and discussion

IJs of nematodes can actively search for potential host. Some substances *eg* fertilizers and pesticides may impair their ability to locate potential host insects. In the present studies an adverse effect of oil derivatives on IJs of *S. feltiae* applied on contaminated agar medium was noted (Fig. 1). Infective juveniles did not migrated from application point on agar medium contaminated with high doses of oil derivatives. The most adverse effect was noted when petrol was added into the medium. Low doses of oil derivatives also negatively affected nematode migration, but to a lesser extent. On control medium (without oil derivatives) IJs were able to move towards test insects, and almost 90 % of them left application point. During 3 hours experiment almost 20 % of applied IJs were able to reach the test insect (Fig. 2). On medium contaminated with diesel oil the migration of IJs was very low even when low doses of pollutants were used and only few percent of IJs were able to reach test insect when low doses of diesel oil and used engine oil were used. The application of high doses of oil derivatives had negative effect on nematodes mortality (Fig. 3). On agar medium contaminated with petrol high mortality rate of IJs was observed. Not all IJs that did not left application point were dead. Basing on the IJs posture of body they were recognized as dead, active or inactive. In uncontaminated control all IJs were active after 3 hours since the time

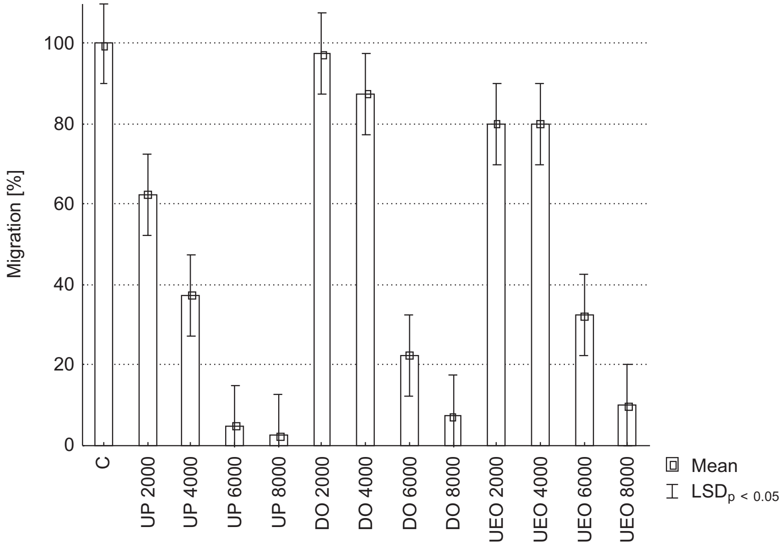

Fig. 1. Migration of *S. feltiae* infective juveniles (IJs) on Petri dish with agar containing oil derivatives in the presence of test insect (*T. molitor* larvae). C – control, UP – unleaded petrol, DO – diesel oil, UEO – used engine oil, 2000–8000 – concentration in $mm^3 \cdot dm^{-3}$

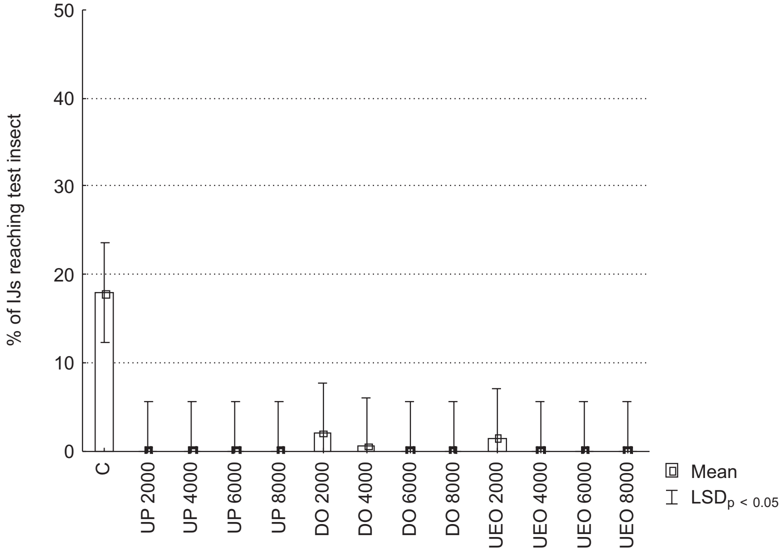

Fig. 2. Ability of *S. feltiae* infective juveniles (IJs) to find test insect (*T. molitor* larvae) on Petri dish with agar containing oil derivatives. The symbols as in Fig. 1

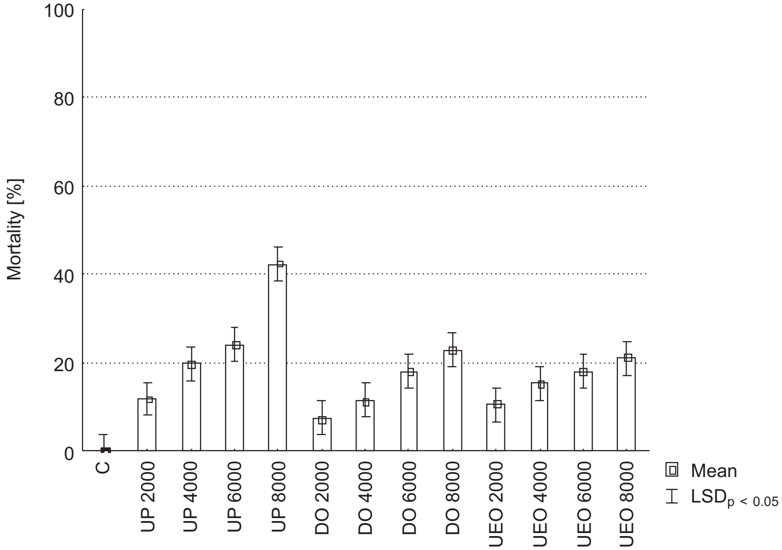

Fig. 3. Mortality of *S. feltiae* infective juveniles (IJs) on Petri dish with agar containing oil derivatives in the presence of test insect (*T. molitor* larvae). The symbols as in Fig. 1

they were applied (Fig. 4). On agar medium contaminated with oil derivatives most of IJs were dead of inactive. The most toxic effect was observed when petrol was added to the medium. Oil derivatives added to the agar medium significantly affected nematodes

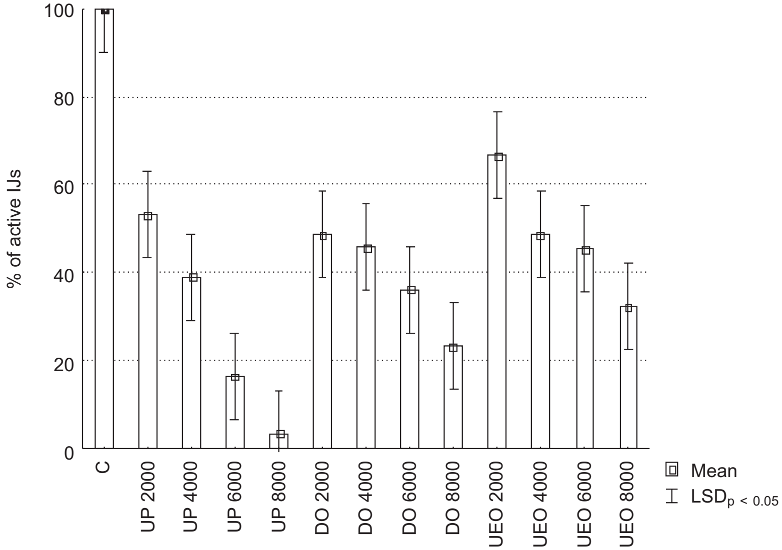

Fig. 4. The effect of oil derivatives on the activity *S. feltiae* infective juveniles (IJs) in the presence of test insect (*T. molitor* larvae). The symbols as in Fig. 1

ability to locate and reach potential host. This method may be useful to assess the toxicity of some substances on entomopathogenic nematodes.

The mortality of test insects in soil contaminated with oil derivatives are presented in Fig. 4. The highest mortality was observed in control (unpolluted soil). The comparison of test larvae mortality revealed that the highest one was observed in soil contaminated with unleaded petrol. The adverse effect of oil derivatives was most visible at higher doses of pollutants. Diesel oil at the concentration of 2000 and 4000 mg · kg^{-1} d.m. of soil did not significantly affected the pathogenicity of *S. feltiae*. In soil contaminated with high doses of unleaded petrol (6000 and 8000 mg · kg^{-1} d.m. of soil) mortality of test insects caused by nematodes was very low: 2.5–5 %.

Infective juveniles of entomopathogenic nematodes are able to find and infest insects in soil environment [15]. Pollutants such as heavy metals may affect nematodes activity and their ability to find host [16]. In soil contaminated with heavy metals nematodes needed more time to locate and invade potential host. Similar result were obtained in present study (Fig. 5). Nematode infective juveniles were placed on the bottom of the containers filled with contaminated soil. In order to reach their victim the larvae had to cover a distance of about 5 cm through contaminated soil. Average time needed to locate and kill test insect was significantly longer in soil contaminated with oil derivatives than in unpolluted control (Fig. 6). Oil derivatives differ in their physical and physiological properties. Petrol is mobile and readily volatile liquid and usually deeply penetrates into soil. Diesel oil is much less volatile than petrol and used engine oil characterize with very low volatility. Diesel oil and used engine oil envelops organisms with thin films, which may be reason of nematodes inability to find test insects.

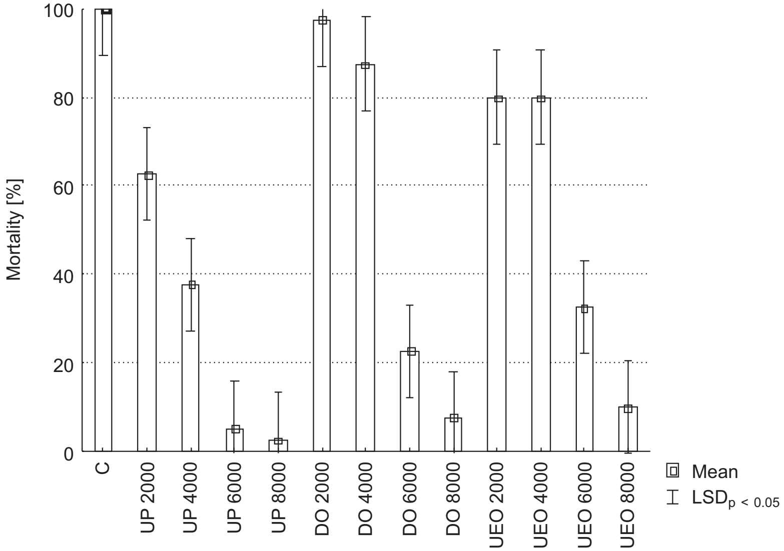

Fig. 5. Effect of oil derivatives on pathogenicity of *S. feltiae* infective juveniles (IJs) against *T. molitor* larvae in contaminated soil. C – control, UP – unleaded petrol, DO – diesel oil, UEO – used engine oil, 2000–8000 – concentration in mg · kg^{-1}

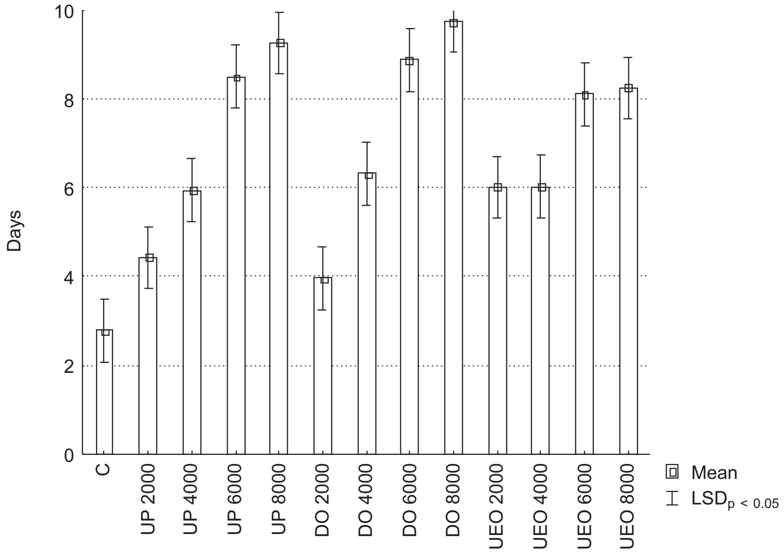

Fig. 6. Ability of *S. feltiae* infective juveniles to locate and kill potential host (*T. molitor* larvae) in soil contaminated with oil derivatives, expressed as time needed to locate, reach and infect test insect. The symbols as in Fig. 5

Oil derivatives impact on the nematode activity has been reflected by the extensiveness of the test insect infestation (Fig. 7). Even low doses of unleaded petrol, diesel oil

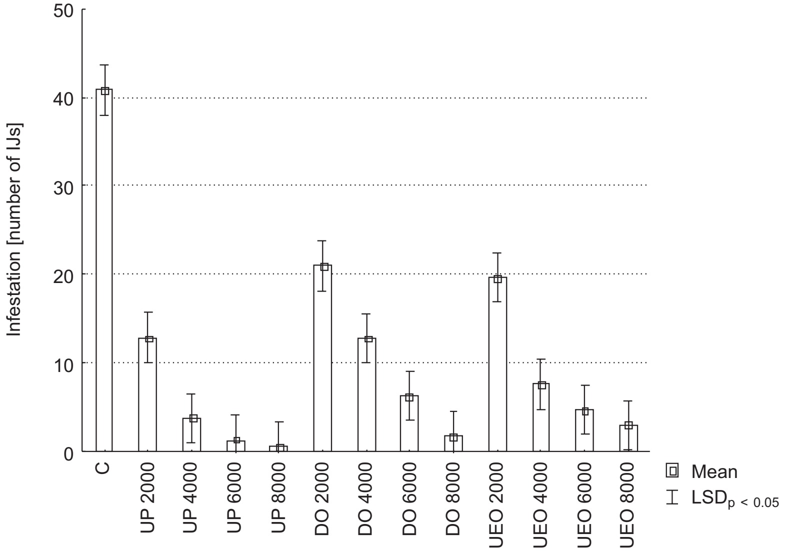

Fig. 7. Intensity of test insect (*T. molitor* larvae) infestation with *S. feltiae* infective juveniles in soil contaminated with oil derivatives measured with the number of individuals isolated from dead insects. The symbols as in Fig. 5

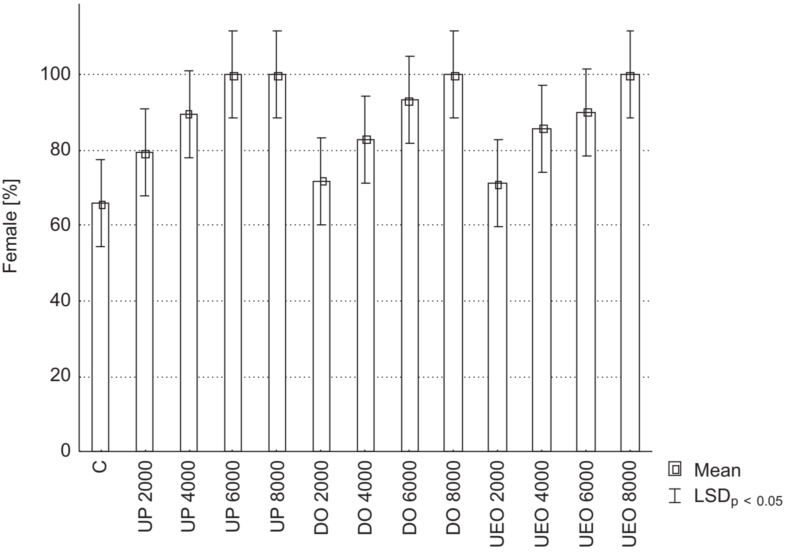

Fig. 8. Female/male ratio of *S. feltiae* isolated from dead insects in soil contaminated with oil derivatives. The symbols as in Fig. 5

and used engine oil caused significant reduction of IJs, which were able to invade test insects. Not only number of IJs was reduced but also the ratio female/male was affected by applied contaminants (Fig. 8). In contaminated soil number of male nematodes isolated from insects was very low. In soil contaminated with high doses of unleaded petrol only female nematodes were able to infest test insects. It suggest that IJs which develop inside insects into males are more sensitive to oil derivatives than the larvae developing into females.

Entomopathogenic nematodes are important group of soil mesofauna, which may be used as indicators of soil pollution *ie* with heavy metals [13]. Present studies shows that these group of invertebrates are sensitive to pollution with oil derivatives and nematode *S. feltiae* can be useful as indicator of soil contamination with such substances.

Conclusions

1. Oil derivatives impaired nematode ability to migrate towards test insect on agar medium, affecting also their activity and mortality. Petrol revealed the most toxic effect on the tested nematode *S. feltiae* among all three applied pollutants

2. In soil contaminated with oil derivatives the ability of IJs to infect test insects was impared. *S. feltiae* revealed the greatest sensitivity to soil contamination with petrol at the dose of 8,000 mg · kg^{-1} soil d.m.

3. The applied oil derivatives had an adverse effect on the female/male ratio of nematodes infesting host insect.

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WPŁYW ROPOPOCHODNYCH NA ZDOLNOŚĆ NICIENIA *Steinernema feltiae* DO ODNAJDYWANIA ŻYWICIELA

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Abstrakt: Celem badań było poznanie wpływu wybranych substancji ropopochodnych na owadobójczego nicienia *Steinernema feltiae*. Badano wpływ benzyny bezołowiowej, oleju napędowego i zużytego oleju silnikowego na zdolność larw inwazyjnych *S. feltiae* do odnajdywania potencjalnego żywiciela. Doświadczenia zostały przeprowadzone w warunkach laboratoryjnych. W pierwszym doświadczeniu badano wpływ ropopochodnych na zachowanie się larw inwazyjnych nicienia na zanieczyszczonym podłożu agarowym w obecności owadów testowych. Substancje ropopochodne zastosowano w następujących stężeniach: 2000, 4000, 6000 i 8000 $mm^3 \cdot dm^{-3}$. Na podłożu agarowym silnie zanieczyszczonym substancjami ropopochodnymi larwy inwazyjne nicienia nie kierowały się w stronę owada testowego, pozostając w miejscu aplikacji. Zastosowanie wysokich stężeń ropopochodnych powodowało również wysoką śmiertelność larw inwazyjnych. W drugim doświadczeniu larwy inwazyjne wprowadzono do skażonego podłoża glebowego. Zastosowano następujące stężenia substancji ropopochodnych: 2000, 4000, 6000 i 8000 $mg \cdot kg^{-1}$ s.m. W glebie skażonej ropopochodnymi śmiertelność owadów testowych była istotnie mniejsza niż w niezanieczyszczonej kontroli. Stwierdzono także, że owady testowe były w mniejszym stopniu infekowane przez nicienie w glebie skażonej. Reakcja nicienia *S. feltiae* zależała od rodzaju substancji ropopochodnej. Zdolność nicieni do odnajdywania żywiciela była najsilniej ograniczana przez skażenie podłoża benzyną bezołowiową. Ropopochodne wpłynęły również na strukturę płciową nicieni, które zdołały wniknąć na owadów testowych.

Słowa kluczowe: *Steinernema feltiae*, ropopochodne

Małgorzata WIDŁAK[1]

EVALUATION OF EXCHANGEABLE ALUMINIUM CONTENT AND SELECTED SOIL PARAMETERS OF SWIETOKRZYSKIE PROVINCE

OCENA ZAWARTOŚCI GLINU WYMIENNEGO I WYBRANYCH PARAMETRÓW GLEB WOJEWÓDZTWA ŚWIĘTOKRZYSKIEGO

Abstract: The work involves the determination of exchangeable aluminum content and parameters such as reaction, soil pH, organic carbon and iodine. Soils on the test sites showed a pH ranging from very acidic to alkaline (pH 3.87–7.65). The content of exchangeable aluminum in the soil was: 0.09–6.3 mg/100 g and 65 % of Al^{3+} mg/100g exceeded the allowable amounts of this element therefore becoming a toxic threat to most plants. Organic matter in the range 1.8–4.3 %, corresponds to 3.1–7.5 % humus content in samples. The soil has a high content of humus, which is crucial for its fertility. For research purposes intensively cultivated areas were selected. In six districts of Swietokrzyskie province which different in terms of geographical location, twenty research points were placed. Soil samples were collected during early spring, before fertilization and sowing.

Keywords: mineral soil, pH, soil acidity, exchaneable aluminum, organic matter.

Introduction

Soil is a non-renewable part of the natural environment, it consists of minerals, organic matter, water, air and living organisms. Organic matter in the environment are all present in the soil, carbon-containing compounds of organic origin. [1, 2].The type and dynamics of the processes occuring in the soil are affected by a number of factors determined as soil-forming: the parent rock, climate, water, living organisms and antropogenic factors. Depending on which of these soil-forming factors was dominated a particular type of soil developed [3–5].

Nearly 80 % of the Polish surface is covered by complex brown soils, podzolic and fawn while podzoilc soils make up about 25 % of all lands [6].

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Podzolic soils have very acidic reaction and low humus content. In acidic soils are easily washed out basic components and their location in the soil solution occupy cations of: hydrogen, aluminum, iron, manganese, which can be a toxic threat to the plant.

Aluminum in the soil solution is in the form of soluble and insoluble, and depending on the pH of the soil takes the form of a cation, anion or neutral molecule. Cationic forms of aluminum called aluminum movable or exchangeable are responsible for the formation of the basic properties of the soil environment. The presence of aluminum in the soil also determines its high sorption capacity [7–11].

Soils of Swietokrzyskie province

Physical and geographical diversity of the region has a direct impact on the quality of the soil, due to the diversity of the geological structure and the rocks from which they created different types and kinds.

Generally, in the north-western, western and northern ends of the province acidic soils predominate. In the eastern and southern parts of the region in arrears loess chernozem and brown soil, which were classified as the most fertile soils of

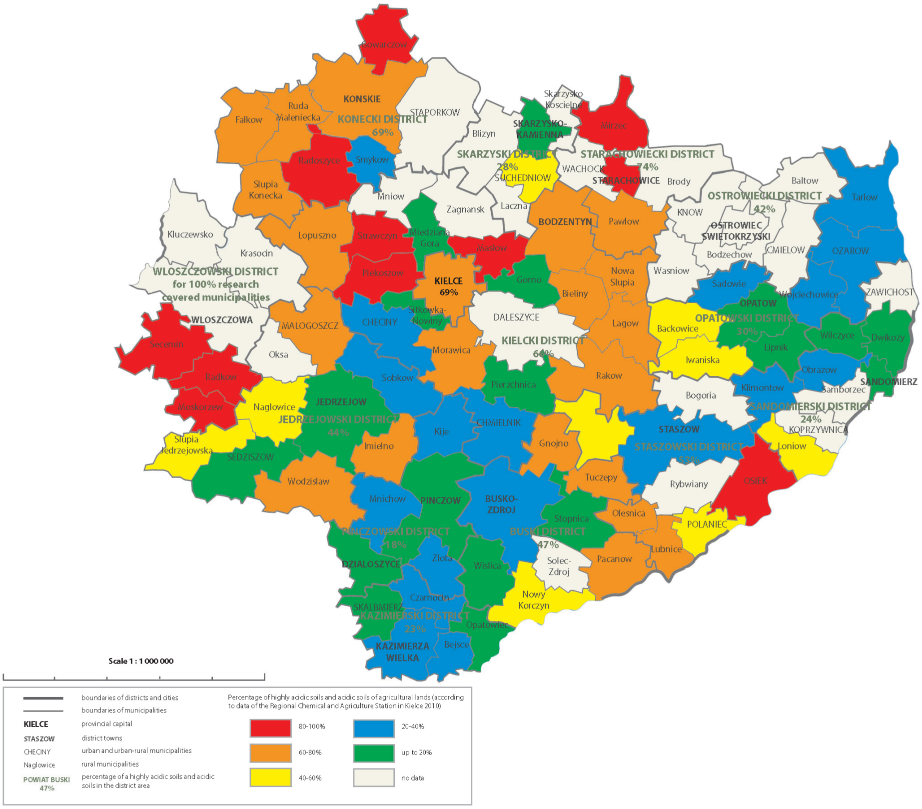

Fig. 1. Soils pH in arable and humus layer in Swietokrzyskie province OSChR Kielce 2010 [6]

Swietokrzyskie province (I and II bonitation class). On the south-west and south of the region on the formations of carbonate and sulfate developed rendzinas and pararendzinas. Within the higher hills and mountains slopes are shallow Embryonic soil. In the central and northern parts of the region are anthropogenic soils (in and around the city of Kielce, Skarzysko, Starachowice, Ostrowiec Swietokrzyski) [6, 12]. The pH of the soils in a arable and humus layer in Swietokrzyskie province shows Fig. 1.

The aim of this study was to evaluate the content of exchangeable aluminum and selected soils parameters of Swietokrzyskie province.

Materials and methods

For study were selected soils areas of intensive agricultural use from six districs of Swietokrzyskie province. Districts are located in the central part of the province, north-west, west-south and east-central. Samples were taken from 20 points representing 10 communities of four province regions, all samples were mineral soils. Location and designation of collected research material is presented in Table 2.

From every research point was collected representative mineral soil sample which includes soil profile of 0–25 cm, dry air samples were analyzed [13]. Following chemical properties of the soil were determined: pH in 1 N KCl-potentiometry, exchangeable acidity and exchangeable aluminum by Sokolow method, organic carbon by Tiurin method, an iodine value according to PN-83/C97555.04 standard [14].

Research material taken from selected areas of Swietokrzyskie province was characterized by varying the percentage share of highly acidic soils and acid soils for agricultural land according to data from the Regional Agricultural Chemical Station in Kielce, 2010. Fig. 1.

Results and discussion

Reaction and pH

In acidic soils of pH_{KCl} < 4.5 a source of hydrogen ions in the soil solutionare aluminum ions. In these soils absorbed aluminum is in equilibrium with Al ions in soil solution, which acidify the soil by the ability to hydrolyze.

Table 1

Characteristics of soil material used in the testing

pH ranges	Soil pH	Percentage of soil within pH range [%]	The lowest value of pH_{KCl} in the pH range	Al^{3+} mg $\cdot 100\ g^{-1}$ content for the lowest pH
< 4.5	very acidic	15	3.87	6.30
4.6–5.5	acidic	35	4.74	0.72
5.6–6.5	slightly acidic	15	5.87	0.90
6.6–7.2	neutral	10	6.76	0.18
> 7.2	alkaline	25	7.34	0.27

In acidic soils of pH_{KCl} ~4.5–6.5 a source of hydrogen ions in the soil solution is primarily hydrogen, present in the soil solution and adsorbed by the absorbent complex [15, 16].

In neutral soils acidic ions (H^+ and $Al^{3+)}$ are not dominant, aluminum hydroxides ions are converted to gibbsyt:

$$Al(OH)_2^+ + OH^- \longrightarrow Al(OH)_3$$

Very acidic, acidic and slightly acidic soils in the pH range 3.87 to 6.50 (Table 2) account for 65 % of soil samples from selected municipalities of Swietokrzyskie province. Acidic soils accounts for 35 % of research areas.

Table 2

Soils pH in arable and humus layer in selected municipalities of Swietokrzyskie province – March 2012

Region number	Location of community in Swietokrzyskieprovince	Designation of research sample	Soil reaction (pH_{KCl})
I	central region	1A	3.87
		1B	4.13
II	north-west region	2A	5.47
		2B	5.33
III	south-west region	3A	7.34
		3C	5.43
		4A	4.74
		4B	7.55
		5A	5.27
		5B	5.87
IV	east-central region	6A	7.46
		6B	4.03
		7A	5.93
		7B	5.18
		8A	7.01
		8B	4.97
		9A	7.57
		9B	6.14
		10A	7.65
		10B	6.76

The first (central region) and the second (north-western region) area of research are soils of pH very acidic and acidic. In the third area (south-western region) and the fourth area (central-eastern region) occur soils of acidic reaction, neutral and alkaline with alkaline soils dominance.

Organic carbon and iodine value

Organic matter is one of the key factors determining soil fertility. It fulfill a variety of direct and indirect functions in the formation of the physical, physico-chemical and biochemical properties of soil. Within the soil organic matter there are two main groups of substances: humus (humus) and non-humic.

Humus has a high water capacity, is characterized by a very high sorption capacity, humic compounds increase the buffer capacity of the soil, regulate the pH of the soil solution and the concentration of nutrients. Humus content in the soil is varied. Soils, due to their content of humus can be divided into poor humus soils (0.1–1 %), low humus soils (1.01–2 %), medium humus soils (2.01–4 %) and humus above 4 %.The humus content in the levels of accumulation of Polish mineral soils, depending on the type of soil and is usually in the range of 0.9 to 6.8 % [4].

Only organic matter has the ability to adsorb exchangeable cations. Richer in humus soils are more resistant to pollution than low humus containing soils [17, 18].

Table 3

Determination of organic matter in arable and humus layer in selected municipalities of Swietokrzyskie province – March 2012

Region number	Location of community in Swietokrzyskie province	Designation of research sample	Organic carbon content [%]	Humus content [%]	Iodine number [mg/g]
I	central region	1A	2.9	5.0	25
		1B	1.8	3.1	13
II	north-west region	2A	3.1	5.4	13
		2B	3.7	6.3	51
III	south-west region	3A	2.9	4.9	38
		3C	2.0	3.5	38
		4A	2.0	3.4	121
		4B	4.3	7.5	32
		5A	2.5	4.4	95
		5B	3.4	5.9	25
IV	east-central region	6A	3.8	6.5	25
		6B	3.4	6.3	51
		7A	2.5	4.4	51
		7B	2.2	3.8	82
		8A	3.6	6.1	44
		8B	2.5	4.4	19
		9A	1.9	3.4	44
		9B	2.4	4.2	76
		10A	2.3	3.9	69
		10B	3.7	6.4	95

The results include the organic carbon content (C_{org}) in the range from 1.8 to 4.3 %, this corresponds to the content of humus in the range 3.1–7.5 %. Tested soils in 55 % showed humus content on the level of medium-humus soils (Table 3).

Sorption ability of the soil is characterized by the iodine value in the range from 25 to 95 mg/g.

Aluminum content

Exchangeable aluminum occupies a large part of the sorption capacity of more than 50 %, changing the share of other exchangeable cations in the sorption complex. In general, it is concluded that the Al content of 0.05 to 0.4 mg per 100 g of the soil affects toxicaly the growth of most plants in tested soils. The largest amount of exchangeable H^+ and Al^{3+} occurs in humus levels. In Polish soils exchangeable ions content of H^+ and Al^{3+} is 0.04–13.4 m.eq H^+ and 0.0 to 10.3 m.eq Al per 100 g of soil [10, 19–21].

Table 4

Exchancheable acidity and exchancheable aluminum in arable and humus layer in selected municipalities of Swietokrzyskie province – March 2012

Region number	Location of community in Świętokrzyskie province	Designation of research sample	K_w [m.eq/100 g]	Al [m.eq/100 g]	Al [mg/100 g]
I	central region	1A	0.85	0.70	6.3
		1B	0.60	0.40	3.6
II	north-west region	2A	0.16	0.08	0.72
		2B	0.20	0.04	0.36
III	south-west region	3A	0.11	0.03	0.27
		3C	0.30	0.15	1.35
		4A	0.16	0.08	0.72
		4B	0.50	0.45	4.05
		5A	0.30	0.10	0.90
		5B	0.12	0.10	0.90
IV	east-central region	6A	0.10	0.01	0.09
		6B	0.45	0.25	2.25
		7A	0.20	0.10	0.90
		7B	0.25	0.15	1.35
		8A	0.11	0.01	0.09
		8B	0.30	0.20	1.80
		9A	0.25	0.15	1.35
		9B	0.20	0.05	0.45
		10A	0.10	0.02	0.18
		10B	0.15	0.02	0.18

Organic carbon and removable Al and pH of tested soil

Analyzing the results of the percentage of organic carbon and removable Al [mg/100g] for the pH (Table 2, 3, 4) of tested soil the following dependence is observed: when the organic carbon content increases the occurrence of removable aluminum is decreasing in acidic, slightly acidic and neutral soil (Fig. 2).

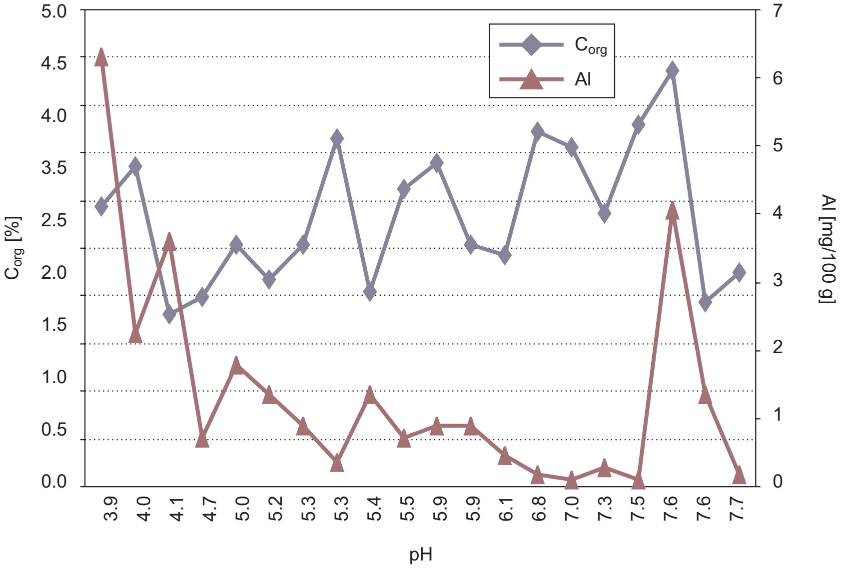

Fig. 2. Correlation between percentage of organic carbon, removable aluminum and pH of tested soil

Summary

1. Soils of Swietokrzyskie province shows in 35 % acidic reaction.

2. Removable aluminum found in tested samples exceeded in 65 % the content which has toxic influance on plant growth.

3. Organic carbon content indicates medium humus soils.

4. Increasing organic carbon content reduces the occurrence of removable aluminum in acidic and neutral soils.

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OCENA ZAWARTOŚCI GLINU WYMIENNEGO I WYBRANYCH PARAMETRÓW GLEB WOJEWÓDZTWA ŚWIĘTOKRZYSKIEGO

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Politechnika Świętokrzyska w Kielceach

Abstrakt: Praca obejmuje badanie nad zawartością glinu wymiennego i parametrów, takich jak: odczyn i pH gleby, węgiel organiczny i liczba jodowa. Gleby na badanych stanowiskach wykazywały odczyn od bardzo kwaśnego do zasadowego (pH 3,87–7,65). Zawartość glinu wymiennego w glebie wynosiła odpowiednio: 0,09–6,3 mg/100g, w 65 % przekroczyła dopuszczalną zawartość tego pierwiastka, stanowiąc toksyczne zagrożenie dla większości roślin. Zawartość materii organicznej w przedziale 1,8–4,3 %, odpowiada zawartości próchnicy 3,1–7,5 % w badanych próbkach. Gleba charakteryzuje się wysoką zawartością próchnicy, która jest decydującym czynnikiem jej żyzności. Do celów badawczych wytypowano obszary intensywnie użytkowane rolniczo. W sześciu powiatach województwa świętokrzyskiego, zróżnicowanych położeniem geograficznym, zlokalizowano 20 punktów badawczych. Próbki gleby pobierano wczesną wiosną, przed nawożeniem i zasiewami.

Słowa kluczowe: gleba mineralna, pH, kwasowość gleby, glin wymienny, materia organiczna

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Sławomir WIERZBA[1]

BIOSORPTION OF COPPER(II) BY LIVE AND DEAD CELLS OF *Yarrowia lipolytica*

BIOSORPCJA MIEDZI(II) PRZEZ ŻYWE I MARTWE KOMÓRKI *Yarrowia lipolytica*

Abstract: The biosorption characteristic of Cu(II) using live and dead cells of *Yarrowia lipolytica* as biosorbents have been investigated in the present research. Biosorption of Cu(II) was enhanced with an increase in pH, temperature, agitation, contact time and initial concentration of the metal ion. It was observed that dead and live biomass efficiently removed copper at 30 min at an initial pH of 5.0. Temperature of 35 °C was optimum at agitation speed of 150 or 200 rpm. For initial copper concentrations of 1–200 mg/dm^3, the adsorption data provide an excellent fit to the Langmuir isotherm. The maximum metal uptake values ($q_{\max}$, mg/g) were found as 9.82 and 12.03 for live and dead biomass, respectively.

Keywords: biosorption, copper, *Yarrowia lipolytica*, live and dead cells

Introduction

Wastewater contaminated with heavy metals is a serious environmental problem because they do not undergo biodegradation and are accumulated into the organism entering into the food chains. During recent years, the intensive industrial activities, such as electroplating, microelectronics, battery manufacture, dyestuff, chemical, metallurgical, pharmaceutical and other, greatly contribute to the increase of heavy metals in the environment [1].

The stringent limits of different pollutant concentrations in industrial and municipal wastewaters, imposed by the environmental legislation, make the treatment to be imperative. Conventional physiochemical methods for metals remediation include precipitation, filtration, coagulation, evaporation, ion exchange, membrane separation and solvent extraction. However, application of such processes is always expensive and ineffective in terms of energy and chemical products consumption, especially at low metal concentrations of 1–100 mg/dm^3 [2]. Ion exchange, membrane technologies and activated carbon adsorption process are extremely expensive when treating large

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amount of water and wastewater containing heavy metal in low concentration, they cannot be used at large scale. Therefore, there is a great need for an alternative technique, which is both economical and efficient. Biosorption, based on live or dead biosorbents, has been regarded as a cost-effective biotechnology for the treatment of complex wastewater containing heavy metals at high volume and low concentration [3]. In the concept of biosorption, several physical or chemical processes may be involved such as physical and/or chemical adsorption, ion exchange, coordination, complexation, chelation and microprecipitation. Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids offer many functional groups which can bind metal ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. In addition to these functional binding groups, polysaccharides often have ion exchange properties [4, 5]. In general, yeasts belonging to the genera *Saccharomyces*, *Candida* and *Pichia* are considered to be valuable adsorbents for metal ions [6]. However, in the recent years, there are reports on different strains of the non-conventional yeast *Yarrowia lipolytica* tolerating, resisting and accumulating metal ions [7]. This microorganism utilizes a variety of renewable carbon sources and its use in the waste water treatment processes generates a large quantity of biomass [8, 9].

The biosorption characteristic of Cu(II) using live and dead cells of *Y. lipolytica* as biosorbents have been investigated in the present research.

Material and methods

In this work, the strain of *Y. lipolytica* Polish Collection of Microorganisms was used. Stationary-phase cells were typically inoculated on YEPD medium (yeast extract, 3.0; peptone, 10.0; dextrose, 10.0 g/dm^3 of distilled water) for 48 h on a rotary incubator shaker. Cells growing in this medium were harvested by centrifugation (6.000 rpm. 10 min) at the end of the exponential phase, while dead cells were fist subjected to autoclaving at 121 ^{o}C for 20 min. After rinsing with distilled water three times, live and dead cells of *Y. lipolytica* were prepared as biosorbents for Cu(II) biosorption.

First of all, effects pH, temperature, agitation speed, contact time and biosorbent dose on copper biosorption and removal by live and dead cells were examined to find optimum conditions. All the samples were incubated for 24 h in 50 mg/dm^3 of Cu(II). Unless otherwise stated, standard conditions for biosorption experiments included an initial pH 5.0, being agitated at 150 rpm and having a dosage of 1.0 g/dm^3. Effects of 3.0–7.0 pH values, temperature from 15–35 ^{o}C, agitation speeds from 50 to 200 rpm, contact time (0–60 min) and different biomass densities (0.5–2.5 g/dm^3) were tested in parallel.

The concentrations of copper solutions were determined in aqueous solutions spectrophotometrically by measuring the absorbance of complex formed between rubeanic acidand Cu(II) at 390 nm. Both the values of biosorption capacity and removal ratio of Cu(II) were evaluated as follows:

$$q_e = \frac{(C_0 - C_e)}{X}$$

where q_e is the equilibrium Cu(II) concentration on the biosorbent (mg/g dry cell); C_0 and C_e is the initial and residual metal concentration [mg/dm^3]; X is the biomass concentration [g dry cell/dm^3].

The Langmuir isotherm model is valid for monolayer sorption onto surface and finite number of identical sites and given by:

$$q_e = \frac{q_{\max} K_L C_e}{1 + K_L C_e}$$

or presented in linear from as follows:

$$\frac{1}{q_e} = \frac{1}{q_{\max}} + \frac{1}{K_L q_{\max} C_e}$$

where $q_{\max}$ is the maximum amount of the metal ion per unit weight of cell to form a complete monolayer on the surface bound at high C_e [mg/dm^3] and K_L the constant related to the affinity of the binding sites, $q_{\max}$ represent a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in case where the sorbent did not reach its full saturation in experiments.

The general Freundlich equation is written as follows:

$$q_e = K_F C_e^{1/n}$$

where K_F and n are Freundlich constants characteristic of the system. K_F and n are indicators of adsorption capacity and intensity, respectively. The values of K_F and n were evaluated from the intercept and the slope, respectively, of the linear plot of ln q_e versus ln C_e based on experimental data. The Freundlich isotherm is also more widely used but provides on information on the monolayer adsorption capacity, in contrast to the Langmuir model [10].

All experiments were run in triplicates and mean values were considered for data analysis. Error bars indicate standard deviation. Mean values were used for fitting the data to different equations. All statistical analysis was done by using the SigmaPlot software.

Results and discussion

The resulted response surface plots shown in Figs. 1–2 indicate the influence of the experimental factors on biosorption process. It was found that the optimum pH for Cu(II) removal by live and dead cells was 5.0. The maximum biosorption of Cu(II) by living cels was 12.56 mg/g while that was 13.41 mg/g for dead cells.

As reported by Li et al [10] and Vasquez et al [11], pH has a significant effect on the solubility, speciation and biosorption capacity of heavy metals. The dependence of metal uptake on pH is related to both the surface functional groups on the biomass and the metal chemistry in solution. At lower pH in this research, the surface charge of the biosorbent is positive, which is not favourable to copper ion biosorption. Meanwhile, hydrogen ions compete strongly with metal ions for the active sites, resulting in less biosorption. With increasing pH from 3 to 5, electrostatic repulsions between copper ions and surface sites and the competing effect of hydrogen ions decrease: consequently, the metal biosorption increases [12]. Other authors [13] have found the same trend for copper sorption by other sorbent materials. Beyond pH 5, insoluble copper hydroxide starts precipitating resulting in lower amount of copper biosorbed at equilibrium.

Figure 1 shows the biosorption of Cu(II) ions at different temperatures for the yeast cells. An increase in temperature showed a positive effect on metal biosorption process. Maximal biosorption occurred at 30–35 ^{o}C. This result suggested that the biosorption process was endothermic in nature. Enhanced biosorption was possible due to the increased number of freely available active sites on the surface of the adsorbent at higher temperatures. Increased metal biosorption efficiencies at elevated temperatures have also been reported Shinde et al [9] and Bankar et al [14].

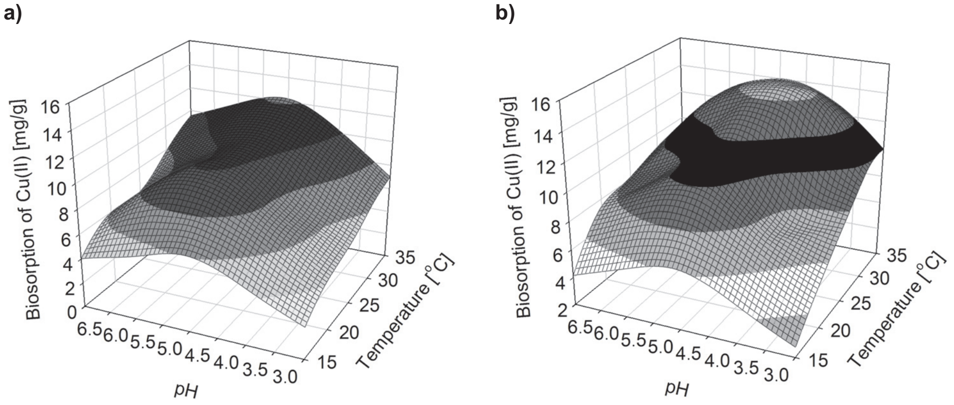

Fig. 1. Effect of pH and temperature on biosorption capacity of Cu(II) by live (a) and dead (b) cells *Yarrowia lipolytica*

As can be seen in Fig. 2, the effect of agitation speed on Cu(II) biosorption by live and dead cells had similar trends, where maximum biosorption capability was found at 150–200 rpm. An increase in agitation speed is known to enhance interactions of metal ions with the binding sites on the yeast biomass [9, 10, 14].

Earlier studies of Li et al [10] and Yao et al [16] have indicated that biosorbent dose was also an important parameter affecting biosorption capacity as well as removal efficiency. Fig. 2 shows the effect of biomass concentration on Cu(II) biosorption. These experiments were carried out for biomass concentrations ranging from 0.5 to 2.5 g/dm^3 at pH 5.0. The maximum uptake of Cu(II) was obtained at a biomass concentration of 1.0 g/dm^3. This observation is possibly due to the enhanced number of

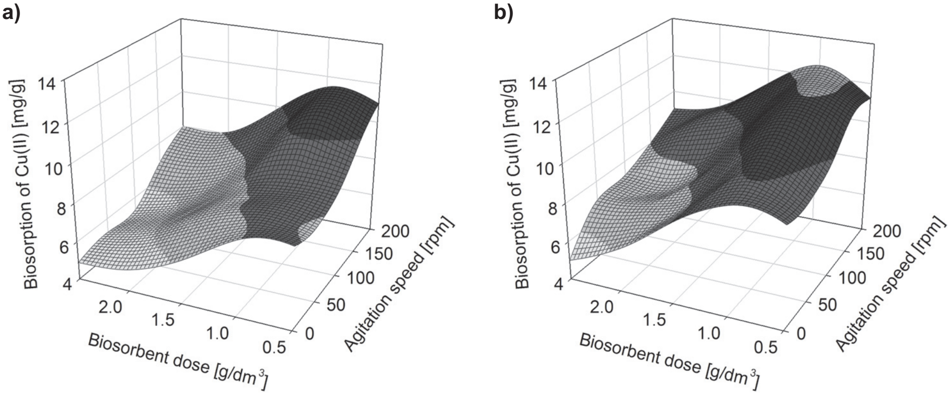

Fig. 2. Effect of biosorbent dose and agitation speed on biosorption capacity of Cu(II) by live (a) and dead (b) cells *Yarrowia lipolytica*

binding sites that were available for complexation of Cu(II) ions and due to increased electrostatic interactions with large quantities of biomass [10, 15, 16].

The effect of contact time on biosorption of Cu(II) ions by live and dead cells was studied under favorable conditions. From Fig. 3, it is evident that the uptake of Cu(II) was rapid during the time frame of 25–30 min. The biosorption process was slower at later stages (30 min – 2 h). There was no significant increment in the biosorption after 2 h and equilibrium was thus attained at this time point.

The biosorption process of heavy metal by yeast cells usually completes rapidly. The biosorption of metals such as copper, zinc, lead and uranium by non-growing cells is

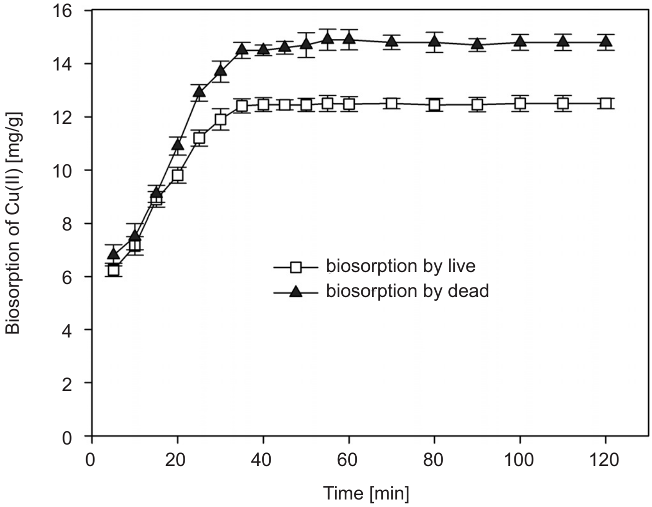

Fig. 3. Effect of contact time on biosorption capacity of Cu(II) by live (□) and dead (▲) cells *Yarrowia lipolytic*

a rapid process and often reaches equilibrium within several hours [2, 9, 10, 17]. Li et al [10] indicates that the first phase of biosorption is always rapid, and it is considered to be a spontaneous process with no energy consumed. This rapid initial uptake of metal ion may be an important parameter for a practical application of biosorption in industrial wastewater [17].

The adsorption isotherms are used to establish the ratio between equilibrium concentration of solute in the solution and equilibrium concentration of solute on the sorbent at constant temperature [10]. Langmuir and Freundlich models are widely applied in the equilibrium analysis to understand the sorption mechanisms. The Langmuir model considers sorption by monolayer type and supposes that all the active sites on the sorbent surface have the same affinity by the sorbate [18]. This equation is used empirically to simulate favorable equilibrium uptake curves. The Freundlich isotherm is an empirical equation which assumes a heterogeneous biosorption system with different active sites.

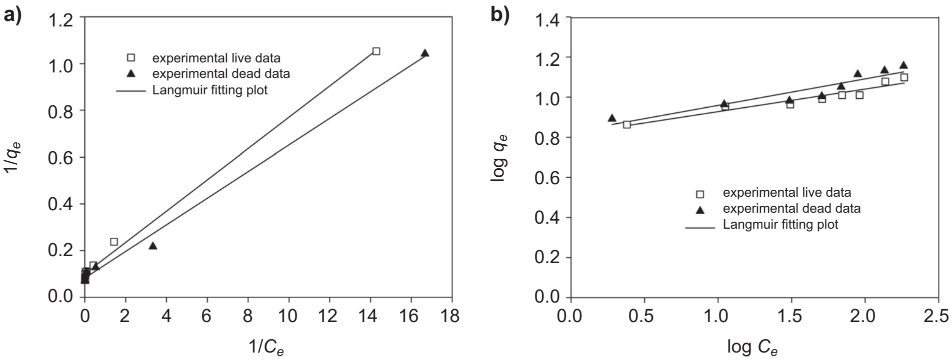

Fig. 4. Langmuir (a) and Freundlich (b) fitting plots of biosorption of Cu(II) by live (□) and dead (▲) cells *Yarrowia lipolytica*

The linearized Langmuir and Freundlich adsorption isotherms on Cu(II) biosorption for live and dead *Y. lipolytica* were show in Fig. 4. The adsorption constants, metal binding constant and correlation coefficients for the metals obtained from Langmuir, Freundlich isotherms analysis are given in Table 1.

Table 1

Constans simulated with Langmuir and Freudlich models for Cu(II) biosorption using live and dead cells *Yarrowia lipolytica*

Strain	Langmuir model			Freudlich model		
	K_L [l/mg]	q_m [mg/g]	R^2	n	K_F [l/g]	R^2
	$q_e = q_{\max}K_LC_e/(1 + K_LC_e)$			$q_e = K_FC_e^{1/n}$		
Live	0.6034	9.8231	0.9967	1.0511	1.7142	0.9139
Dead	0.5935	12.0336	0.9941	1.1511	1.8254	0.8762

Former research of Shinde et al [9] and Benad'ssa et al [12] have showed that the biosorption process is defined by two Langmuir constants $q_{\max}$ and K_L together. Generally speaking, high $q_{\max}$ and high K_L are desirable for good biomass. However, sometimes at low metal concentration, a biosorbent with low $q_{\max}$ and high K_L could outperform that with high $q_{\max}$ and a low K_L. So K_L is also an important parameter which is related to the initial sorption isotherm slope [10]. In the present work, the affinity constant K_L for dead biomass was 0.5935 and 0.6034 for live biomass, respectively. Maximum metal uptake capacities of *Y. lypolitica* ($q_{\max}$) were found as 9.82 mg/g and 12.03 mg/g for lives and dead biomass, respectively. Thus, with little higher affinity towards Cu(II) than lives ones, dead cells of *Y. lypolitica* seemed to be better adsorbents. Machado et al [19] found the maximum Cu(II) uptake values for live and dead *Saccharomyces cerevisiae* cells as 7.81 and 9.98 mg/g, respectively. Velkova et al [20] found the maximum copper uptake capacity of *Aspergillus awamori* as 35.97 mg/g.

In the case of Freundlich model, the adsorption feature is defined by both K_F and n values, where K_F represents the adsorption coefficient and $1/n$ is related to the effect of concentration of metal ions. On average, a favorable adsorption tends to have the constant n between 1 and 10. Larger value of n implies stronger interaction between biosorbent and heavy metal [10]. The K_F value of dead biosorbents was 1.8254, indicating a large biosorption capacity comparing to live ones, for which the K_F value was 1.742. The n value for live biomass was 1.0511 while that for dead one was 1.1511, from which it could be derived that the effect of metal ions on dead biomass was stronger than that on living material. Besides, with the n values between 1 and 10, the biosorption by both types of biomass were favorable under studied conditions. Hence, the results underlines that the adsorption of Cu(II) onto the dead cells of biomass is favorable under all conditions considered in this work.

The correlation coefficients obtained for live biomass *Y. lypolitica* from the Langmuir model and Freundlich equation were 0.9976 and 0.9139, respectively. For dead biomass *Y. lypolitica*, the correlation coefficients for the Langmuir model and Freundlich equation were 0.9941 and 0.8762, respectively. Langmuir equation was more in correlation with the experimental data. According to coefficients of correlation, the model of Freundlich is not adequate for modelling the isotherm of copper biosorption by live and dead cells of *Y. lypolityca* in all the studied concentration domain.

Conclusion

The biosorption of Cu(II) by live and dead cells of *Yarrowia lypolitica* has been investigated at optimum conditions determined in advance The favorable conditions for biosorption included a pH of 5.0, temperature of 35 ^{o}C, agitation at 150–200 rpm, biomass dose of 1.0 g/dm^3, and contact time of 30 min. The adsorption of Cu(II) onto the biomass of *Y. lyppolitica* is an endothermic process and become more favorable with the increasing of temperaturę. The Langmuir adsorption model and Freundlich equation were used for mathematical description of the biosorption of Cu(II) ions onto live and dead yeast biomass and it was found that the adsorption equilibrium data fitted well to the Langmuir model. According to Langmuir isotherm, the maximum adsorption capacity of investigated dead biomass was found to be 12.03 mg/g. Taking into consideration of present findings, dead cells of *Y. lypolitica* proved to be more efficient and low-cost biosorbents than live ones, which can be utilized as an alternative for the treatment of wastewater.

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BIOSORPCJA MIEDZI(II) PRZEZ ŻYWE I MARTWE KOMÓRKI *Yarrowia lypolitica*

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Uniwersytet Opolski

Abstrakt: W pracy przedstawiono charakterystykę biosorpcji Cu(II) przy użyciu żywych i martwych komórek *Yarrowia lipolytica* jako biosorbentu. Biosorpcja Cu(II) zwiększała się wraz ze wzrostem pH, temperatury, szybkości mieszania, czasu kontaktu i początkowego stężenia jonu metalu. W pracy zaobserwowano, że żywa i martwa biomasa skutecznie usuwa miedź w ciągu 30 minut, przy początkowym pH 5,0. Temperatura 35 ^{o}C była optymalna przy szybkości mieszania wynoszącej 150–200 rpm. Dla początkowego stężenia miedzi z zakresu 1–200 mg/g uzyskane dane biosorpcji były doskonale dopasowane do modelu Langmuira. Maksymalne wartości sorpcji (q_{max}, mg/g) dla żywej i martwej biomasy wynosiły odpowiednio 9,82 i 12.03.

Słowa kluczowe: biosorpcja, miedź, *Yarrowia lipolytica*, żywe komórki, martwe komórki

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Andrzej BUTAREWICZ[1] and Tadeusz ŁOBODA[1]

INFLUENCE OF SEWAGE SLUDGE ON THE CONTENT OF SELECTED METALS AND CHLOROPHYLL IN LAWN GRASS MIXTURES

WPŁYW OSADU ŚCIEKOWEGO NA ZAWARTOŚĆ WYBRANYCH METALI I CHLOROFILU W MIESZANKACH TRAW GAZONOWYCH

Abstract: The aim of this study was to analyze the influence of municipal sewage sludge from the Municipal Wastewater Treatment Plant in Sokolka on the content of magnesium and selected micronutrients such as zinc, manganese, copper and iron in the soil and lawn grasses and on the content of chlorophyll *a* and *b* in the analyzed plants. These elements are especially important in the process of photosynthesis. In addition, the paper discusses the relationship between the studied elements and the content of assimilation pigments.

The experimental plots were sown with two lawn grass mixtures: Eko and Roadside, and three doses of sewage sludge 0.0 (control), 7.5 $kg \cdot m^{-2}$ and 15.0 $kg \cdot m^{-2}$ were used. Chlorophyll *a* and *b* content was determined using spectrophotometer by measuring absorbance at $\lambda = 663$ and 645 nm. Metals concentrations in soils with sewage sludge and in plant material were determined using Atomic Absorption Spectrometry. It was found that the sludge dose had a significant effect on the manganese content in the soil taken from the experiment in Piastowska Street. In the experiment in Popieluszki Street the application of sewage sludge had a significant impact on the content of zinc and copper in the studied mixtures of grasses and in Raginisa Street – on the contents of zinc, copper and manganese.

The analysis of correlations for the results obtained from experiment in Hetmanska Street revealed strong positive correlations between the content of Zn and Mn in the soil and the content of chlorophyll *a* in plants ($r = 0.84$ and $r = 0.83$, respectively) and strong negative correlations between the content of Zn in the soil and the content of chlorophyll *b* in lawn grasses collected in June and August ($r = -0.81$ and $r = -0.85$, respectively). Strong correlations between the concentration of zinc and the content of chlorophyll *b* in grass samples collected in Hetmanska Street, Piastowska Street and Popieluszki Street ($r = 0.85$, $r = 0.86$ and $r = 0.98$, respectively) and the Cu content in plants and chlorophyll *b* for plants collected in Hetmanska Street ($r = 0.97$), Piastowska Street ($r = 0.86$), Popieluszki Street ($r = 0.90$) and Raginisa Street ($r = 0.82$).

The content of chlorophyll *b* was differentiated by dose of sludge and sampling time. The highest average chlorophyll *b* content in the grass samples collected in June and July was found (1.10 $mg \cdot g^{-1}$ of f.m.), and the lowest in samples collected in October (0.39 $mg \cdot g^{-1}$ of f.m.). The content of chlorophyll *a* in the analyzed

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samples of grasses decreased depending on the sampling time. As with the chlorophyll *b* content the lowest average content of chlorophyll *a* was in samples collected in October (0.45 mg · g^{-1} of f.m.). The average content of chlorophyll *a* in samples collected in June and July were similar independently of the fertilization of sewage sludge (0.64 mg · g^{-1} of f.m.).

Keywords: chlorophyll, grasses, magnesium, micronutrients, sewage sludge

A side effect of the rapid development of civilization is the emergence of ever-increasing amounts of sewage sludge and its management is a serious problem [1]. According to Central Statistical Office [2] 519.2 thousand Mg d.m. of municipal sewage sludge were produced in 2011 in Poland, and it is estimated that by 2018 over 700 thousand Mg d.m. of sewage sludge will be produced. Generated in wastewater treatment sludges require management not only for legal reasons, but also practical and aesthetic and, if possible, sludges after processing should return to the environment [3]. Many authors [4–6] report that sludge contains valuable nutrients, therefore, can be used as fertilizer. It contains also organic matter, which improves soil structure. It is confirmed by Szwedziak's studies [6] in which sludge placed on the upper layers of the soil were the source of many nutrients for plants, influenced soil formation processes and contributed to increased soil biological activity. However, as noted by Bien et al [3] for large sewage treatments plant the pathway for agricultural use is virtually closed. This is due to inadequate physico-chemical properties of sediments, mainly abnormal concentrations of heavy metals. In Poland the limits of heavy metals in terms of the natural use of sewage sludge are regulated by the Regulation of the Minister of the Environment of 13 July 2010 on municipal sewage sludge [7].

The lawns in parks and in passageways meet a number of key ecological safety and aesthetic functions [8] and may also influence the well-being of man. They are usually established on the poor soils having defective physical and chemical properties and poor trophic character, what contributes to the bad conditions for plants [9]. According to Kalembasa and Malinowska [10], one of the ways of use of sludge is to use it in perennial crops, which gradually releases the nutrients for providing plant growth at a satisfactory level. Therefore, sludge can be a good base of nutrients for growing grass in urban areas.

In order to effectively clean soil environment of metals, the plants should be of high resistance to harsh environmental conditions, have the capacity to accumulate xenobiotics from soil, as well as to grow fast and to produce a great quantity of biomass. Such great phytoremediative potential is characteristic for grasses [11], which collect metals mainly from soil solution through the root system [10], but they also absorb them from the atmosphere (*eg* in the area of traffic pathways) through stomata and leaf surfaces in gaseous form or as dissolved metal ions in rainwater [12]. These heavy metals can negatively affect physiological processes such as photosynthesis, respiration and transpiration. Metals such as Fe, Cu, Mn, Zn, etc. are essential for normal growth and development of the plant as they are constituents of many proteins, including enzymes. Both excess and deficiency of these elements have a negative impact on the development of plants. According to Prasad [13], the occurrence of Fe deficiency in soil contributes to the development of leaf chlorosis, i.e. the inability to produce chlorophyll and therefore reduces the photosynthetic rate and the consequences of the scarcity of assimilates is inhibition of shoot growth. Iron deficiency causes anatomical deformities of roots. The most important physiological function of manganese is its participation in the oxygen-evolving comlex in light reactions of photosynthesis [14]. Toxicity of trace metals also consists of substituting the appropriate metal in the structures of metalloproteins and other substances (*eg* Mg in chlorophyll, Ca in calmodulin), for connecting to the preferential oxygen, nitrogen and sulphur of many different molecules, their interaction with the functional groups, for example, phosphate and ATP or ADP with carboxyl groups [13].

Chlorophylls are essential molecules for light-harvesting and energy transduction in oxygenic photosynthesis, which comprises a porphyrin ring and hydrocarbon tail, in most cases phytol [15]. The major functions performed by chlorophylls in photosynthetic reactions are: absorbing light efficiently in the light-harvesting complexes; transferring the excitation energy with high quantum efficiency to the reaction centers; and performing the primary charge separation across the photosynthetic membranes and generating membrane potential that leads to ATP and strong reductants (NADPH) production [16].

The aim of this study was to analyse the influence of municipal sewage sludge from the Municipal Wastewater Treatment Plant in Sokolka on the content of magnesium and selected micronutrients such as zinc, manganese, copper and iron in the soil and lawn grasses and on the content of chlorophyll *a* and *b* in the analyzed plants**.**

Materials and methods

The study was conducted on four specially prepared test areas along main streets of Bialystok (Hetmanska Str., Piastowska Str., Popieluszki Str. and Raginisa Str.). Each test area was divided into 3 blocks (30 m^2 each) and each of them was divided into 6 plots of 5 m^2 area. In Fall of 2010 test areas were fertilized with stabilized municipal sewage sludge with three doses 0.0 (control), 7.5 and 15.0 $kg \cdot m^{-2}$ (sewage sludge contains 19.3 % of dry matter) from the Municipal Wastewater Treatment Plant in Sokolka. The doses of sewage sludge were established according to Kiryluk [17] who found in several years study that the most effective doses for turfing of municipal waste disposal areas were those above 40 $Mg \cdot ha^{-1}$.

Before the establishment of the experiment both sewage sludge and soil from each combination were analyzed according to the Regulation of the Ministry of the Environment of July 13th, 2010 concerning municipal sewage sludges [7]. The analyses were done by the Regional Chemical and Agricultural Station in Bialystok (Tables 1 and 2).

In the experiment two grass mixtures were used: Eko from Nieznanice Plant Breeding Station which included 30.0 % of *Lolium perenne* Niga cv., 15.0 % of *Poa pratensis* Amason cv., 22.6 % of *Festuca rubra* Adio cv. and 32.4 % of *Festuca rubra* Nimba cv., and Roadside from Barenbrug which included 32.0 % of *Lolium perenne* Barmedia cv., 5.0 % of *Poa pratensis* Baron cv., 52.0 % of *Festuca rubra rubra* Barustic cv., 5.0 % of *Festuca rubra commutata* Bardiva (BE) cv. and 6.0 % of *Festuca rubra commutata* Bardiva (NL) cv.

Table 1

Selected properties of municipal sewage sludge

Properties	Magnitude
pH	6.7
Dry weight [%]	19.3
Organic matter [$g \cdot kg^{-1}$ d.m.]	584.0
Total P [$g \cdot kg^{-1}$ d.m.]	27.0
Total N [$g \cdot kg^{-1}$ d.m.]	40.0
Ammonium N [$g \cdot kg^{-1}$ d.m.]	1.0
Mg [$g \cdot kg^{-1}$ d.m.]	7.0
Cu [$mg \cdot kg^{-1}$ d.m.]	194.0
Zn [$mg \cdot kg^{-1}$ d.m.]	1459.0

Table 2

Selected physical and chemical properties of soils at four studied locations

Properties	Hetmanska Str.	Piastowska Str.	Popieluszki Str.	Raginisa Str.
pH	7.9	7.7	7.6	7.4
Sand [%]	75.9	71.9	75.7	84.4
Silt [%]	22.0	25.4	22.3	14.7
Clay [%]	2.1	2.7	2.0	1.0
Textural class	loamy sand	sandy loam	loamy sand	sand
Cu [$mg \cdot kg^{-1}$ d.m.]	9.5	16.8	17.9	8.8
Zn [$mg \cdot kg^{-1}$ d.m.]	40.9	195.0	82.9	36.6

In October 2011 samples of soil (0–20 cm) were collected. Heavy metals concentrations in soils with sewage sludge and in plant material were determined using Atomic Absorption Spectrometry Varian Spectra AA-100. The samples of soil were mineralized in temperature at about 450 ^{o}C and remains were dissolved in *aqua regia* (HCl and HNO_3 mixture, 3:1 *v/v*) in 80 °C according to PN-ISO 11047: 2001 [18]. The samples of mixtures of grasses were mineralized in temperature at about 450 °C and remains were dissolved in concentrated HNO_3 [19].

For chlorophyll determination fresh plant material was homogenized in a mortar with addition of $CaCO_3$ and quartz sand. Chlorophyll was extracted with 80 % acetone. Chlorophyll *a* and *b* content was determined using HACH DR5000 spectrophotometer by measuring absorbance at $\lambda = 663$ and 645 nm.

$$\text{Chlorophyll } a = (12.7 \cdot D_{663} - 2.7 \cdot D_{645}) \cdot V \cdot (1000\ W)^{-1}$$
$$\text{Chlorophyll } b = (22.9 \cdot D_{645} - 4.7 \cdot D_{663}) \cdot V \cdot (1000\ W)^{-1}$$

where: D_{645} and D_{663} – optical density at $\lambda = 645$ and 663 nm, respectively,
V – volume of the solution [cm^3],
W – fresh weight of the leaves sample [g].

The results were statistically analyzed using analysis of variance with Tukey test at significance level at a = 0.05. The correlations between analysed parameters were calculated using Statistica 9.0.

Results and discussion

It seems reasonable the use of sludge in remediation urban soils as they require remediation treatment because of their progressive chemical degradation that leads to a sustainable and progressive deterioration of their properties [20]. The use of sludge has a double benefit; on the one hand, sewage sludge is utilized, on the other hand, one returns to use land transformed by human activity or not used at all [21]. According to Kalembasa and Malinowska [10], such organic substance introduced into the contaminated soil may increase the mobility of heavy metals as a result their complexation with the low molecular organic compounds. It may lead to the situation that one will not be able to determine the actual risk they pose to the environment or their availability to living organisms, the risk to human health, of water pollution and uptake by plants [22]. Plants absorb metals from the soil solution mainly through the root system [23], but they also absorb them from the atmosphere (*eg*. in the area of transportation pathways) through stomata and leaf surfaces in gaseous form or as metal compounds dissolved in rainwater [12]. In this study, the dose of sewage sludge differentiated the content of the analyzed elements in soils and plants, but in most cases the differences were not statistically significant. Only in the case of Piastowska Str. sludge dose had a significant impact on the content of manganese in the soil, while in the case of Raginisa Str. affected the content of zinc in plants. In both cases, with increasing doses of sewage sludge the contents of the studied elements in soil and grass mixtures increased. The study of Wisniewska and Kalembasa [24] suggests that increasing doses of sewage sludge application resulted in increased accumulation of zinc in Italian ryegrass. Authors obtained similar results for the copper content. According to Kalembasa and Malinowska [25], uptake and use of ions by plants from sewage sludge is dependent on many factors *ie*. holding water capacity, redox potential, soil temperature as well as, microbial activity in the rhizosphere. Each of these factors (either alone or in combination with the others) can stimulate or inhibit mineral nutrient uptake by the plant and can influence plant chemical composition.

The highest coefficient of variation of heavy metals in soil was found for zinc (64.5 %), its content in the soil ranged from 27.5 to 207.3 mg $\cdot$ kg^{-1} d.m., while the lowest coefficient of variation in the soil were calculated for magnesium and zinc (3.6 and 3.5 %, respectively), which content in the soil ranged from 14652.0 to 16395.0 for magnesium and from 9.3 to 23.5 mg $\cdot$ kg^{-1} d.m. for zinc. In plants the greatest variability was found for manganese (24.8 %), its content in the above ground parts of grass mixtures ranged from 32.3 to 73.7 mg $\cdot$ kg^{-1} d.m. The lowest variability was observed in the case of plant magnesium content (2.0 %), its content in the test plants ranged from 3419.4 to 3712.1 mg $\cdot$ kg^{-1} d.m. Heavy metals leaching from leaf litter penetrate back into the soil, and thus they re-enrich soil. They become more available and more mobile than the metals present in the soil solution, and they can be re-absorbed by the plant [26]. Toxicity of trace metals also consists of substituting the appropriate metal in the structures of proteins and other substances (*eg* chlorophyll Mg, Ca in calmodulinie), for connecting to the preferential oxygen, nitrogen and sulphur of many different molecules, their interaction with the functional groups, for example, phosphate and ATP or ADP with carboxyl groups [13].

Zinc, copper, manganese and iron are considered necessary for the proper growth and development of plants. Both deficiency and excess of these elements in plant can have a negative impact on the plant. Zinc activates many plant enzymes. For example zinc ions activate carbonic anhydrase, alcohol dehydrogenase and dehydrogenases depending on NADH and NADPH. Zinc also regulates the proportions of components, which affect the permeability of cell membranes. Zinc ions also determine the processes of formation of functional ribosomes. Deficiency of this element impairs the synthesis of tryptophan, which directly affects the production of auxin, and indirectly leads to reduced growth rate of the plant. Zinc ions have a high capacity to translocate to the above ground of plant [27].

In our study the concentration of zinc in soils enriched with sewage sludge was in the range of limits values according to the Regulation of the Ministry of the Environment of September 9th, 2002 [28] on standards for soil quality and earth quality for urban soils (Table 3). The zinc content in the tested mixtures of lawn grasses ranged from 45.5 in the control plants to 68.9 mg $\cdot$ kg^{-1} d.m. in plants grown on plots with the highest addition of sewage sludge at Raginisa Str. According to Wilk and Gworek [1], the average content concentration of zinc in crop plant is 10–100 mg $\cdot$ kg^{-1} d.m. To cover plant demand for zinc, its adequate content concentration is 15–30 mg Zn $\cdot$ kg^{-1} d.m. However, the zinc can be accumulated in the plants even in large amounts (more than 1 %) with no apparent toxicity symptoms. It is also worth noting that the most sensitive to the deficiency of this nutrient are herbaceous grasses.

Many authors [1, 27, 29] point out that the solubility of zinc compounds and related with this bioavailability to plants decreases with increasing pH. In the present study it is not confirmed. However, Bjelkova et al [30] suggest that zinc content in the grasses could be also affected by the location of the experimental plots. The source of zinc in plants and soils near the routes are also dust resulting from wear of tires and other parts of vehicles that get into the soil and plants along with rainfall and as a dry precipitation.

Another essential trace element for plants is copper, which activates a number of enzymes in plants and is a component including catechol and ascorbate oxidases and copper flavoproteids. This element is involved in major processes of photosynthesis and respiration, in the processes of synthesis of proteins, in the metabolism of nitrogen compounds, in the transport and metabolism of carbohydrates and in cell membranes metabolism (copper affects their permeability). Furthermore, copper regulates synthesis of DNA and RNA as well as, affects the sexual reproduction of plants. However, as microelement it is absorbed by plants in trace amounts. Accumulates mailny in roots, but under conditions of severe pollution of the environment its concentration increases in the above ground parts of the plant [27].

The concentration of copper in soils amended with sewage sludge was in the limits values according to the Regulation of the Ministry of the Environment 2002 [28]. The

Table 3

Mean metals concentration in the soil amended with sewage sludge and in grasses

Street	Degree of sewage sludge [kg · m^{-2}]	pH$_{KCl}$	Cu	Fe	Mg	Mn	Zn	Cu	Fe	Mg	Mn	Zn
			Soil [mg · kg^{-1} d.m.]					Plant [mg · kg^{-1} d.m.]				
Hetmanska	0	7.3	13.1	8746.7	15271.8	261.2	59.8	9.8	347.0	3673.1	49.9	49.8
	7.5	7.4	14.3	8582.3	15601.8	235.7	54.0	10.3	339.8	3632.7	45.0	61.0
	15	7.3	13.4	9418.5	15378.0	254.3	51.5	10.8	368.2	3712.1	50.0	63.7
Piastowska	0	7.3	18.7	12245.7	16395.0	304.9	123.8	9.6	364.3	3596.4	38.3	55.2
	7.5	7.2	23.3	13656.3	16329.0	323.3	188.0	13.5	413.5	3575.6	39.1	65.2
	15	7.0	23.5	14330.7	16251.0	353.4	207.3	10.7	326.5	3599.0	32.3	60.3
Popieluszki	0	7.4	18.7	11970.0	15964.8	400.0	87.8	9.6	312.5	3556.9	43.6	49.5
	7.5	7.2	18.2	10744.9	15660.0	395.6	80.7	11.1	330.3	3590.4	40.3	57.8
	15	7.2	19.1	11668.1	15691.8	379.9	89.5	10.6	426.9	3601.0	38.4	64.2
Raginisa	0	7.6	9.3	7328.4	14652.0	232.8	27.5	7.1	245.3	3419.4	37.7	45.5
	7.5	7.5	11.7	7657.4	15033.0	200.4	44.8	8.7	351.8	3561.5	58.2	58.6
	15	7.3	12.1	7287.8	14947.8	203.0	48.7	9.6	298.0	3639.3	73.7	68.1
Mean		7.3	16.3	10303.1	15598.0	295.4	88.6	10.1	343.7	3596.5	45.6	58.2
Standard deviation		0.2	4.6	2471.3	566.8	74.0	57.1	1.5	48.7	72.0	11.3	7.0
Relative standard deviation [%]		2.1	3.5	23.9	3.6	25.1	64.5	15.1	14.2	2.0	24.8	12.1

copper content in plants can be highly differentiated depending on the part of the plant and stage of development, on the variety and species. Its average physiological content in the above ground parts is from 5 to 30 mg · kg^{-1} [27]. The toxic content of copper in these plants is estimated to be 20–100 mg · kg^{-1}, but in plants from highly contaminated soils, the concentration of copper may be several thousand mg · kg^{-1} [30]. In our study the average content of this element in the above ground parts of lawn grasses was in the range 7.1–13.5 mg · kg^{-1} of d.m. (Table 3), and therefore it is within the range of content recognized as physiological.

Correlation analysis showed a negative correlation between soil pH and copper content in the plant at Popieluszki and Raginisa Streets ($r = -1.0$, $r = -0.8$, respectively), significant at $p < 0.05$. Generally, the copper solubility decreases with increasing pH of the soil, as reported by several authors [1, 31].

Manganese activates many enzymes, forming chelate bonds between the proteins and their substrates. It activates decarboxylases and dehydrogenases in the process of mitochondrial respiration. Manganese takes part in the light phase of photosynthesis (in oxygen-evolving complex) and enzymes involved in the reduction of nitrates. Level of manganese in plants is in the range 3.6–15.0 mg · kg^{-1} d.m. [31], while Ostrowska et al [32] reported that its content in the grass can be from 50.0 to 147.0 mg · kg^{-1} d.m. Our findings indicate rather low and moderate level of accumulation of this element in the mixtures of grasses (Table 3), despite fertilization with sewage sludge and location of plots along the transport routes.

Iron is involved in the process of photosynthesis and metabolism of nucleic acids, stimulates the synthesis of chlorophyll, is involved in nitrate reduction and fixation of free nitrogen and regulates oxidative-reductive reactions. The iron content in plants usually ranges from several mg Fe · kg^{-1} of d.m. to several hundred or even several thousand mg Fe · kg^{-1} of d.m. An excess of iron in the environment is toxic for plants by interaction with other compounds. Iron can be accumulated in large amounts by plants without apparent adverse effects [31]. Each metal has a specific pattern of uptake, transport and accumulation in the plant, whereas in the presence of other ions in the soil interactions between the ions can change this pattern. The same combinations of metals may interact synergistically with each other in one plant species, but antagonistically in other one [33].

Correlation analysis of the results from the Hetmanska Str. showed positive correlation between the content of manganese in the soil and the copper and iron in plants ($r = 0.6$, $r = 0.7$) and content of iron in the soil and zinc in plants ($r = 0.6$), while the negative between content of manganese in the soil and magnesium in plants ($r = -0.8$). In the case of Piastowska Str. there were obtained strong correlations between soil copper content and zinc content in plants ($r = 0.8$) and content of manganese in the soil and iron in plants ($r = -0.7$). For Popieluszki and Piastowska Streets there was correlation between content of copper in soil and zinc in plants ($r = 0.6$) with $\alpha = 0.05$. In the case of Raginisa Str. there was positive correlation between the contents of copper and zinc in the soil and of iron in plants ($r = 0.7$ and $r = 0.6$, respectively), and the content of copper in the soil and magnesium in plants ($r = 0.6$).

Macronutrients in the plant play a building role. Magnesium is an important component of chlorophyll. About half of the magnesium contained in the leaves occurs in chloroplasts. Magnesium is also a component of protopectin in the cell walls. Magnesium ions are activators of many enzymes involved in the synthesis of DNA and RNA, kinases transfering phosphate groups, enzymes involved in photosynthesis and respiration. This element is very mobile and is involved in the regulation of acidity (pH) in the cell.

Each of the analyzed elements had an impact on the content of chlorophyll *a* and *b* in the above ground parts of lawn grass mixtures. In most cases, the ratio of chlorophyll *a* to *b* was relatively low (usually less than 1). The reason for this may be the growth conditions of mixtures of grasses sown along the traffic routes exposed to pollution caused by heavy traffic, which can be an additional stress factor. This may explain the particularly low ratio of chlorophyll *a* to *b* in the case of plants collected from control plots in the first months of the study. According to Pinto et al [34], some heavy metals are essential to the metabolism of living organisms at very low content, they may become toxic at higher levels. Some heavy metals, such as Cu^{2+}, Cd^{2+}, Zn^{2+} and Ni^{2+}, are known to substitute the central Mg^{2+} atom in the chlorophyll molecule, a process that lowers the fluorescence quantum yield and results in a shift in the fluorescence spectrum. Moreover, metal excess can lead to binding of metals to proteins thus disturbing protein structures [35].

Our study shows that municipal soil amendment sewage sludge influenced chlorophyll *a* and *b* content and their ratio in lawn grass mixtures. The content of chlorophyll *a* in aboveground parts of grasses was dependent on the used grass mixture, harvest time, location and to a lesser extent on the dose of sewage sludge (Fig. 1).

Toxicity can also be related to oxidative stress induced in living systems either by increasing concentrations of reactive oxygen species or by reducing cellular antioxidant capacity [34]. In primary producers these effects may result in inhibition of chlorophyll production, photosynthesis and growth [36]. They may cause physiological and anatomical changes in plants. The same reaction took into account Prasad [13], discussing the negative impact of metals present in the cell for biochemical processes, such as photosynthesis, respiration, and transpiration. The presence of heavy metals influences the cell nucleus and cell division, causing a decrease in mitotic activity and abnormal cytokinesis, damage to DNA, RNA, decrease in transcriptional activity, chromatin condensation, chromosomal aberrations and destruction of nuclear envelope [37].

The content of chlorophyll *b* was differentiated by dose of sludge and sampling time. The highest average chlorophyll *b* content in the grass samples collected in June and July was found (1.10 $mg \cdot g^{-1}$ of f.m.), and the lowest in a samples collected in October (0.39 $mg \cdot g^{-1}$ of f.m.). The increase in content of chlorophyll *b* was proportional to the dose of sewage sludge. The content of chlorophyll *a* in the analyzed samples of grasses decreased depending on the sampling time (Fig. 1). As the chlorophyll *b* content, the lowest average content of chlorophyll *a* was in samples collected in October (0.45 $mg \cdot g^{-1}$ of f.m.). The average content of chlorophyll *a* in samples collected in June and

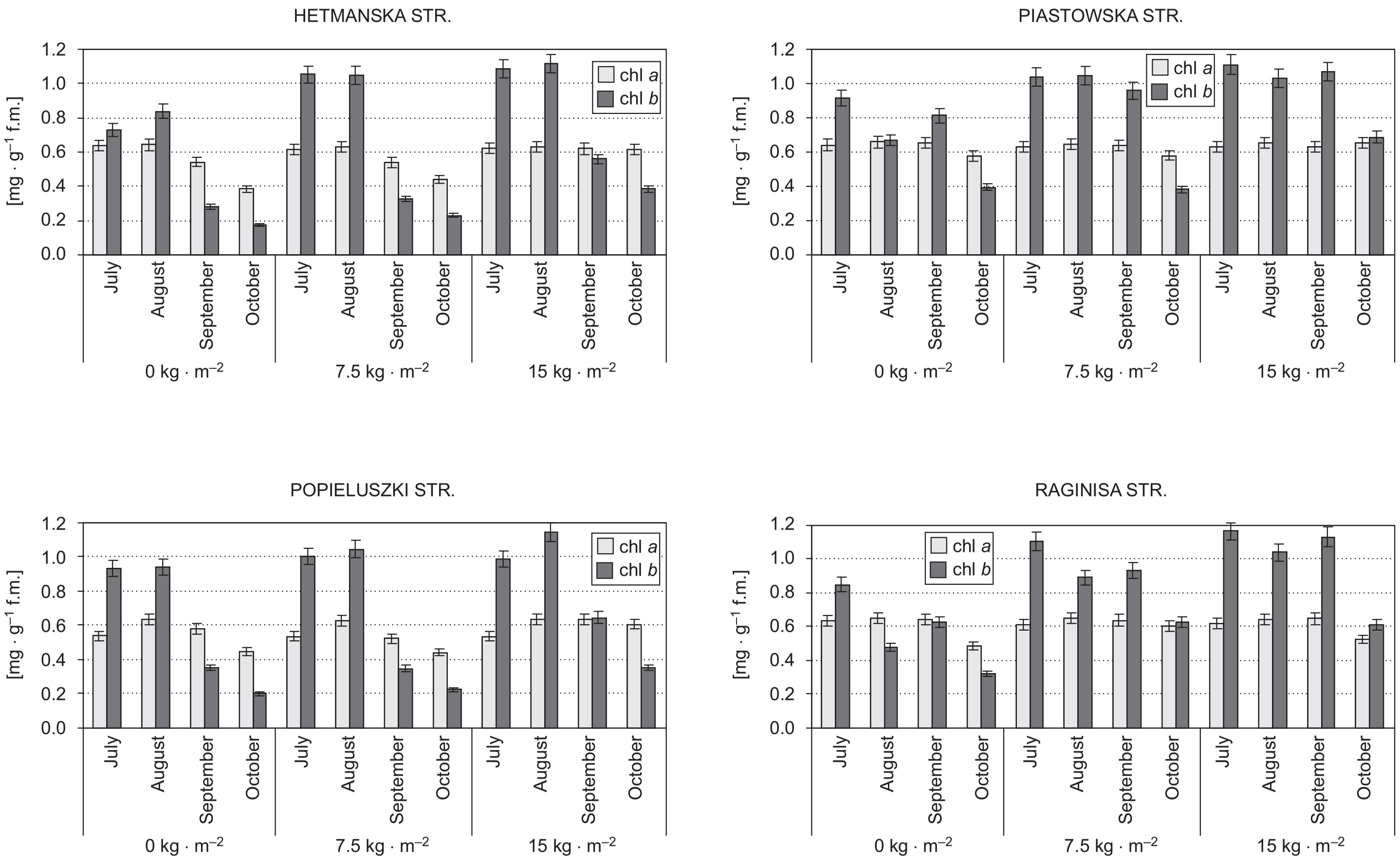

Fig. 1. Influence of doses of sewage sludge, time of sampling and localization on leaves' chlorophyll *a* and *b* content [mg · g^{-1} f.m.]

July were similar independently of addition of sewage sludge (0.64 mg · g^{-1} of f.m.) (Fig. 1).

The analysis of correlations for the results obtained from Hetmanska Street revealed strong positive correlations between the content of Zn and Mn in the soil and the content of chlorophyll *a* in plants (r = 0.84 and r = 0.8, respectively) and strong negative correlations between the content of Zn in the soil and the content of chlorophyll *b* in lawn grasses collected in June and August (r = –0.8 and r = –0.8, respectively). Strong correlations between the concentration of zinc and the content of chlorophyll *b* in grass samples collected in Hetmanska Street, Piastowska Street and Popieluszki Street (r = 0.8, r = 0.8 and r = 0.9, respectively) and the Cu content in plants and chlorophyll *b* for plants collected in Hetmanska Street (r = 0.9), Piastowska Street (r = 0.9), Popieluszki Street (r = 0.90) and Raginisa Street (r = 0.8). Moreover, it was found positive correlations between the content of Fe in the soil and the content of chlorophyll *a* and *b* in lawn grasses collected at Piastowska Street (r = 0.8 and r = 0.7, respectively). According to Hänsch and Mendel [37] iron, like copper is also of great importance for life plants. As redox-active metal it is involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis (ethylene, gibberellic acid, jasmonic acid), production and scavenging of reactive oxygen species, osmoprotection, and pathogen defense. Up to 80 % of the cellular iron is found in the chloroplasts that is consistent with its major function in photosynthesis.

Conclusions

1. Amendment with sewage sludge has contributed to an increase in the content of manganese in the soil at Piastowska Str. and zinc in plants at Raginisa Str. In other cases, the municipal sewage sludge did not affect the accumulation of the analyzed elements in soil and grass mixtures.
2. The content of the analyzed elements in plants was at a moderate level, and was far from toxic levels.
3. Alkaline pH of soils caused the limited availability of Cu, Mn and Fe by lawn grass mixtures.
4. Soil manganese in Piastowska and Hetmanska Streets caused lower uptake of iron and magnesium by studied plants.
5. The sewage sludge amendment significantly influenced chlorophyll *b* content in lawn grass mixtures in the first month of the growing season, which resulted in a low ratio of chlorophyll *a* to *b*. This may indicate that the applied dose of sludge caused some kind of stress in the studied plants.
6. The availability of metals for plants should be concidered taking into account not only dose of applied sewage sludge but also the correlations between different metals and environmental factors.

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WPŁYW OSADU ŚCIEKOWEGO NA ZAWARTOŚĆ WYBRANYCH METALI I CHLOROFILU W MIESZANKACH TRAW GAZONOWYCH

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Abstrakt: Celem badań było określenie wpływu komunalnego osadu ściekowego, pochodzącego z Miejskiej Oczyszczalni Ścieków w Sokółce, na zawartość magnezu i wybranych mikroskładników, tj. cynku, manganu, miedzi i żelaza w glebie i mieszankach traw gazonowych oraz na zawartość chlorofilu *a* i *b* w analizowanych roślinach. Wybrane składniki mineralne mają ważne znaczenie w procesie fotosyntezy. Ponadto w pracy określono zależności pomiędzy badanymi pierwiastkami a zawartością barwników asymilacyjnych.

Na poletkach wysiano dwie mieszanki traw gazonowych: Eko i Roadside oraz zastosowano trzy dawki osadu ściekowego: 0 (kontrola), 7.5 kg · m^{-2} i 15 kg · m^{-2}. Całkowitą zawartość metali w glebie i w roślinach określono metodą absorpcyjnej spektrometrii atomowej, natomiast zawartość chlorofilu *a* i *b* za pomocą spektrofotometru, mierząc absorbancję przy długościach fali λ = 663 i 645 nm.

Stwierdzono, że dawka osadu ściekowego miała istotny wpływ na zawartość manganu w glebie w doświadczeniu przy ul Piastowskiej. W doświadczeniu przy ul. Popiełuszki dawka osadu ściekowego wpłynęła istotnie na zawartość cynku i miedzi w badanych mieszankach traw, natomiast przy ul. Raginisa na zawartość cynku, miedzi i manganu.

Analiza korelacji wykazała silną współzależność pomiędzy stężeniem cynku a zawartością chlorofilu *b* w próbkach traw pobranych przy ul. Hetmańskiej, Piastowskiej i Popiełuszki, (r = 0,85, r = 0,86 i r = 0,98) oraz zawartością miedzi i chlorofilu *b* w roślinach pobranych przy ul Hetmańskiej (r = 0,97), Piastowskiej (r = 0,86), Popiełuszki (0,90) i Raginisa (r = 0,82).

Termin poboru próbek oraz zastosowany dodatek osadu ściekowego istotnie różnicowały zawartość chlorofilu *b*. Największą zawartość chlorofilu *b* stwierdzono w próbkach traw pobranych w czerwcu i w lipcu (1,10 mg · g^{-1} ś.m.), natomiast najmniejsze w próbkach z października (0,39 mg · g^{-1} ś.m.). Z kolei zawartość chlorofilu *a* w analizowanych próbkach traw zmniejszała się w zależności od terminu poboru. Podobnie jak w przypadku chlorofilu *b* najmniejszą zawartość chlorofilu *a* stwierdzono w próbach zebranych w październiku, średnio ok. 0,45 mg · g^{-1} ś.m, natomiast od lipca do czerwca zawartość chlorofilu *a* utrzymywała się na podobnym poziomie średnio ok. 0,64 mg · g^{-1} ś.m. bez względu na zastosowany dodatek osadu.

Słowa kluczowe: chlorofil, trawy, magnez, mikroelementy, osad ściekowy

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COMPOSITE BASED ON FOAM LIME MORTAR WITH FLAX FIBERS FOR USE IN THE BUILDING INDUSTRY

KOMPOZYT NA BAZIE SPIENIONEJ ZAPRAWY WAPIENNEJ Z WŁÓKNAMI LNIANYMI DO ZASTOSOWANIA W BUDOWNICTWIE

Abstract: Building industry as an important branch of the economy of each country consumes significant amounts of energy and emits greenhouse gases into the environment (CO_2). These negative effects which affect on the environment have a contribution to make many of the restrictions recorded in the documents of sustainable development in the construction industry. The most significant are: environmentally friendly building products, energy efficiency of buildings, organized management of construction wastes including demolition materials. However, in relation to a building and its surroundings can be distinguished: reduction of impurities emissions resulting from the combustion of the heating facility, reduction of the energy demand for heating, possibility of disposal of waste and effluents.

These considerations leads to the exploration and initiation of new construction materials and technologies, and in the final stage for the implementation of low-energy and passive buildings.

This paper presents the results of preliminary studies of new composite material based on foam lime mortar, packed with natural flax fibers and additives and admixtures. The paper presents the physico-mechanical characteristics of the composite (strength parameters, absorption, coefficient of thermal conductivity, etc.) of different composition mix output. It is expected that the composite will be applied in the implementation of low-energy and passive building as parts fulfilling the structural and insulation function. The proposed material solution from natural ingredients meets the requirements of sustainable development in the construction industry.

Studies are carried out under a cross-border grant from the Technical University in Brest and the material as the original solution is submitted to the Polish Patent Office.

Keywords: flax, organic fibers, lime binder, sustainable development

Introduction

Although ecological building is a relatively new phenomenon, it is growing rapidly in the construction sector. Regulations on the reduction of greenhouse gas emissions

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(CO_2) and waste production accelerate the development of sustainable construction. Public awareness of the conventional energy sources depletion and the negative effects of environmental impact contribute further to the progress in the field of construction. For this reason, natural, eco-friendly materials are still being sought since they are recyclable and can also be used in the construction industry as a construction material. In addition, and at the same time fulfilling the function of thermal insulation in accordance with the specifications of the heat transfer coefficient [1, 2].

In France and the United Kingdom, a study was carried out on a composite consisting of cut pieces of hemp stalks and lime binder [3]. In Slovakia, in turn, a composite containing hemp was tested, for thermal loads effects on the mechanical properties [6]. Apart from hemp, other fibers were also used in tests. In Brazil for instance, sisal fiber was added to concrete blocks [4]. More importantly, the largest use of natural composites has been observed in France where about 2,000 houses have been built from eco-friendly materials.

In Poland, however, especially in agricultural regions for instance, in Lublin surroundings, an alternative is flax-plant, once popular in cultivation, yet forgotten due to low profitability which mainly depends on the garment industry. Linen products (oil, straw) are widely used in construction industry, for example in the manufacture of flaxboards, linoleum, flax-seed oil [7].

The purpose of this article is to present a proposal for a composite material with a natural composition with flax applied to the low buildings, and to develop proposals of technological solutions. The material was tested for physico-mechanical and thermal properties, test results and analysis are presented in the further part of the article.

Own research and used materials

The laboratory tests included the execution of composites based on lime binder involving fiber and straw from flax. The material properties were modified by adding additives and admixtures. Flax was obtained from a farm in the province of Lublin. Processing flax (containing straw and fiber inside) consisted of cutting it into lengths of about 25 mm (Fig. 1). The diameter of flax straw was about 1–1.5 mm. A flax fiber itself was also used which was sliced into three fractions, the length of 10 mm, 15 mm and 20 mm (Fig. 2). During the preparation of flax straw, its high water absorption was found, which could affect the destruction of the thermal properties of a finished composite. It was noted that flax straw swelled quickly, and after an hour of soaking in water, its weight increased twice. In order to reduce the absorption of straw hydrophobization, natural impregnation (flax-seed oil) was applied.

Furthermore, hydrated lime and in some cases, lime with the addition of Portland cement CEM I 42,5R were used as a binder. Lime as a natural binder is an ecological product, hence affects positively a microclimate in the building, as well as the health of residents.

The aggregates used in small quantities include sand fraction of 0–1 mm and powdered limestone. In order to accelerate the binding of the lime binder, a pozzolanic admixture in the form of microsilica (Woerosil U-P) was applied to the mixture. In addition, such an admixture causes the tight fill of voids in the mix, and improves its

Fig. 1. Chopped straw with fiber inside used in the study

Fig. 2. Cut flax fiber used in the study

workability. Another admixtures include sodium bicarbonate ($NaHCO_3$) and citric acid ($C_6H_8O_7$). These compounds react together in the presence of water, which results in the emission of large amounts of carbon dioxide that accelerates the carbonation of lime. It is worth mentioning that even Ancient Romans added young fig wine to lime, which led to the fermentation and the consequent emission of considerable amounts of CO_2 that contributes to faster bonding of lime [5].

In order to improve the thermal properties of the final product, composites containing foam, which was obtained by the mechanical mixing foaming agent with water, were also made. Foam introduced into the mix causes the closure of air bubbles in the bulk and increases the volume of the finished product, which leads directly to the decrease in the density of the composite. Moreover, the structure of cutted capillary pores facilitates the distribution of ready-mix in shuttering.

Four composites, differing in composition, were executed (K1, K2, K3, K4) (Table 1). In the composites K1 and K2, flax straw was impregnated by using flax-seed oil, while in the others, flax components did not hydrophobize. In the case of K3 and K4, cement was used in the amount of 30 % and 25 % by volume of the lime. The quantity of pozzolanic admixture was 5 % by the weight of lime.

Table 1

The components included in the various composites

Components / Composites	Water	Lime	Cement	Sand	Lime powder	Micro-silica	Flax straw	Flax fiber	Foam
K1	X	X	—	X	—	—	X	—	—
K2	X	X	—	X	—	X	X	—	—
K3	X	X	X	X	—	—	X	—	X
K4	X	X	X	X	X	X	X	X	X

After mixing all components, samples were formed by light, hand-held tamping successive layers. After disassembling samples that maturated at air-dry conditions, tests on physical-mechanical and thermal properties of K1 and K2 composites were held after 90 days of maturation, and of K3 and K4 after 28 days.

The basic physical and mechanical properties of the material, such as bulk density, tightness, porosity, water absorption and compressive strength, were examined. All the tests were performed on cubic samples with the dimension of 10 × 10 × 10 cm. Thermal conductivity coefficient was measured on the basis of thermal parameters in Heat Flow Meter (HFM) instrument, on samples having the dimension of 25 × 25 × 5 cm. For each test, an outcome was defined as the arithmetic mean of the three samples. In order to evaluate the microstructure of the composite, a material was viewed by using a Scanning Electron Microscope (SEM).

Analysis of the results

Results of physical parameters are shown in Table 2. Bulk density values of composites are in the range from 0.44 to 1.29 kg/m^3, with the lowest values obtained by K3 and K4. This is caused by the presence of foam in the mixture, whose application minimizes the use of other ingredients. Bulk density differs by 20 % between K1 and K2 which is caused by the use of a larger amount of sand in the composite K1. The differences in bulk density between the composites indicate that there is a wide range of options to modify the material, depending on the application (construction, construction and insulation, insulation).

Table 2

Examples of the physical properties of tested composites

Composites Physical properties	K1	K2	K3	K4
Bulk density [kg/dm^3]	1.29	1.05	0.5	0.44
Tightness [%]	50.5	48.3	23.0	17.5
Porosity [%]	49.5	51.7	77.0	82.5
Mass absorption [%]	28.0	39.0	67.0	77.0

While analyzing the results of the porosity test, it was observed that the examined composites can be divided into two groups. Hence K3 and K4 composites obtained higher porosity of about 60 % in comparison with K1 and K2. The differences in these values are due to the foam used in K3 and K4 which forms a structure of closed pores filled with air. The high porosity of K3 and K4 is equivalent to the low tightness of these composites.

Composites, as a result of relatively high porosity in the case of conventional building materials (such as concrete, brick), and the presence of straw and flax fibers show absorption of 35 % for K1 and K2, and 70 % for K3 and K4. During a binding process, samples in which flax component was impreganted (K1 and K2) obtained the solid mass in air-dry conditions in three times longer period than K3 and K4. It was found that the hydrophobization of flax straw (K1 and K2) accelerates the process of lime binding in relation to samples in which impregnation was not applied (K3 and K4).

In subsequent studies, currently underway, a mixture is also secured by adding impregnating admixture and a mature material is secured by surface hydrophobization in order to reduce the absorption of the composite. This analysis shows that a hydrophobized flax component should be used by contractors, which translates into improving physical and mechanical properties (rapid growth of strength, reduced absorption, improved thermal insulation properties).

Strength tests showed that the compressive strength changed in the range from 0.45 MPa to 0.65 MPa (Fig. 3). The mechanism of the destruction of all the samples proceeded slowly and caused a large plastic deformation. Such a phenomenon is consequent upon the presence of straw, and flax fibers which together with a binder,contained in the matrix, increase the cohesion of the material. This feature affects the safety of the structure in case of emergency, which translates into the comfort of a used building, made of a tested material. Samples of the lime binder or cement-lime binder without fibers deteriorate dramatically due to large discontinuity in the structure.

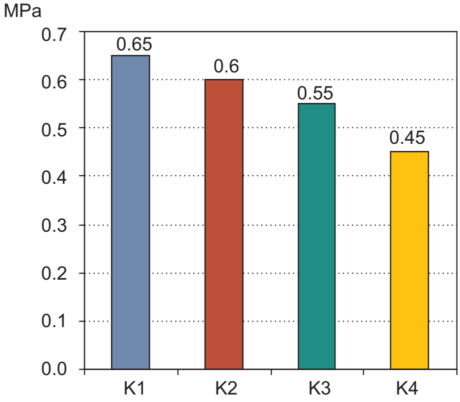

Fig. 3. The compressive strength of test samplesy

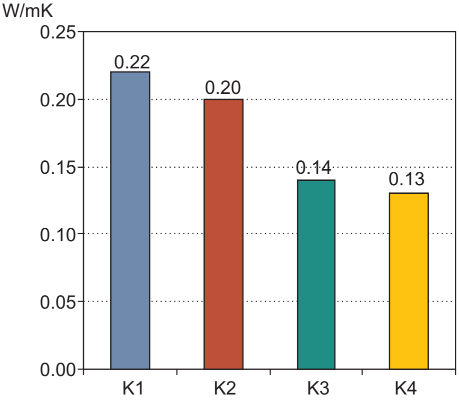

Fig. 4. The thermal conductivity of composites

In Fig. 5, the sample after reaching about 15 mm deflection is shown. After the research, the breakthrough of the sample was made, which showed the arrangement of the fibers in the matrix (Fig. 6).

The analysis of the results allowed to observe the relationship of the strength of the material and density. If composite K1 obtains the highest strength which is also characterized by the highest bulk density, the quantity of the sand is also the highest compared with other composites. More importantly, the lowest strength and density reached K4, in which foam was applied. A percentage difference in strength between K1 and K4 is about 30 %. Composite K4 has approximately three times lower bulk density than K1. It was concluded that by adjusting the composition of the mixture, strength properties of a composite can be increased, simultaneously trying to reduce the density of the material.

Measured values of λ coefficient of tested composites are shown in Fig. 4. Composite K3 and K4, despite lower content of fiber and straw in the volume of

Fig. 5. Compressive strength test

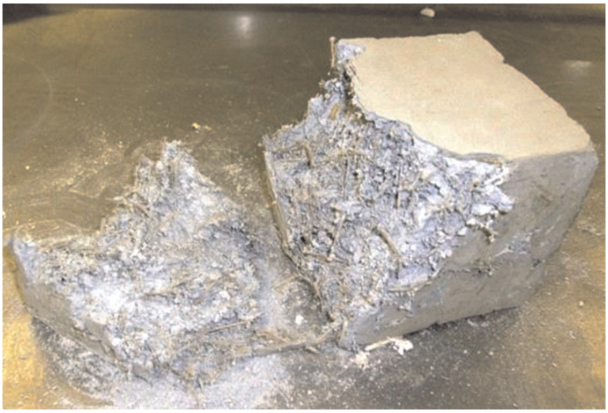

Fig. 6. Breakthrough of flax straw composite

material in comparison with K1 and K2, showed higher thermal resistance due to the large amount of air voids that resulted from the application of foam. The λ coefficient for K3 and K4 amounted respectively to 0.14 and 0.13 W/mK. The wall of this material would meet current thermal requirements for a traditional building (U-value ≤ 0.3 W/m^2K) with a thickness of 45 cm. Composites K1 and K2, althgouh having the highest bulk density, the thermal conductivity is relatively low (about 0.2 W/mK). It was noted that the increase of the amount of straw and flax fibers and the use of foam result in lowering of the thermal conductivity coefficient.

In Figs. 7 and 8 the fiber and the structure of mortar K3 composite were shown respectively with the use of a SEM. Moreover, relatively little energy was used, approximately of 100x-5000x due to the combustible nature of the individual natural fibers.

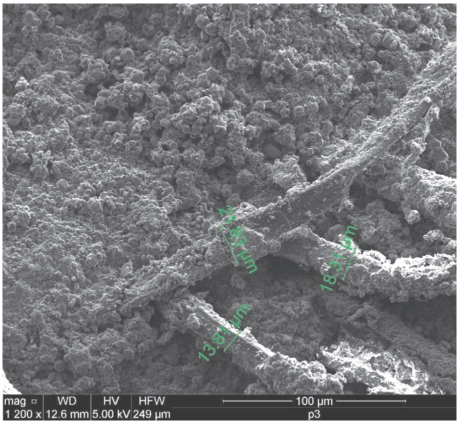

Fig. 7. Flax fiber

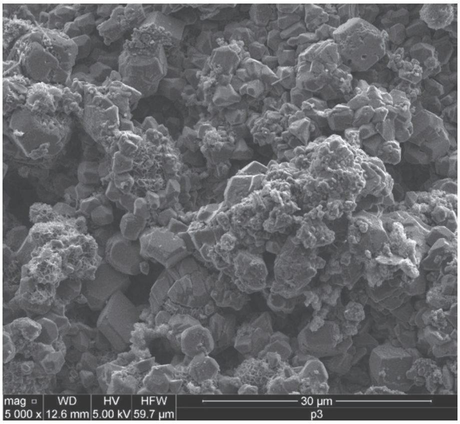

Fig. 8. The structure of mortar with foaming component

On the basis of the material structure analysis, it was observed that there occurred very good adhesion of flax fibers into the lime-cement matrix, which was consequent upon the rough surface of a fiber. In addition, in comparison with steel fibers used in conventional structural reinforced concrete, flax fibers exhibit high plasticity. This property is beneficial for the cohesion of material, which is due to better "matching" flax micro-reinforcement to the discontinuity of the composite structure, as opposed to steel fibers. Figure 8 shows the porous nature of the material and the calcium carbonate crystals in surroundings of partially hydrated cement grains. Calcite appears in the form of flat cubic tiles, which shows the final stage of carbonation of calcium hydroxide. Studies were performed by using scanning electron microscopy in the laboratory of the Faculty of Civil Engineering and Architecture of Lublin University of Technology.

Possible applications of composites

The target application of the composite is the construction of walls. The components in the form of blocks, fill timber frame (Fig. 9) and monolithic wall (Fig. 10) are taken into consideration. As described in the previous section, the lowest obtained value of thermal conductivity was 0.13 W/mK. Table 3 presents the achievable U-values for a wall in different configurations of insulation and composite thicknesses.

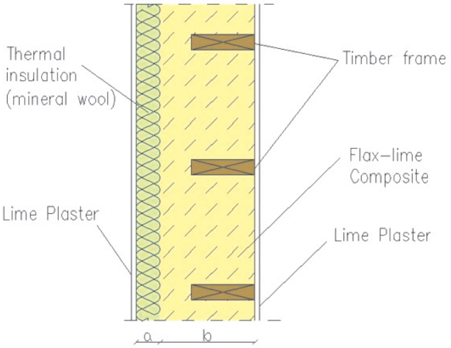

Fig. 9. Frame construction filled with the flax-lime composite and insulated with mineral wool

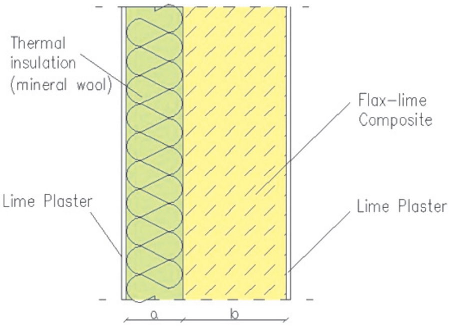

Fig. 10. TMonolithic wall made of the flax-lime composite and insulated with mineral wool

Results 1–4 in Table 3 are related to the thermal requirements of the walls of conventional buildings, where the U-value ≤ 0.3 W/m^{2}K. Options 5–8 are intended for a passive and low-energy construction, where the required heat transfer coefficient is 0.15 W/m^{2}K, while recommended amounts to U ≤ 0.10 W/m^{2}K. Due to the ecological character of the composite, mineral wool was deliberately used instead of foamed polystyrene as a thermal insulation. To meet the standard thermal requirements it is possible to eliminate completely the additional insulation by using a 45 cm thick wall made of flax-lime composite. In the case of a passive house, the additional insulation is necessary. Further studies aim at decreasing the obtained value of thermal conduction coefficient, in order to minimize the thickness of the mineral wool.

Table 3

Configurations of wall layers thickness and the corresponding value of "U" (alternative solutions)

Option	a) Mineral wool $\lambda = 0.042$ W/mK	b) Flax-lime composite $\lambda = 0.13$ W/mK	U-value [W/m^2K]
1	10 cm	20 cm	0.26
2	8 cm	30 cm	0.24
3	5 cm	40 cm	0.23
4	—	45 cm	0.29
5	20 cm	40 cm	0.13
6	20 cm	45 cm	0.12
7	25 cm	40 cm	0.11
8	30 cm	40 cm	0.10

Conclusion

The pilot study demonstrated the applicability of flax of composites. This natural ingredient can be the future of green building in Poland. The binder is mostly a natural product – which is lime. It is planned to reduce gradually the amount of cement to the zero level in the composite to increase the ecological nature of the product. This material is completely biodegradable and after demolition it can be used again for building. Also the foam-forming component is made of a blend of natural ingredients, harmless to humans and the environment; in contrast to the aluminium powder and the process of autoclaving in the case of aerated concrete.

The article shows that the reduction of CO_2, produced in the process of building, can be influenced by using natural materials, locally available, to implement various elements of the building, where the quality of use is not inferior to materials commonly used.

Studies continue to search for the most optimal formula which gives the best strength and thermal results, according to the principles of sustainable development in the construction industry. The pilot study is simultaneously carried out, with the possible uses of additives that significantly accelerate the binding process of lime binder, which results in a rapid increase of strength of the material.

The research is carried out on the basis of cross-border grant with Brest State Technical University.

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KOMPOZYT NA BAZIE SPIENIONEJ ZAPRAWY WAPIENNEJ Z WŁÓKNAMI LNIANYMI DO ZASTOSOWANIA W BUDOWNICTWIE

Wydział Budownictwa i Architektury
Politechnika Lubelska

Abstrakt: Budownictwo jako ważna gałąź gospodarki każdego kraju zużywa znaczące ilości energii i emituje do otoczenia gazy cieplarniane (CO_2). Te negatywne skutki wpływające na środowisko były przyczynkiem do wprowadzenia wielu ograniczeń zapisanych w dokumentach zrównoważonego rozwoju w budownictwie. Do najważniejszych można zaliczyć: wyroby budowlane przyjazne środowisku, efektywność energetyczna obiektów budowlanych, zorganizowane zarządzanie odpadami budowlanymi w tym rozbiórkowymi. Natomiast w odniesieniu do obiektu budowlanego i jego otoczenia można wyróżnić: obniżenie emisji zanieczyszczeń powstałych ze spalania przy ogrzewaniu obiektu, redukcję zapotrzebowania na energię do ogrzewania, możliwość utylizacji odpadów i ścieków.

Powyższe uwarunkowania prowadzą do poszukiwania i wprowadzenia nowych rozwiązań materiałowych i technologicznych, a w końcowym etapie do realizacji budynków niskoenergetycznych i pasywnych.

W artykule przedstawiono wyniki badań pilotażowych nowego materiału kompozytowego opracowanego na bazie spienionej zaprawy wapiennej, z wypełnieniem naturalnych włókien lnianych oraz z dodatkami i domieszkami. W opracowaniu przedstawiono cechy fizykomechaniczne kompozytu (wytrzymałość, nasiąkliwość, współczynnik przewodzenia ciepła itd.) o różnym składzie mieszanki wyjściowej. Przewiduje się, że kompozyt będzie miał zastosowanie przy realizacji budynków niskoenergetycznych i pasywnych jako elementy spełniające funkcję konstrukcyjną i izolacyjną. Zaproponowane rozwiązanie materiałowe z naturalnych składników spełnia wymogi zrównoważonego rozwoju w budownictwie.

Badania realizowane są w ramach grantu transgranicznego z Uniwersytetem Technicznym w Brześciu, a materiał jako oryginalne rozwiązanie zgłoszono do Urzędu Patentowego RP.

Słowa kluczowe: len, włókna organiczne, spoiwo wapienne, zrównoważony rozwój

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ORGANIC COMPOUNDS EMISSION FROM MODIFIED, BIOMASS FUELED SMALL-SCALE BOILER

EMISJA ZWIĄZKÓW ORGANICZNYCH ZE ZMODYFIKOWANEGO KOTŁA MAŁEJ MOCY ZASILANEGO BIOMASĄ

Abstract: The low emission problem, visible not only in cities but also in agriculture areas, strongly concerns quality of human life and it is connected with small-scall boilers with are heat-suppliers for individual households. The exhaust gases emitted during incorrect fuel combustion process consist mutagenic, genotoxic, irritant and carcinogenic organic substances like volatile organic compounds (VOCs) or polyaromatic hydrocarbons (PAHs). Those substances are dangerous for human health, part of them are very reactive becoming source of secondary pollution, part of them have ability to cumulate in environment. To avoid health problems connected with low emission and toxic organic compounds pollution it is necessary to develop the solutions for non-effective fuel combustion process prevention.

The paper presents the results of research work which aim was investigation of small-scall, biomass fueled boiler modifications in aspect of volatile organic compounds emission reduction. The special catalytic--activated ceramic and metal construction was applied inside the combustion space. VOCs and PAHs in exhausts was determined by Varian 450 gas chromatograph. The results, presented in tables and figures, show that applied modification based on ceramic and ceramic activated fittings change significantly emission characteristic reducing VOCs and PAHs concentration in the exhaust but the changes trends are discussible.

Keywords: small-scall boiler, low emission, volatile organic compounds, inner catalyst

Introduction

The solid, biomass fuel combustion is complicated process that needs to be well controlled to achieve maximal efficiency and low production of toxic gaseous

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pollutants. Small-scale biomass combustion is a specific field of emission regulations issues because of the fact that combustion process in so small reactor is almost impossible to control [1].

The term “small-scale” is often interpreted differently in the literature. Here it is represented by the boilers not exceeding power output of about 50 kW.

Compared to relatively stable coal or oil combustion process in large boilers, small-scale biomass boilers are responsible for higher emission of unburned hydrocarbons, especially volatile aromatic and polyaromatic compounds. Emission limits for those heat sources are also less restrict than for boilers characterized by higher nominal power output (Table 1) [1].

Table 1

Emission limits according to EN-303-5

Nominal power output [kW]	Fuel type: biologic. Fuel feeding: automatic		
	CO	TOC	Particles
	mg/m^3 at 10 % O_2, dry gas, standard conditions		
≤ 50	3000	100	150
50–150	2500	80	150
150–300	1500	80	150

The exhaust gases emitted during incorrect fuel combustion process from small-scall boilers consist mutagenic, genotoxic, irritant and carcinogenic organic substances like volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs). Those substances are dangerous for human health, part of them are very reactive becoming source of secondary pollution, part of them have ability to cumulate in environment [2]. To avoid health problems connected with low emission and unburned hydrocarbons pollution it is necessary to develop the solutions for non-effective fuel combustion process prevention [3, 4].

One of the most important features on field of small-small boilers market is also cost effectiveness. It means that any kind of control system improvement should be done with the lowest possible additional costs [1].

The authors of the article presents the results of research work which aim was investigation of small-scall, biomass fueled boiler low-cost modifications in aspect of volatile organic compounds and polyaromatic compounds emission.

Materials and methods

Research object

Small-scale, 10 kW nominal power output, biomass boiler was applied as an research object. The unit is composed of a circular steel heat exchanger, tubular type of high efficiency and burner, which is designed to combust pellets with a thickness of 6–8 mm, and also cereals (oats) as an additional option.

The geometry and mechanics of the boiler is shown on Fig. 1.

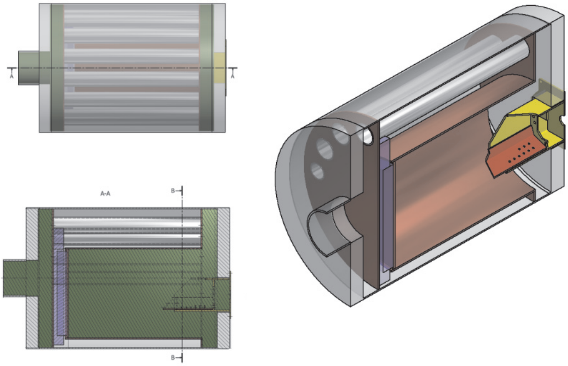

Fig. 1. The geometry and mechanics of the research object

The boiler was located in special-design laboratory equipped with constant heat receiving system in laboratories base of Institute of Machine Design and Operation of Wroclaw University of Technology.

Analytic method

VOCs and PAHs samples were up taken by tubes with active coal (SKC lot 2000). The analysis was done according to polish standard: PN-EN ISO 16017-1: 2006 and Emission Research Laboratory procedures 1/2010 and 2/2010 based on ISO directives. The qualitative and quantitative analysis was proceed on Varian 450 GC gas chromatograph with FID detector and capillary column was used for quantity and quality analysis. Carbone disulfide was used for VOCs, and dichloromethane for PAHs extraction from active coal. The chromatography conditions were: column temperature (110 ^{o}C) for VOCs and 60 ^{o}C up to 280 ^{o}C, dozers (250 ^{o}C) and detectors (250 ^{o}C).

The sampling scheme is presented in Fig. 2.

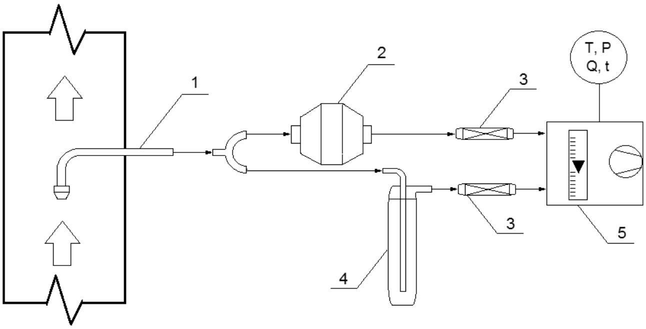

Fig. 2. The geometry and mechanics of the research object, where: 1 – aspiration lead, 2 – PM filter, 3 – active coal tube, 4 – absorption bulb, 5 – aspirator

For on-line measurement of basic exhaust components and combustion performance Kane May-9106 Quintox Upgradeable Combustion Analyser was applied.

Combustion chamber modifications

According to the aim of the research the combustion chamber was modified. The modifications based on applying special steel frame as a ceramic and further active ceramic fittings carrier (Fig. 3).

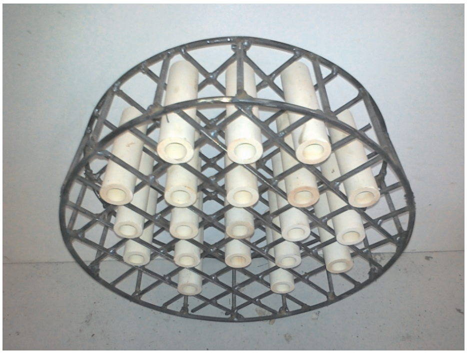

Fig. 3. The frame with ceramic fittings

As an active factor cuprum, manganese and potassium salts (solution of $Cu(NO_3)_2 \cdot 3H_2O$, $Mn(NO_3)_2 \cdot 4H_2O$ and KNO_3) was chosen (separate, laboratory research was provide for catalyst selection) and applied.

The tests was provided on 75 % of nominal boiler power load. As a fuel wooden pellets was applied. Four combustion chamber states was chosen for the research: chamber without any modification ("zero" state), chamber with the steel frame, chamber with the frame and ceramic fittings and chamber with the frame and active-ceramic fittings (Table 2).

Table 2

Combustion chamber states chosen for the tests

Combustion chamber state	Symbol
Chamber without any modification ("zero" state)	I
Chamber with the steel frame	II
Chamber with the frame and ceramic fittings	III
Chamber with the frame and active-ceramic fittings	IV

Results

In Table 3 average value of oxygen, carbon oxides, nitric oxides, sulfur dioxide and total hydrocarbons concentration is presented for all stage of the research.

Table 3

The average value of combustion gases compounds for each stage of the tests

Combustion chamber state	Concentration					
	O_2 [%]	CO [ppm]	CO_2 [%]	NO_x [ppm]	SO_2 [ppm]	C_xH_y [ppm]
I	11.22	583.75	8.94	72.37	36.46	170.73
II	10.71	1063.88	9.61	67.11	52.79	344.34
III	10.43	1178.25	9.70	70.84	63.39	358.22
IV	10.88	1043.32	9.21	70.50	55.84	311.03

It can be seen that combustion chamber modification were disadvantageous for combustion process. This phenomena is caused because of combustion gases flow disturbances effect. Only nitric oxides concentration slightly decrease what was correlated with insignificant decrease in combustion temperature of the process.

To investigate impact of implemented changes on VOCs and PAHs emission the analysis of this compounds was provided.

In Table 4 VOCs concentrations are presented in all chosen combustion chamber state.

Table 4

The VOCs concentration in combustion gases for each stage of the tests (two samples for one stage)

VOC	Concentration [mg/m^3]							
	I		II		III		IV	
	1	2	1	2	1	2	1	2
n-Pentane	2.93	3.38	2.63	2.99	1.69	1.70	4.15	3.62
2-Propanol	81.8	79.3	50.6	91.8	40.5	30.2	35.3	27.1
Benzene	14.2	15.9	0.00	0.00	0.00	4.32	17.7	16.8
2-Butanol	0.00	0.00	0.00	0.00	0.00	0.00	3.95	3.11
Toluene	15.9	17.0	1.29	9.82	0.828	2.62	10.2	9.68
1-Butanol	2.90	2.94	0.243	1.43	0.00	0.00	1.09	3.19
Ethylbenzene	0.00	0.00	0.00	0.00	0.00	0.00	1.94	0.00
Mesitylene	0.189	0.013	0.013	0.123	0.00	0.00	0.359	0.277
Xylene isomers	0.028	0.069	0.00	0.00	0.00	0.00	0.049	0.00
Unidentyfied VOCs	0.112	0.05	0.00	0.00	0.00	0.00	0.327	0.493
Total VOCs	118	119	54.8	106	43.0	38.8	75.1	64.4

According to the achieved results of the analysis the modifications seems to be advantageous in aspect of VOCs emission. The results of the analysis of VOCs group share (average for both samples for each state – Fig. 4) indicates that catalyst

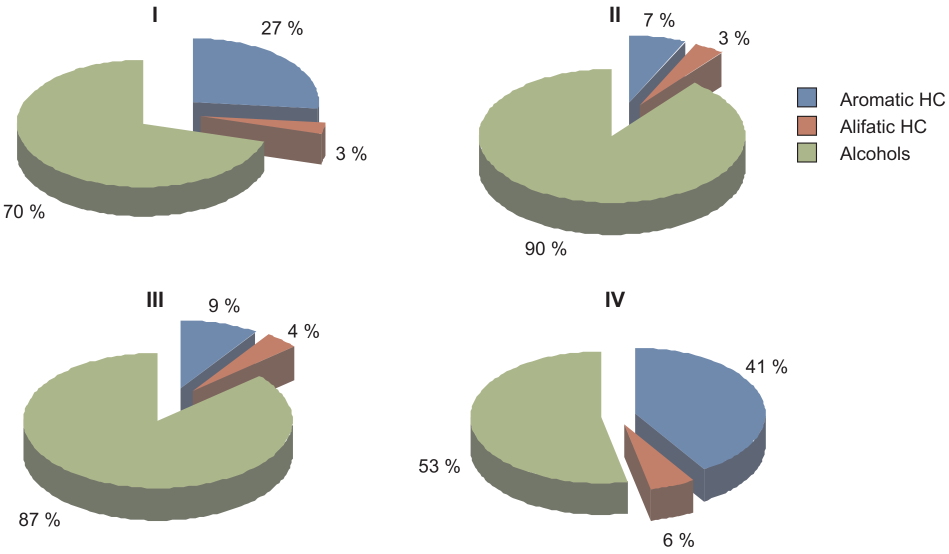

Fig. 4. The group share of VOCs concentration in various states of the combustion chamber

implementation (state IV) cause increase in aromatic compounds group. This group of compounds is most hazardous for humans [2].

In comparison analysis of total VOCs concentration (Fig. 5) the most advantageous configuration seems to be steel frame with ceramic fittings application inside combustion chamber, which cause about 60 % decrease in sum of volatile organic compounds concentration in the exhaust.

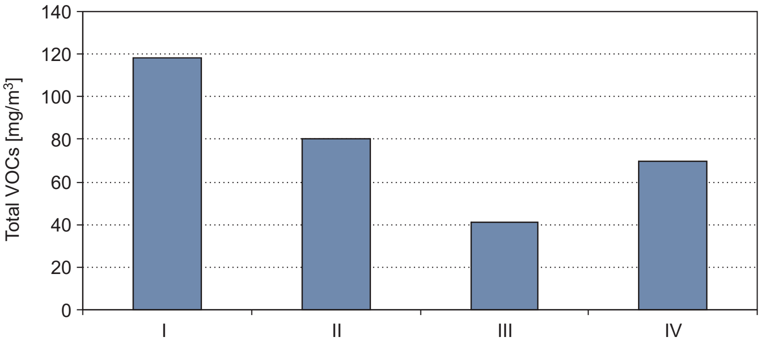

Fig. 5. The total VOCs concentration in various states of the combustion chamber

In Table 5 polyaromatic hydrocarbons concentrations are presented. Because of very toxic benzo(a)antracene and antracene appearance in some states, based on the chemical analysis results, Toxicity Equivalent Factor of individual composition was calculated (using Nisbet and LaGoy factors estimated for PAHs).

Table 5

The PAHs concentration in combustion gases and I_{TEF} for each stage of the tests

PAH	Concentration [mg/m^3]				TEF [Nisbet and LaGoy]	Individual Toxicity Equivalent Factor (I_{TEF}) I_{TEF} = TEQ × C			
	I	II	III	IV		I	II	III	IV
Naphthalen	0.00	20.0	5.14	0.00	0.001	0.004	0.021	0.000	0.015
Acenaphthylene	3.81	21.0	0.00	14.8	0.001	0.011	0.000	0.006	0.000
Acenaphtene	11.1	0.0	5.71	0.00	0.001	0.020	0.063	0.016	0.034
Fluorene	19.8	63.0	15.5	33.8	0.001	0.016	0.031	0.044	0.015
Phenanthrene	15.6	31.0	44.0	14.8	0.001	0.009	0.024	0.033	0.016
Anthracene	8.76	24.0	33.1	16.2	0.01	0.000	0.040	0.011	0.000
Fluoranthene	0.00	4.00	1.14	0.00	0.001	0.001	0.000	0.002	0.001
Pyrene	0.76	0.00	1.50	1.43	0.001	0.000	0.000	0.001	0.000
Benzo[a]antracene	0.00	0.00	0.50	0.00	0.1	0.000	0.000	0.000	0.048
Chrysenes	0.00	0.00	0.00	0.48	0.01	0.060	0.179	0.112	0.129
Total PAHs	59.8	162	107	81.0	—	0.060	0.179	0.112	0.129

The total PAHs concentration and Individual Toxicity Equivalent Factors for each PAHs mixture are shown in Fig. 6.

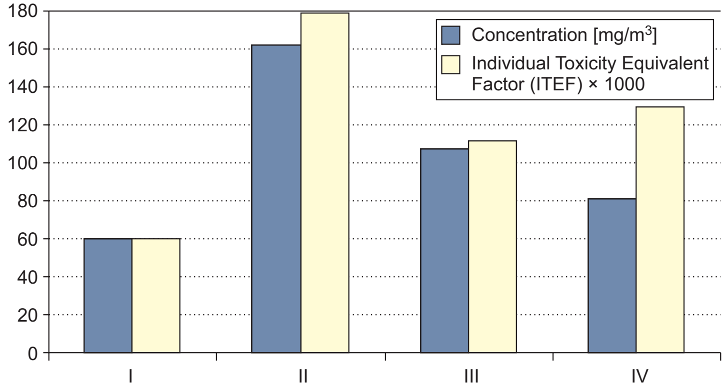

Fig. 6. The total PAHs concentration and Individual Toxicity Equivalent Factors for each PAHs mixture

The analysis of the results indicates that any modification of the combustion chamber cause increase in PAHs concentration and significantly increase mixture toxicity. Ceramic fittings implementation seems to impact on polyaromatic compounds decrease in comparison to state with "clear" steel frame. It may direct on ceramic implementation positive impact on those toxic hydrocarbons emission. Catalytic factor cause appearance of extremely hazardous for human health benzo[a]antracene.

Conclusions

The results of the research issue modification of the small-scale boiler combustion chamber by implementation active ceramic fittings of steel frame are ambiguous. The effect of aggravation of main emission parameters is probably caused by interferences of the combustion gases flow and distribution. Ceramic and active ceramic implementation into combustion space significant impact on combustion process and VOCs and PAHs emission. This impact is discussible and indicates on the need of further researches which should be focused especially on frame and fittings shape as a factor determining combustion process proceeding.

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EMISJA ZWIĄZKÓW ORGANICZNYCH ZE ZMODYFIKOWANEGO KOTŁA MAŁEJ MOCY ZASILANEGO BIOMASĄ

[1] Wydział Mechaniczny, Politechnika Wrocławska
[2] Ove Arup & Partners International Ltd Sp. z o.o.
[3] Wydział Inżynierii Środowiska, Politechnika Wrocławska

Abstrakt: Problem niskiej emisji, dotykający w szczególny sposób jakości życia człowieka zarówno w aglomeracjach miejskich, jak i osadach wiejskich, związany jest nieodłącznie z kotłami małej mocy zasilającymi indywidualne gospodarstwa domowe w ciepło. Spaliny emitowane podczas niepoprawnego (niepełnego) prowadzenia procesu spalania paliw w tzw. małych kotłach zawierają groźne dla zdrowia ludzkiego związki z grup Lotnych Związków Organicznych (LZO). Substancje te charakteryzuje mutagenne oraz kancerogenne działanie na organizmy żywe i całe ekosystemy, nawet przy niewielkich poziomach stężeń oraz zdolność do kumulowania się w środowisku. W celu ochrony środowiska i zdrowia ludzi niezbędne jest opracowanie rozwiązań umożliwiających redukcję, tak charakterystycznych dla procesów spalania w nieefektywnych kotłach grzewczych, stężeń szkodliwych substancji węglowodorowych.

W artykule przedstawiono wyniki pracy badawczej, której celem było badanie wpływu modyfikacji przestrzeni spalania w kotle małej mocy zasilanego biomasą na emisję lotnych związków organicznych. W przestrzeni spalania kotła wprowadzono zmiany konstrukcyjne polegające na wprowadzeniu kształtek o własnościach katalitycznych. Oznaczenie LZO w spalinach wykonywano metodą chromatografii gazowej za pomocą aparatu Varian 450 GC. Wyniki zaprezentowano w postaci tabel i rysunków.

Słowa kluczowe: kotły małej mocy, niska emisja, lotne związki organiczne, katalizator wewnętrzny

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