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OCCURRENCE OF HEAVY METALS IN SELECTED MADE GROUNDS

WYSTĘPOWANIE METALI CIĘŻKICH W WYBRANYCH GRUNTACH NASYPOWYCH

Abstract: This paper presents the analysis of selected urban and industrial areas containing made grounds in the superstratum. The studied soils were characterized by exceeded quality standards only for the presence of heavy metals. The specificity of the occurrence and the visible regularity of the presence of heavy metals in the geological profile were determined. Preliminary scenarios of the action in the event of the exceeded standards for soil quality were presented, showing also the type and scale of the problem. The quality of the soil was analysed taking into account the already existing legislation regarding standards for soil quality. The analysed soils originated from the Silesian Voivodeship.

Keywords: heavy metals, embankment for non-construction purposes, standards for soil quality

Geochemical state of soils in Poland is regulated by legal standards for soil quality [1]. Analysis of the problem of soil pollution is reduced practically to the soil ranging from 0.0 to 0.3 m below the land surface (bls), or subsoil (to a depth of 1.0 m), while the soil beneath this depth is not the main focus of scientific research. It is often the case that land owners learn about the exceeded contaminants concentrations no sooner than at the onset of the investment process. In Poland, the attention is mainly focused on soils contaminated with petroleum substances, which for obvious reasons are remediated, among others, due to the risk of migration of hydrocarbons from the identified sources of pollution such as old military bases, petrol stations, oil tanks, etc. Such contaminants can be economically and effectively removed by in-situ methods, using biochemical techniques [2]. On the other hand, a land contaminated with only heavy metals remains a problem because of the lack of effective and economical methods for in-situ treatment and due to complicated and uneconomical methods of

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ex-situ remediation (also disputable from the environmental perspective). In most cases these are solidification and immobilization methods, which do not remove contaminants but only immobilise them, which does not solve the problem of the final disposal. In addition, due to the nature of the industry in the Silesian Voivodeship, which was developed mainly in the nineteenth and twentieth centuries, excessive amounts of metallurgical and foundry wastes (slag and dross) were deposited in heaps. These waste materials were readily available for levelling the surrounding areas by creating embankments for non-construction purposes. The type and condition of such embankments do not meet the requirements for earthworks or construction groundwork [3]. At that time, there were no legal limits for control and the environmental considerations were overridden by the need for industrial development. The above-mentioned production wastes, which occur in the form of slag, dross and ash (usually mixed with debris, stones and soil), remain embedded in the geological profile in a substantial area of land of Silesia. Due to the current local regulations for the protection of the land surface, which in accordance with the definition constitutes landform features, soil and the underlying soil to a depth of human impact [...] [4], one should consider embankments for non-construction purposes as an integral part of land surface. This implies the necessity to apply the quality standards for soil also to embankments for non-construction purposes, and after the planned change in legislation – the maximum allowable content of substances causing the risk in the soil.

Virtually all of the analyzed samples, which were found to exceed allowable metals concentrations, contained slag and metallurgical or foundry dross. Metallurgical waste is one of the most diverse groups of industrial waste. Depending on the process by which the by-products are formed, they are characterized by a variable structure, technical properties, and the chemical and mineralogical composition [5–9]. Based on the example of the slags originating from two slag heaps in Silesia, the content of selected metals is as follows [7]: the slag from the Zn-Pb ores smelting heap – manganese from 430 to 2223 mg/kg d.m., zinc from 6270 to 83700 mg/kg d.m., lead from 5340 to 29385 mg/kg d.m., cadmium from 32 to 262 mg/kg d.m., chromium from 16 to 69 mg/kg d.m., copper from 66 to 1859 mg/kg d.m., nickel from 25 to 146 mg/kg d.m., molybdenum from 6 to 25 mg/kg d.m., in the waste from the steel slag heap – manganese from 873 to 86 600 mg/kg d.m., zinc from 1 to 10 800 mg/kg d.m., lead from 6 to 3200 mg/kg d.m., cadmium from < 0.3 to 136 mg/kg d.m., chromium from 15 to 2920 mg/kg d.m., copper from 3 to 837 mg/kg d.m., nickel from < 1 to 102 mg/kg d.m., molybdenum from < 1 to 8 mg/kg d.m.

The aim of this study was to analyse the selected areas characterized by exceeded maximum allowable concentrations of heavy metals in the man-made fill layer as regulated by the already existing legislation and in correlation with the capacity to meet the legal requirements for such areas of land in Silesia.

Materials and methods

Table 1 presents the Polish standards for soil quality for different groups of land including urban, industrial and transportation areas, which were the basis for this study.

Table 1

Soil and land standards in the range of the accepted concentrations of heavy metals [1]

Metal	Group A ^a	Maximum allowable concentrations in soils [mg/kg d.m.]						Group C ^c	
		Group B ^b			Depth [m bsl]				
		0.0–0.3	0.3–15.0	> 15.0	up to	up to	below		
					1 · 10 ⁻⁷	1 · 10 ⁻⁷	not applicable		
								1 · 10 ⁻⁷	
Arsenic (As)	20	20	20	25	25	55	60	25	
Barium (Ba)	200	200	250	320	300	650	1000	300	
Chromium (Cr)	50	150	150	190	150	380	500	150	
Tin (Sn)	20	20	30	50	40	300	350	40	
Zinc (Zn)	100	300	350	300	300	720	1000	300	
Cadmium (Cd)	1	4	5	6	4	10	15	6	
Cobalt (Co)	20	20	30	60	50	120	200	50	
Copper (Cu)	30	150	100	100	100	200	600	200	
Molybdenum (Mo)	10	10	10	40	30	210	250	30	
Nickel (Ni)	35	100	50	100	70	210	300	70	
Lead (Pb)	50	100	100	200	100	200	600	200	
Mercury (Hg)	0.5	2	3	5	4	10	30	4	

^a Group A – landed property included in the area subject to protection under the provisions of the Water Act, and areas subject to protection under the provisions of the Nature Conservation Act; ^b Group B – land classified as agricultural land except land under ponds and ditches, forests and wooded land, barren land, and urban areas with the exception of industrial land, surface mining land in use, transportation land; ^c Group C – industrial land, surface mining land in use, transportation land.

The cases selected for this study included the soils with exceeded maximum allowable concentrations for heavy metals only. The analysed soil samples were collected from 12 industrial/urban areas of the Silesian Voivodeship. The presented results are the maximum values of heavy metals concentrations in the analysed made grounds. All of the studied cases concerned the areas designated for construction investments (land belonging to groups B and C) in accordance with local zoning plans. The soils were compared with the limit values specified separately for each metal, depending on the permeability of the soil and the type of soil (land use). Determinations of metals present in the soil samples were carried out by atomic absorption spectrometry after previous digestion of the samples.

Quality standards were set at two thresholds, *ie* separately for the soil of permeability $< 1 \cdot 10^{-7}$ m/s and separately for the soil with permeability $> 1 \cdot 10^{-7}$ m/s, depending on the depth. The land to be used according to the specifications for group B was analysed in the range of the depth from 0.3 to 15.0 m, and the land belonging to group C was analysed both in the range of 0.0 to 2.0 m, and also from 2.0 to 15.0 m. Hence, the cases within group A and group B (in the range of 0.0 to 0.3) were not analysed. The type of land in which the exceeded standards were found was assessed within the set of identified cases of exceeded standard values. Subsequently, it was examined whether the exceeded values were also found in the layer situated directly beneath a layer in which the exceeded values were found initially, taking into account the geological structure of the land.

The comparison of soil samples was carried out for the samples taken from boreholes in the same area of study and also for the samples taken in the other regions of Silesia. Each of the analyzed area was considered in terms of meeting soil quality standards specified for a particular land zoning: B or C (in accordance with applicable regulations). In addition, however, to illustrate the scale of the problem, all the results were also compared with the second category.

The legal aspect of the case of land exceeding the maximum allowable concentrations was analysed in an attempt to evaluate action strategy for areas in the Silesian Voivodeship. This analysis was performed with respect to the legal situation – *ie* soil and earth quality standards being in force since 2002 and which due to changes in the legislation in September 2014 are to be replaced by “allowable content of risk causing substances in the soil or in the earth” as defined by the regulation to be introduced by September 2016.

Results and discussion

Table 2 shows the observed maximum concentrations of selected heavy metals in the made grounds collected from urban and industrial areas of the Silesian Voivodeship.

All of the made grounds were qualified as highly water-permeable (up to $1 \cdot 10^{-7}$ m/s). The embankments, in each of the analysed cases were a mixture of slag or construction debris and soils, sand or clay or stones. In each of the embankment sample at least one of the identified components was of anthropogenic origin (construction debris, slag, crushed dross, bricks, sinter, etc.).

Table 2

Maximum concentrations of heavy metals in selected made grounds in the Silesian Voivodeship

Metal	Group A ^a	Observed maximum metal concentration [mg/kg d.m.]						Group C ^c	
		Group B ^b			Depth [m bsl]				
		0.0–0.3	0.3–15.0	> 15.0	up to (embankments)	below (native soil)	up to below		
Arsenic (As)			82	14.1			412	378 < 0.2	
Barium (Ba)			1420	197			3280	2200 36.8	
Chromium (Cr)			38.4	18.1			1060	53.0 28.3	
Tin (Sn)			125	9.0			51.2	20.0 8.1	
Zinc (Zn)			5540	89.0			50500	86000 206	
Cadmium (Cd)	Not analysed	11.8	0.64		Not analysed	Not analysed	398	239 < 0.3	
Cobalt (Co)	Not analysed	9.4	12.7				23.7	15.1 8.80	
Copper (Cu)		3000	18.3				7991	57.4 8.89	
Molybdenum (Mo)		< 5.00	< 5.00				216	< 5.00 < 5.00	
Nickel (Ni)		52.6	32.7				188	45.5 14.9	
Lead (Pb)		1330	20.6				2810	9460 13.7	
Mercury (Hg)		0.345	2.11				0.320	< 0.1 < 0.1	

^a Group A – landed property included in the area subject to protection under the provisions of the Water Act, and areas subject to protection under the provisions of the Nature Conservation Act; ^b Group B – land classified as agricultural land except land under ponds and ditches, forests and wooded land, barren land, and urban areas with the exception of industrial land, surface mining land in use, transportation land; ^c Group C – industrial land, surface mining land in use, transportation land.

The soil directly below the embankments was observed to be both highly permeable (mostly sand) and poorly permeable (mainly clay and silt).

The analyses of soil showed that in all the cases in which the exceeded standards for metals were observed in man-made fill layer, the layers situated directly beneath the embankment contained no contaminants, regardless of the type of those formations, this is, highly permeable or poorly permeable. This demonstrates the absence of a noticeable vertical migration of contaminants from the embankment layer to the native soil. Tables 3 and Table 4 list selected examples of geological profiles with exceeded standards for metals in the embankment layers layer and in the underlying native soil – highly and poorly permeable. Table 3 presents selected profiles from the area B (urbanized), and Table 4 presents selected profiles from the area C (industrial).

It was observed that every soil sample from the area B, in which the exceeded standard value was found for zinc, contained also exceeded concentrations of lead, and most of the samples contained additionally barium and arsenic. In addition, some samples showed exceeded maximum allowable concentrations of tin and copper, and only in few cases of cadmium and chromium. Only a single case was found when the maximum allowable concentration of nickel was exceeded.

For cobalt and mercury no exceeded values were found in the embankment samples, and the exceeded value of molybdenum was found only in one sample. The concentrations of molybdenum and mercury were in most cases below the level of quantification.

The same set of exceed standard values in terms of the presence of a given metal and its concentration range was found for each set of samples taken from all of the tested boreholes within a given area (*eg* within one or several interconnected cadastral land plots). This proves the uniform structure of embankments of the same origin in the area of interest. Comparing a group of samples from different locations in Silesia it was observed that the composition of embankments varied. However, it can be presumed with high probability, that in all of the analysed areas in which metals concentrations were exceeded, the land was levelled using materials containing varying percentage of metallurgical and foundry wastes, mainly from the processing of non-ferrous metals.

The important aspect of the analysis proved to be the classification of land by groups of application. The research shows that the same land classified as category B is considered to be contaminated and by classifying it in category C it would be completely unpolluted, and conversely: no exceeded standard values due to category C of the studied area may be inaccurate in the case of a change of the category to category B. This situation poses a problem resulting mainly in a manner in which the polluted soil is handled. In accordance with the already applicable regulations, the land was considered to be polluted when the concentration of at least one substance exceeds the limit value (standard) [1]. This poses, on the other hand, a problem during the investment process, when the ground has to be excavated, mainly for the foundation. The legal aspect of this situation was examined to find that the polluted soil excavated during earthworks has to be considered as a dangerous waste and is qualified under the code 17 05 03*. According to the hierarchy of methods of waste handling, the soil excavated during earthworks (waste), should be first prepared for re-use or recycled

Table 3

Selected geological profiles with exceeded metals standards in the embankment layers and in the underlying native soils
– highly and poorly permeable from an urbanized area (group B)

Metal	Metal concentrations in the selected ground profiles for the area B [mg/kg d.m.]				
	Profile 1 Embankment	Profile 2 Silt clay occurring directly beneath the embankment	Profile 3 Sandy loam occurring directly beneath the embankment	Profile 4 Medium sand with coarse sand intercalations occurring directly beneath the embankment	Silt clay occurring directly beneath the embankment
Arsenic (As)	82*	11.0	20.7*	< 0.200	17.0
Barium (Ba)	734*	1.97	249	58.1	577*
Chromium (Cr)	18.0	14.5	14.9	10.6	10.0
Tin (Sn)	125*	8.7	5.40	1.95	14.2
Zinc (Zn)	5540*	89	1040*	45.5	580 *
Cadmium (Cd)	10.6*	< 0.300	5.52	< 0.300	2.19
Cobalt (Co)	9.4	5.37	4.79	3.93	6.45
Copper (Cu)	96.2	7.70	19.8	10.70	33.0
Molybdenum (Mo)	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00
Nickel (Ni)	35.2	10.4	9.5	11.3	17.0
Lead (Pb)	1330*	20.6	696*	19.9	160*
Mercury (Hg)	0.148	< 0.100	< 0.100	0.152	< 0.100

* Exceedance of the standard.

Table 4
Selected geological profiles with exceeded metals standards in the embankment layers and in the underlying native soils
– highly and poorly permeable from an industrial area (group C)

Metal	Metal concentrations in the selected ground profiles for the area C [mg/kg d.m.]							
	Profile 5		Profile 6		Profile 7		Profile 8	
	Embankment	Sandy loam	Embankment	Fine sand	Embankment	Fine sand	Embankment	Fine sand
Arsenic (As)	< 0.200	< 0.200	6.6	< 5.0	5.32	< 5.0	< 5.0	< 5.0
Barium (Ba)	450	36.8	424	< 20.0	181	21.1	378	< 20.0
Chromium (Cr)	1060*	15.1	51.5	< 10.0	128	28.3	33.4	< 10.0
Tin (Sn)	51.2	8.1	< 20.0	< 20.0	< 20.0	< 20.0	< 20.0	< 20.0
Zinc (Zn)	3730*	30.1	2560*	30.3	2740*	42.8	1570*	206
Cadmium (Cd)	15.2*	< 0.300	9.4	< 2.0	8.50	< 2.0	8.1	< 2.0
Cobalt (Co)	6.33	4.80	23.7	8.8	15.8	7.50	21.8	2.4
Copper (Cu)	135	8.50	44.5	< 5.0	17.1	< 5.0	58.8	< 5.0
Molybdenum (Mo)	< 5.00	< 5.00	< 5.00	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Nickel (Ni)	43.3	13.7	32.8	< 10.0	23.7	14.9	49.7	< 10.0
Lead (Pb)	65.0	10.7	362	< 20.0	889*	< 20.0	373	< 20.0
Mercury (Hg)	< 0.100	< 0.100	< 0.100	< 0.100	< 0.3	< 0.3	< 0.3	< 0.3

* Exceedance of the standard.

when the re-use method is unavailable, if both of these methods are unavailable the soil should be subjected to other methods of recovery and finally disposed of (including landfilling) [10]. The current version of the document "Waste Management Plan for the Silesian Voivodeship 2014" (hereinafter PGO) was analysed to find only three functioning installations in the Silesian Voivodeship which could handle the waste denoted as 17 05 03*. These installations include: an incineration plant; solid fuel production plant (accepts only the waste which is contaminated with petroleum compounds), which rules out processing of soil contaminated with heavy metals; and the third installation which is a plant producing pellets and aggregates, where pollutants are not removed but solidified by the Geodur method. The production capacity of this plant is 10 000 Mg (in total for several types of waste). In addition to these installations, PGO of the Silesian Voivodeship does not suggest any other installation that could handle waste bearing the code 17 05 03* (such as soil contaminated by metals only). It is noted that none of the landfills in the Silesian Voivodeship permit landfilling of this type of waste. Taking into consideration the observed area of pollution in the investigated areas of investment, it becomes clear that it is virtually impossible to respect the "proximity principle" applicable to waste producers (waste transfer to the nearest sites where they can be processed [10]). The assumption of an average thickness of the made grounds (depth 2.2 m), and the occurrence of an area with exceeded standard metal concentration with surface of 20 m × 20 m, yields at least 640 Mg of waste to be handled from only one construction site. Moreover, the observed thickness of non-conforming made grounds can be even 5.5 m, as well as the area of investment is much more extensive, so the mass of soil that has to be disposed of in this case is much greater.

The amended regulations of the Environmental Protection Law (Act of 11 July 2014. Amending the Act – Environmental Protection Law and other laws; Dz.U. 2014 No. 0 pos. in force since 05.09.2014) regarding the pollution of land, change the approach in dealing with contaminated soil or earth. They announce that in two years a regulation determining the manner of assessing the contamination of the surface of the earth will come into force. This assessment will include the identification of substances causing a risk particularly important to protect the surface of the earth, their allowable content in soil and their allowable content in earth, differentiated for individual soil properties and land groups, distinguished depending on their usage. The amended regulation will include specific requirements for determining the maximum allowable content in the soil of a risk-causing substance, including the analysis of its impact on human health and the environment. Therefore, the current standards of soil quality, which are used as a reference of all the soil contamination analyses, will be withdrawn no later than by September 2016. Long-term observations of the land (with the exceeded standards for metals only) in Silesia suggest urgent need for changes in the decision-making on the treatment of the soil. The existing legislation, which mandates that in every single case of such land the standards established depending on the usage of the area (A, B or C) have to be met, is irrational. All the cases studied indicate no noticeable metal migration to the layers underlying the embankments. Additionally, taking into consideration the problems with the management of contaminated soil as a waste (which in accordance to

the legislation is a hazardous waste) and no reasonable means of technical metal removal from the soil (especially in the areas of investment), the necessity to cease referring to the quality standards (determining the decision to remove the contaminants) in favor of risk assessment of the impact of these pollutants on human health and the environment becomes essential.

Furthermore, it seems inappropriate to decide that the contaminants should be removed only because of the exceeded allowable values, which are significantly different for the urbanized areas and industrial areas, as the land zoning is often subjected to changes.

A review of the local zoning plans indicates that the planned function of the land use often combines industrial and commercial/service uses. In such a case, the assessment of the area as polluted or unpolluted depending on the current or planned land use becomes an absurd procedure.

A comparison of the standards shown in Table 1 indicates great difference between the allowable concentrations established for heavy metals in the areas B and C.

For example, there is a several-fold difference for two the most often exceeded metal contents in embankments which are Zn and Pb (Zn standard for the area B is 350 mg/kg d.m. and for the area C it is 1000 mg/kg d.m.; the standard value for Pb for the area B is 100 mg/kg d.m. and for the area C it is 600 mg/kg d.m.).

This causes that the embankment originating from the same source and having the metal content between the B and C standards would be considered to be polluted and to be a hazardous waste when excavated (in the areas B), and in the strictly industrial areas it would be treated as unpolluted soil.

It also distorts the overall analysis of the soil, especially in the areas C, because often high concentrations of Zn and Pb indicating their unnatural origin and proportions (metallurgical source of soil components) do not exceed the standard C.

An example is the analysis of embankments indicated in Tables 3 and 4 where it can be seen that in the area B (Table 3) every sample containing slags in the embankment is characterized by exceeded zinc but also by exceeded lead, but for the areas C (Table 4) most of the samples with exceeded zinc do not exceed the standards for lead.

This is due to the fact that the current standards for metals have been established disproportionately – for example, the standard for Pb in the areas B is 3.5 times lower than the standard Zn concentration. On the other hand, for the areas C high standard value for Pb (only 1.7 times smaller than the standard for Zn), often does not show its increased content in the slags (in which it occurs with zinc), which interferes with the assessment of the situation.

Table 5 provides an example of made grounds in the area C (industrial land use) where, in accordance with the existing regulations, they would not be considered as polluted, but in the area B (commercial/service land use) this land should be subjected to reclamation by removing the contaminants to the depth specified by the quality standards.

Table 5 clearly shows that the same land with the quality exceeding the standards for the area B, depending on the planned investment (eg construction of a shopping mall in one case or a manufacturing plant in another), would have to be subjected to costly

Table 5

Comparison of the metal content of the embankments to the standards for areas B and C

Metal	Metals concentrations in the selected made grounds [mg/kg d.m.]													
	Soil 1			Soil 2			Soil 3			Soil 4			Soil 5	
embank- ment	Group B	Group C	embank- ment	Group B	Group C	embank- ment	Group B	Group C	embank- ment	Group B	Group C	embank- ment	Group B	Group C
Arsenic (As)	28.6	+	-	0.433	-	-	20.1	+	-	2.30	-	-	24.1	+
Barium (Ba)	470	+	-	364	+	-	141	-	-	208	-	-	682	+
Chromium (Cr)	63.0	-	-	289	+	-	53.0	-	-	8.93	-	-	17.5	-
Tin (Sn)	22.1	-	-	15.3	-	-	<20.0	-	-	4.69	-	-	42.5	+
Zinc (Zn)	920	+	-	451	+	-	828	+	-	477	+	-	657	+
Cadmium (Cd)	3.53	-	-	0.930	-	-	<2.00	-	-	3.85	-	-	3.17	-
Cobalt (Co)	10.5	-	-	8.70	-	-	15.1	-	-	2.77	-	-	8.22	-
Copper (Cu)	122	+	-	39.7	-	-	37.6	-	-	19.3	-	-	249	+
Molybdenum (Mo)	< 5.00	-	-	95.0	+	-	<5.00	-	-	<5.0	-	-	<5.00	-
Nickel (Ni)	28.9	-	-	188.0	+	-	45.5	-	-	6.68	-	-	21.7	-
Lead (Pb)	166	+	-	67.3	-	-	196	+	-	11.7	+	-	213	+
Mercury (Hg)	<0.100	-	-	<0.100	-	-	<0.10	-	-	<0.10	-	-	0.320	-

“+” exceedance of the standard; “-” non-exceedance of the standard.

disposal and would be treated as a hazardous waste when excavated (in the first case), would be treated as an unpolluted soil, which could be used for levelling the investment area (in the second case). So far, the other factors such as leachability, geological structure, toxicity, pH, metals speciation etc. were neglected. The announced new regulations should take into account the risk analysis of the identified pollutants to human health and the environment as a basic condition for further handling of the ground.

Conclusions

Observations of the cases of made grounds characterized by exceeded standard metals concentrations allow the following conclusions:

– Proper diagnosis of the geological structure plays an important role in carrying out any ground assessment. Careful attention should be devoted to distinguish between soil and embankment built with anthropogenic materials when taking samples of the top layer. This can be of great importance when the results are analysed in terms of sources of metal contamination.

– As evidenced by geological cross-sections no exceeded standard values for metals were observed in the layers situated directly below the man-made fill layer in which metals concentrations were found to be higher than the maximum allowable concentrations. This was observed regardless of their permeability, which suggests no noticeable migration of metals from the embankments containing steel slags.

– There is a certain regularity, this is, in the case of made grounds with exceeded standard value for zinc it was also observed that the concentration of lead was also elevated, and often also in order of: barium and arsenic, sometimes copper and tin, which allows determination of the origin of materials used to build the embankments: metallurgy/foundry of a given type of non-ferrous metal.

– The Silesian Voivodeship does not have any installations which could be used to dispose of the soil with exceeded maximum allowable concentrations of heavy metals.

– The legal regulations governing the treatment of polluted soil should be amended to take into account the case of made grounds with elevated concentrations of metals.

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WYSTĘPOWANIE METALI CIĘŻKICH W WYBRANYCH GRUNTACH NASYPOWYCH

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Abstrakt: W pracy dokonano analizy wybranych terenów zurbanizowanych oraz przemysłowych zawierających w stropowej strefie podłoża grunty nasypowe. W badanych gruntach występowaly przekroczenia w zakresie standardów jakości jedynie pod względem występowania metali ciężkich. Określono specyfikę występowania przekroczeń oraz zauważalną prawidłowość obecności metali ciężkich w profilu geologicznym. Przedstawiono wstępne scenariusze dopuszczalnych działań w przypadku stwierdzenia przekroczeń standardów jakości gleby i ziemi, wykazując rodzaj i skalę problemu. Stan gruntów analizowano przy uwzględnieniu obowiązujących przepisów prawnych w zakresie standardów jakości gleby i ziemi. Rozpatrywane grunty pochodziły z obszaru województwa śląskiego.

Słowa kluczowe: metale ciężkie, nasyp niebudowlany, standardy jakości gleby

Renata GNATOWSKA¹

THE ANALYSIS OF GASEOUS POLLUTANTS DISPERSION IN HILLY TERRAIN

ANALIZA ROZPRZESTRZENIANIA SIĘ ZANIECZYSZCZEŃ GAZOWYCH NA TERENIE PAGÓRKOWATYM

Abstract: The emission of a dangerous substance overlaps in vicinity of wide-range ground-based objects. Consequently, part of the process of dispersion pollution is determined by the flowing conditions and is shaped by their presence and distribution. However, under real conditions, complex systems of terrain obstacles exist. A subject of interest among many authors is the flow and dispersion of pollutants surrounding single elements such as a hill or a building. This type of research enables a better understanding of the flow and propagation of pollutants on terrain with complex topography. The aim of the present work is the investigation of the influence of the complex character of a velocity field, particularly its periodic composition (non-stationary blowing) as well as rotating structures generated by obstacles such as hills on the propagation of various types of gaseous pollutants. The base of analysis represents the evolution of the carbon dioxide concentration in profiles surrounding hills or different locations in relation as well to the height of the source emission. By propagating a gaseous tracer in a oscillating flow from a source located in a flush zone, a source's location relative to circulation zones of increased level turbulent fluctuations of flow velocity can be determined.

Keywords: pollutant dispersion, gaseous pollutions, experimental and numerical modeling, hilly terrain

Introduction

The experimental and numerical simulation of flow over hilly terrain have concerned significant scientific interest during the last decades [1, 2] because of the important implications of the problem in many fields (suitable site for wind power plants, fire propagation, pollutant dispersion, erosion processes). The dispersion process of pollution takes place in flow conditions formed by presence the various ground-based objects. However, in complex systems and exist due to terrain conditions, a subject discussed by many authors is flow and pollution dispersion of small elements in a surrounding (natural topological features like hill, building) [1–3]. Such researchers

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are the first step to better understanding the flow and dispersion on terrain consisting of complex topographies. The tests of structured wind flow in mundane zones, which are comprised complex topographies, contribute series of information relevant for the most recent aerodynamics problems. Among other things, they relate to the dispersion of pollution, fire propagation, wind erosion and local condition researches which favor wind energetic. The last herein mentioned aspect forms the particular motive for wind flow analysis in the oscillating flow. Flow features around single hills have both an influence conditions as inlet conditions and medium geometry. The collection of the mentioned factors can cause big differences in the kinematics within the jet stream which in general case sets detachment phenomenon, recirculation and adherence. The problem of wind flow over oscillating flow was taken in a series of work which involved both researches led in natural all-terrain condition, experimental modeling, and also numerical simulations. Most quoted work belong to Jackson and Hunt [2], presents analytical solutions for the hill shape, numerical Peterson models [4], Lenelin and others [3] and experimental results of Ferreira [5], Cao [6], Lubitz [7] and Kim [8–9]. Several studies have been reported on the flow over hills using RANS and LES methods [10–15]. Turbulent flow over a steep hill contains relatively complex mean-flow characteristics such as separation and reattachment. The center of this research was mainly focused on deformation profiles of wind velocity flow according to apparition of symmetrical hills a low inclination [10, 13]. Geometry which was taken in most analyses does not appear to lead to strong detachment and recirculation zone. This constrains compliance of offered calculation methods [5] and experimental model consistency with a real wind field along the hill. Practical value of results of research denigrates the lack of information about general currents in nature, unfavorable aerodynamic modeled flow features. The study of flow and dispersion over two- and three-dimensional hills are presented in publications of Araya [16, 17], Tsai [18], Chatzipanagiotidis [19] and others. The present analysis undertakes the problem of the detachment and recirculation cause strong fluctuations in velocity zones and change flow wind directions. The main attention has been put on an effect of oncoming wind oscillations on the velocity field structure around the single sinusoidal 2D hill in the context of pollution dispersion.

The methods of experiments

The program of the study consists of wind-tunnel experiments carried out in an open-circuit wind tunnel at the Institute of Thermal Machinery of the Czestochowa University of Technology. The geometries of the analyzed cases are sketched in Fig. 1. The role of tap gas source, from the scoring source (pipe) from pointing source was played by carbon dioxide with a flow outlet velocity equal in approximation of undisturbed flow velocity, above layer zone. To measure the concentration of CO₂ analyser Guardian plus was used. Analyzed model of the hill was installed on a medium aerodynamic tunnel in the Aerodynamic Laboratory of Czestochowa University of Technology upon which the layer zone of thickness $\delta = 0.1$ m was generated, and profile velocity shape typical for an open ground with poor vegetation.

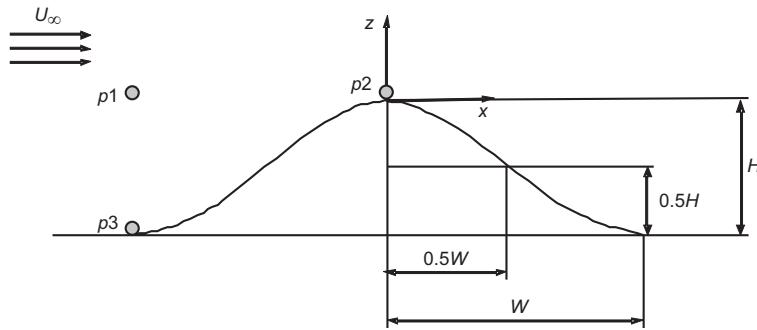


Fig. 1. Scheme of hill model with marked location of emission CO₂ source

The mean velocity of the boundary layer zone amounted to $\bar{U}_\infty = 13$ m/s with profile described by the power law (1):

$$\bar{U}_0(z) = \bar{U}_\infty \cdot \left(\frac{z}{\delta} \right)^\alpha \quad (1)$$

where δ is the depth of boundary layer, $\alpha = 0.166$ is the power law exponent, which corresponds to the velocity profile for open terrain with low vegetation.

The shape of tested hill model is described by the relationship (2):

$$z_s = \frac{H}{2} \left\{ 1 + \cos \left[\left(\frac{\pi}{2} \right) \left(\frac{x}{0.5W} \right) \right] \right\} \quad (2)$$

where $H = 60$ mm, $W = 100$ mm (Fig. 1). Measurements were conducted for three different locations ($x = x_s$, $z = h_s$) and their emissions (Fig. 1), namely:

(p1) $x_s = -W$, $h_s = 1H$;

(p2) $x_s = 0$, $h_s = 1H$;

(p3) $x_s = -W$, $h_s = 0$.

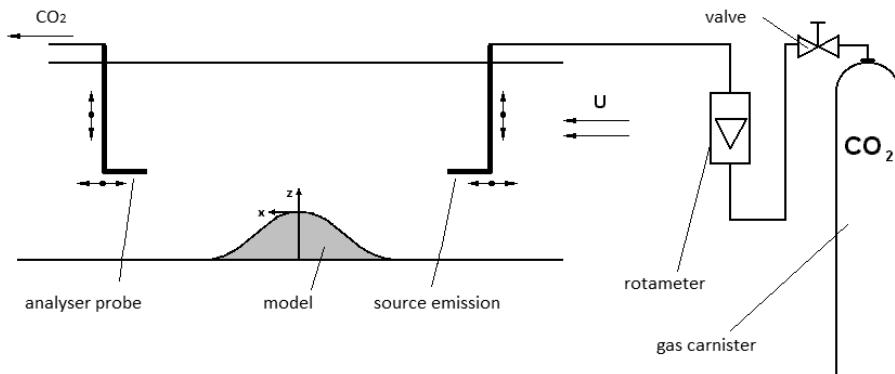


Fig. 2. Scheme of measuring testing bench with mounted researched object

The measuring probe was mounted on a support, which enables establishment of vertical concentration CO₂ profiles for chosen stream $x = \text{const}$ set in localizations: $x = 0; 0.6W; W; 2W; 3W; 4W$ (Fig. 2). All measurements were done in a tunnel axis.

The flow and dispersion characteristics

The aerodynamic outline of the object which enabled the detection of surrounding characteristic zones with strong diversified features, namely the area of increased velocity flow above the top of the hill and recirculation region of flow behind the hill (Fig. 3). Significant from the point of view of gaseous pollutants dispersion, the increase of wind speed over the hill surface is observed in their top zone.

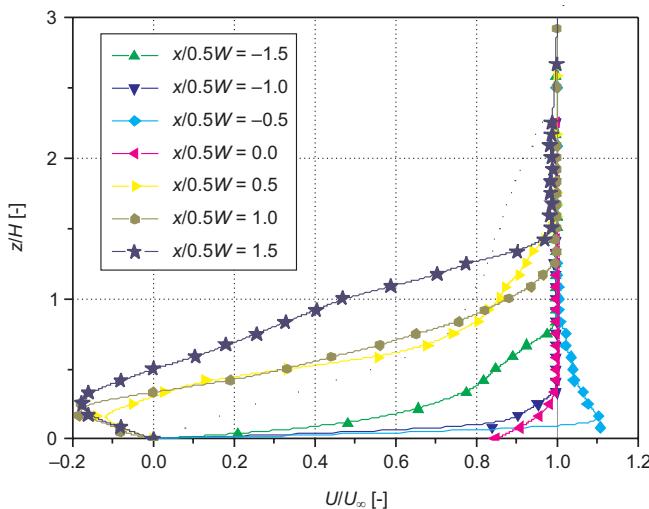


Fig. 3. Evolution of average velocity profile component in flow around hill

Important information for wind engineering is also the distribution of the turbulence intensity in the flow around object. Maximum turbulence intensity occurs at the surface of hill in top and recirculation zone. The value of the turbulence intensity here is several times higher than in the inflow region. It is worth noting the experimental studies confirmed that this high level of velocity fluctuation is maintained at a small range of values of z/H . Obtained during the measurement data allowed estimation of backflow zone, which extends to a distance close to $x/0.5W = 9$. This fact is confirmed by, among others, visualization results [1, 5].

The evolution of the concentration profile of carbon dioxide in a hilly environment for different locations and height source emissions is shown on Fig. 4–6. As shown in the aforementioned diagram, courses differ on a particular drawing both qualitatively and quantitatively, but generally speaking, the presence of the terrain obstacle, which modifies the shape of emitted in environment trail of pollution in a relevant way could be ascertained.

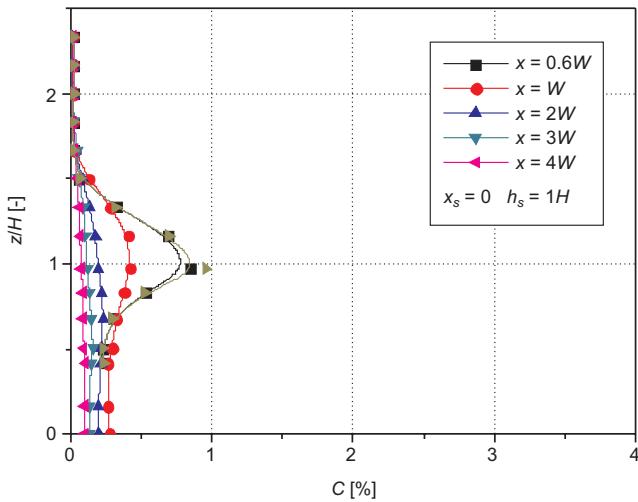


Fig. 4. Concentration profiles of carbon dioxide in surroundings of a hill for a source emission in a location (1)

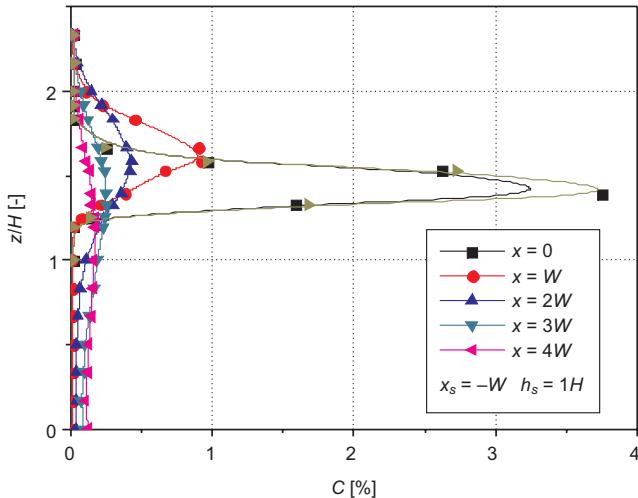


Fig. 5. Concentration profiles of carbon dioxide in surroundings of a hill from a source emission in a location (2)

This stands to features of velocity fields linked to an aerodynamic object in a fluid stream, but in a particularly closed environment in which recirculation zones rise from the zone of increased turbulence zone behind the hill.

For the dispersion process of substances emitted from the source responsible are both mass diffusion mechanism, caused by concentration gradients and advection, transported pollution in a flow direction with help of average air flow and a turbulent transport process in which their own part has own turbulent velocity fluctuations.

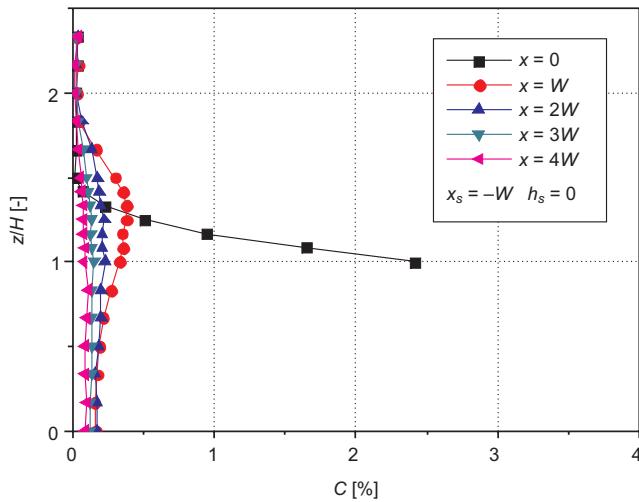


Fig. 6. Concentration profiles of carbon dioxide in surroundings of a hill for a source emission in a location (3)

This is confirmed by comparison the Concentration profiles of carbon dioxide measured on flat ground (without hills) – Fig. 7.

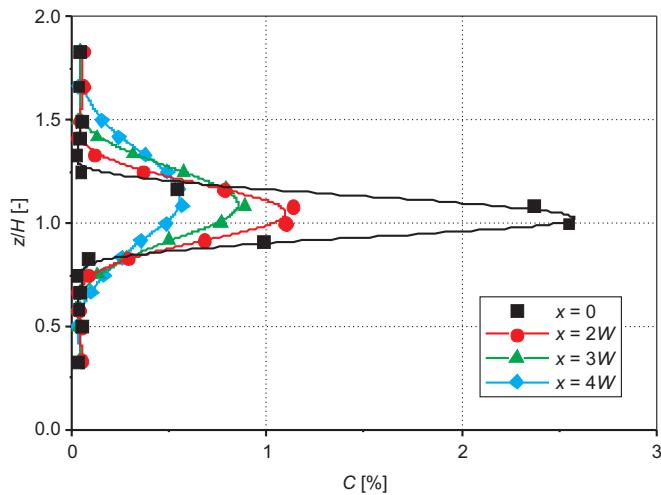


Fig. 7. Concentration profiles of carbon dioxide on flat ground for a source emission in a location (1)

The characteristic of analyzed concentration fields is the different localization of gaseous tab concentrations. The local maxima of the curve of concentrations move with the surrounding flow around an object according to the curves shown in Fig. 8 for two different locations of source emission.

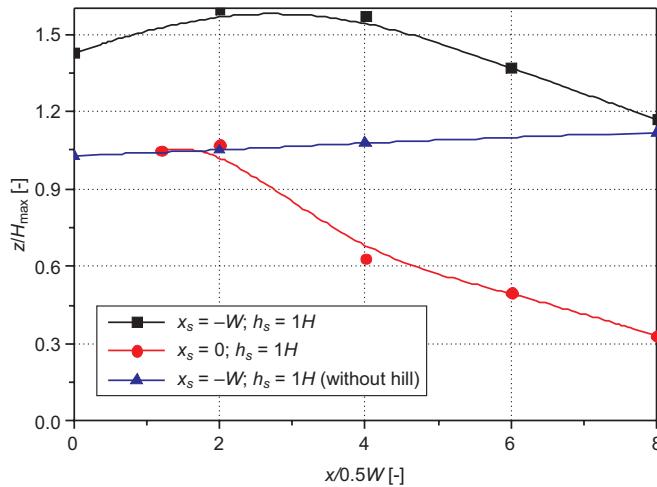


Fig. 8. The changes in concentration of CO_2 to ground along with wandering from the hill for different situated source emissions

For comparison, the drawing also contains data concerning the evolution of an axis of the tracer's plume's location floating in an undisturbed zone which is not affected by the obstacle presence. Appropriate maximum concentration values of carbon dioxide (C_{\max}) shown on a Fig. 9a. As is shown, the holding on the plume axis in every considered case varies. As the maximal values of distributions show qualitative similarity, they decreased with the distance from the source emission in every case, as long as the plume axis locations depend on a location at a predetermined distance from source location.

As an effect of deflection of the stream which is emitted from a source in a location, ($p2$) ($x_s = 0, h_s = 1H$) an increased value of CO_2 concentration at ground level is obtained (C_g), this is shown in drawing draw Fig. 9b. In that case of the location of source the stream of carbon dioxide is provided almost directly into a recirculation zone behind the hill, where dilution and dispersion appears.

The effect of that is mainly movement at ground level. In the case where the source was located on a height $h_s = 1H$ it was found in a distance W in front of the hill, then the dominant transport mechanism of emitted gas is advection, which causes the maxima of concentration to rise above the recirculation zone. At the same time the concentration of CO_2 is measured at ground level is practically equal to normal atmospheric values at close distances behind the hill. At long distances the influence of the situated source in terms of tab gas concentration at ground level disappears.

The last considered case is when a source which is located at the foot of a hill ($x_s = -W, h_s = 0$). In that case the location of source of emission, the flow CO_2 is directed upwards along the surface of the hill (maximal concentration profile measured on a top of a hill exists near hill surface), it then gets into the intensive mixing zone behind the hill, which effects are very flat concentration profiles measures in a forwards distances and big concentration values at ground level.

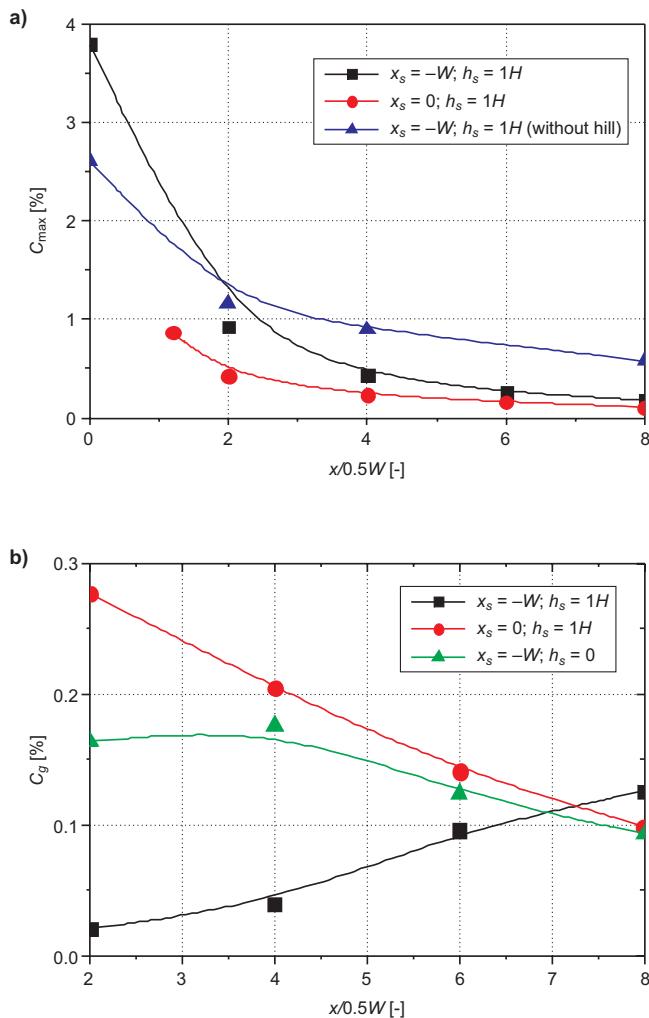


Fig. 9. a) Maximum concentration values above flat medium in a presence of a hill (source of emission in locations (1) and (2); b) Concentration values in a plume axis emitted over a flat medium and in a presence of the hill

Summary and conclusions

The dispersion of the CO₂ emitted from point sources located in a flow flush on element of oscillating flow and on its top performs in the different flow conditions, which are responsible for different character of CO₂ concentration in modeled wind field. The source location in relation to the recirculation zone and the area of increased fluctuated turbulence velocity flow level is critical.

In the article, the initial results of researcher which suggest the significant influence of oscillation component of the velocity on pollution dispersion is shown. It is also acknowledged here that this theme still requires further research.

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ANALIZA ROZPRZESTRZENIANIA SIĘ ZANIECZYSZCZEŃ GAZOWYCH NA TERENIE PAGÓRKOWATYM

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Abstrakt: Proces rozprzestrzeniania się zanieczyszczeń zachodzi w warunkach przepływowych kształtowanych obecnością i rozmieszczeniem różnorodnych obiektów naziemnych. W warunkach rzeczywistych proce-

sy te występują w otoczeniu złożonych układów przeszkoł terenowych, jednak przedmiotem zainteresowania wielu naukowców jest przepływ i dyspersja zanieczyszczeń w otoczeniu pojedynczych elementów. Badania tego typu służą lepszemu zrozumieniu przepływu i rozprzestrzeniania zanieczyszczeń w terenach o złożonej topografii. W niniejszej pracy analizowano wpływ złożonego charakteru pola prędkości, w tym składowej okresowej (wygenerowanej w wyniku niestacjonarnych podmuchów wiatrowych) oraz struktur wirowych generowanych w otoczeniu wzgórza na rozprzestrzenianie się zanieczyszczeń o charakterze gazowym. Na koncentrację znacznika gazowego emitowanego ze źródła usytuowanego w strefie napływu na faliste podłożo ma wpływ przede wszystkim położenie źródła względem strefy recyrkulacji oraz obszaru o podwyższonym poziomie turbulentnych fluktuacji przepływu.

Słowa kluczowe: rozprzestrzenianie się zanieczyszczeń, zanieczyszczenia gazowe, eksperimentalne i numeryczne metody modelowania, teren pagórkowaty

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TOXICOLOGICAL AND ECOTOXICOLOGICAL PROPERTIES OF AN IRON BASED COMBUSTION MODIFIER FOR LIQUID FUELS

WŁASNOŚCI TOKSYKOLOGICZNE I EKOTOKSYKOLOGICZNE MODYFIKATORA ŻELAZOWEGO SPALANIA PALIW CIEKŁYCH

Abstract: It is necessary to use specific modifiers in order to reduce harmful emissions arising in the combustion of liquid fuels into the atmosphere. Such modifiers include organic metal salts which are soluble in fuels and tend to form metal oxides under combustion process conditions, improving the oxidizing properties of fuels. The modifier, described in this paper was used in liquid fuel combustion tests, showing a desirable effect of reducing CO, NO_x and hydrocarbon emissions.

For such modifiers to be approved for use, examination of their physico-chemical, toxicological and ecotoxicological properties is required according to REACH Regulation. REACH is intended, first of all, to provide appropriate protection to the environment and human health, while striving to maintain competitiveness of European enterprises in the global market.

Discussed in this paper are the results of selected tests of the effect of an iron-based modifier for liquid fuels on human health and on the land and air and the aquatic environment. The modifier was subjected to physico-chemical analyses, and toxicological and ecotoxicological tests in accordance with good laboratory practice and OECD guidelines.

The test results indicate that the modifier is a safe substance, posing no hazard to human health or the environment.

Keywords: fuel modifiers, toxicology, ecotoxicology, combustion, liquid fuels

Introduction

Fuel combustion processes generate harmful emissions into the atmosphere. Specific additives are used commercially in the power industry to reduce the emissions. Such additives include modifiers based on organic metal salts, dispersed in organic solvents. Soluble without limitation in the combusted liquid fuels, they are added directly to the fuel. The combustion reaction environment generates metal oxides which catalyze the

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oxidation of fuel components. The advantages of the use of modifiers include: maintaining stable boiler performance, longer boiler operation and reduced costs of maintenance and repairs, more effective combustion due to reduced levels of hydrocarbons in flue gas, lower emission of harmful gases into the atmosphere (CO, NO_x, SO₂, dust, polycyclic aromatic hydrocarbons), and absence of carbon deposit in the combustion chamber, resulting in improved boiler efficiency [1].

The manufacturing and marketing of more than 100 Mg per year of the above mentioned modifiers is planned, therefore, it is necessary to evaluate the toxicological and ecotoxicological properties of the product according to REACH [2]. Manufacturers and importers of chemicals are required under REACH to provide registration documents comprising information about the chemical substance, specifically its toxicological and ecotoxicological properties [3].

The toxicological tests are intended to assess any undesirable or harmful effect of chemical substances or other factors on living organisms, and to perform a probability analysis for their occurrence in various exposure conditions [4, 5].

Ecotoxicological properties are established in examinations of organisms, populations, communities, biocenoses, and ecosystems in the aspect of their exposure to chemical factors, their penetration from the environment into the organisms, as well as any toxic effect that may occur [6].

Discussed in this paper are the results of selected toxicological and ecotoxicological tests of an iron-based modifier for liquid fuels. The findings will subsequently be indispensable in preparing a chemical safety assessment report. The necessary tests were selected on the basis of the planned production volume and data incorporated in REACH Annexes VII–IX [2]. Tests, enabling the assessment of risks to human health and the environment, were carried out in accordance with Good Laboratory Practices at the Institute of Industrial Organic Chemistry, Pszczyna, Poland. Toxicological and ecotoxicological tests were made in the second quarter of 2014.

Methodology

Assessment of environmental risks

Ecotoxicological tests were discussed on the example of respiration inhibition in activated sludge micro-organisms according to OECD Guideline No. 209 (Method C.11) [7, 8] and acute immobilization of *Daphnia magna* according to OECD Guideline No. 202 (EU Method C.2) [9, 10].

The respiration inhibition test according to OECD Guideline No. 209

The test was intended to assess the toxic effect of the iron-based modifier on micro-organisms in activated sludge. The test system was a mixture comprising water, a synthetic sewage feed, activated sludge, and a reference material solution.

Two sets of test samples were prepared in the initial experiment, one set comprising a nitrification inhibitor. A N-allylthiourea (ATU) solution at a concentration of 2.32 g/dm³ was used for inhibiting nitrification.

Every test system includes abiotic control (sample F_A), experimental control (samples F_{B1}–F_{B2}), test material control (samples F_{T1}–F_{T5}) and reference material control (samples F_{R1}–F_{R5}). Activated sludge from a biological waste-water treatment plant was used as a microbial inoculum. The respective amounts of the test material components are shown in Table 1.

Table 1
Amounts of components in the respective mixtures (test material: iron-based modifier)

Components of mixtures	Amounts in test vessels				
	F _{T1}	F _{T2}	F _{T3–5}	F _{B1–2}	F _A
Test material [mg]	5	50	500	0	500
Synthetic sewage feed [dm ³]	0.016	0.016	0.016	0.016	0.016
Activated sludge [dm ³]	0.25	0.25	0.25	0.25	0
Water [dm ³]	A volume of water was added to obtain a total of 0.5 dm ³ in every test vessel				
Total volume of mixture [dm ³]	0.5	0.5	0.5	0.5	0.5
Concentrations in the mixtures:					
of test material [mg/dm ³]	10	100	1000	0	1000
of activated sludge (suspended solids) [g/dm ³]	1.5	1.5	1.5	1.5	0

All mixtures were aerated intensely and incubated for three hours. The test material was used at the following concentrations: 10.0; 100.0; 1000.0 mg/dm³. The reference material was used at the following concentrations: 0.5; 5.0; 10.0; 20.0; 50.0 mg/dm³. Each sample was transferred into a BOD bottle after 3 hours and the concentration of oxygen was measured within 10 minutes using an oxygen electrode.

Oxygen consumption rate (R), as expressed in milligrams per liter per hour [mg/dm³ · hr], and specific respiration rate (R_S), as expressed by the amount of oxygen consumed by 1 gram of dry weight of the microbial activated sludge per hour [mg/g · hr], were calculated using the following formula (Equations 1–2):

$$R = (Q_1 - Q_2) / \Delta t \cdot 60 \quad (1)$$

where: Q_1 – is the oxygen concentration at the beginning of measurement [mg/dm³];
 Q_2 – is the oxygen concentration at the end of measurement [mg/dm³];
 Δt – is the duration of measurement.

$$R_S = R / SS \quad (2)$$

where: SS – suspended solids concentration, as found at the beginning of the experiment [g/dm³].

Based on the data obtained from Equations 1 and 2, the inhibitory effect of the test material on micro-organisms in the activated sludge was measured. This enables the calculation of the value of EC₅₀, defined as the median effective concentration which induces in the environmental sewage feed a respiration inhibition of 50 % [1].

$$I = \left[1 - \frac{(R - R_A)}{R_B} \right] \cdot 100 \% \quad (3)$$

where: I – is the percentage of respiration inhibition;

R – is the oxygen consumption by the test sample [mg/dm³ · hr];

R_A – is the oxygen consumption by the abiotic control [mg/dm³ · hr];

R_B – is the oxygen consumption by the experimental control [mg/dm³ · hr].

The acute immobilization of *Daphnia magna* according to OECD Guideline 202

It was the objective of the acute immobilization of *Daphnia magna* to determine, after 24 and 48 hrs of exposure: the concentration that causes immobilization of 50 % *Daphnia magna* (EC₅₀) and the values of LOEC and NOEC. The LOEC is understood as the minimum concentration of the toxicant (iron-based additive) that causes an observable effect on the test organisms within the prescribed time of testing. The NOEC is the highest concentration of the toxicant that causes no observable effect on the test organisms during the test [1].

This semi-static acute toxicity test was done on young daphnids (*Daphnia magna*) aged less than 24 hours at the start of the test. The organisms were exposed, for 48 hrs, to the effect of the test material (iron-based modifier) at the following concentrations: 0.09, 0.20, 0.43, 0.94, 2.06, 4.54 and 10.0 mg/dm³ and the control (0.0 mg/dm³). The concentrations were used in groups comprising five daphnids each, in four repetitions. The reference material, used for comparing the results, was potassium dichromate.

It was the objective of the test to observe immobilization of the test daphnids after 24 and 48 hours of exposure. The organisms which are not able to swim within 15 seconds after gentle agitation of the test vessel are considered to be immobilized.

Assessment of risks to human health

Toxicological tests are discussed according to OECD Guideline No. 405 (Method B.5) for the acute irritation/corrosion of the eye in rabbit [11, 12] and OECD Guideline No. 406 (Method B.6) for skin sensitization [13–16].

Test for the acute irritation/corrosion of the eye in rabbit according to OECD Guideline No. 405

It was the objective of the test to provide information about the potential health risks, caused by the impact of the iron-based modifier on the eye.

In the experiment, 0.1 cm³ of the test material (iron-based modifier) was applied into the conjunctival sac of one eye of a test animal; the other eye, which remained untreated, served as the control. The test was carried out in three animals to confirm the actual irritant effect or absence of irritation.

For the duration of the experiment, the animals were subjected daily to general clinical observation in respect of disease incidence and lethality. Detailed clinical observations for any lesions in the cornea, iris and conjunctiva were assessed after the lapse of 1, 24, 48 and 72 hours from the application of the test material.

The scoring of acute irritation/corrosion of the eye was defined using the grading of ocular lesions, as shown below (Table 2). The grading concerns lesions in the cornea, iris and conjunctiva.

Table 2

Grading of ocular lesions [6, 7]

Cornea (opacity: degree of density)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity, details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous area; no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris is not discernible through the opacity	4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate hyperaemia or injection; iris is reactive to light	1
Hemorrhage, gross destruction, or no reaction to light	2
Conjunctiva – redness (refers to palpebral or bulbar conjunctiva; excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic	1
Diffuse, crimson color; individual vessels not easily discernible	2
Diffuse, beefy red	3
Conjunctiva – swelling (refers to eye lids and/or nictitating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4

Skin sensitization test according to OECD Guideline No. 406

The skin sensitization test according to OECD Guideline No. 406 was started with a pilot test to establish the test substance concentrations for use in the principal test. In

step I of the principal test (induction exposure by intradermal injections) the selected test material concentration was 6 % and caused a moderate effect on the skin. In step II of the principal test (induction exposure by topical application) the selected test material concentration was 30 % and caused a moderate effect on the skin. In step III of the principal test (challenge exposure – topical application) the selected test material concentration was 0.2 % and caused no effect on the skin. In the principal experiment, 20 test animals (guinea pigs) and 8 control animals were used.

The principal test comprised three steps: induction exposure in two steps and challenge exposure. In step I of the principal test, the test animals were exposed by intradermal injection to a 6 % solution of the iron-based modifier dissolved in peanut oil with Freunds Complete Adjuvant (paraffin or mineral oil emulsion with tubercle bacilli suspended in it) [17, 18]. In step II of the principal test, 30 % of the iron-based modifier solution was applied on the skin in the intradermal injections region after being dissolved in peanut oil. In the control animals, peanut oil (medium) was applied instead of the test material.

The challenge exposure was performed by applying a 0.2 % iron-based modifier solution in peanut oil to the right flank of the test and control animals. Pure peanut oil (medium) was applied to the left flank. The skin reaction in the test and control animals was evaluated after 24, 48 and 72 hours from the end of the challenge exposure.

During the principal test, all the animals were subjected to general clinical observations. Detailed observations of the skin reactions were made after 24, 48 and 72 hours from the end of exposure. The animals were examined for any symptoms of erythema and swelling of the skin in the exposed region.

The challenge patch test reactions were evaluated according to OECD Guideline No. 406/Method B.6.:

- | | |
|-----------------------------------|---|
| – no visible change | 0 |
| – discrete or patchy erythema | 1 |
| – moderate and confluent erythema | 2 |
| – intense erythema and swelling | 3 |

Sensitization reaction was understood as a reaction of the skin observed in the animals 48 and/or 72 hours after the exposure, because the maximum intensity of the sensitization reaction is observed at that time. The changes observed after 24 hours only were considered as changes caused by irritation of the skin.

The intensity of sensitization was classified based on the percentage of animals which showed sensitization changes. That percentage was found from the following formula:

$$X = A / B \cdot 100 \% \quad (4)$$

where: X – percentage of sensitized animals;

A – number of animals in which sensitization changes were observed;

B – number of animals in the exposed group.

The following classification was adopted (Table 3):

Table 3

Classification of the intensity of sensitization according [19, 20]

% sensitized animals	Intensity of sensitization
> 0–8	very weak sensitization
9–28	weak sensitization
29–64	moderate sensitization
65–80	strong sensitization
81–100	very strong sensitization

Results

Assessment of environmental risks

The respiration inhibition test according to OECD Guideline No. 209

After the experiment, the following calculations were made for every sample: oxygen consumption (R), specific respiration rate (R_S) and percentage of respiration inhibition (I) in the activated sludge micro-organisms by the test material (iron-based modifier). The test results are shown in Table 4.

Table 4

Results of tests for various concentrations of the iron-based modifier

Concentration of test material	Oxygen consumption [mg/dm ³ · hr]	Specific respiration rate [mg/g · hr]	Percentage of respiration inhibition in micro-organisms from activated sludge [%]
Control	32.46	21.64	—
Test material concentration: 10 mg/dm ³	32.70	21.80	0.74
Test material concentration: 100 mg/dm ³	32.28	21.52	2.03
Test material concentration: 1000 mg/dm ³	31.94	21.29	3.08

It was established that the concentration of the iron-based modifier, causing 50 % percentage of respiration inhibition in micro-organisms from the activated sludge (EC₅₀) was higher than 1000 mg/dm³.

The acute immobilization of *Daphnia magna* according to OECD Guideline 202

Initially, immobilization of *Daphnia magna* was assessed after 24 and 48 hours of exposure. No immobilization was observed after 24 hours of exposure in the control and at the test material concentrations of 0.09; 0.20; 0.43 and 0.94 mg/dm³. At the test

substance concentrations of 2.06; 4.54; 10 mg/dm³, immobilization was 10, 5 and 45 %, respectively. No immobilization was observed after 48 hours of exposure in the control and at the test substance concentrations of 0.09 and 0.20 mg/dm³. At the test substance concentrations of 0.43; 0.94; 2.06; 4.54 and 10 mg/dm³, immobilization was 10, 85, 80, 95 and 100 %, respectively. The results of immobilization after 24 and 48 hours are shown in Fig. 1.

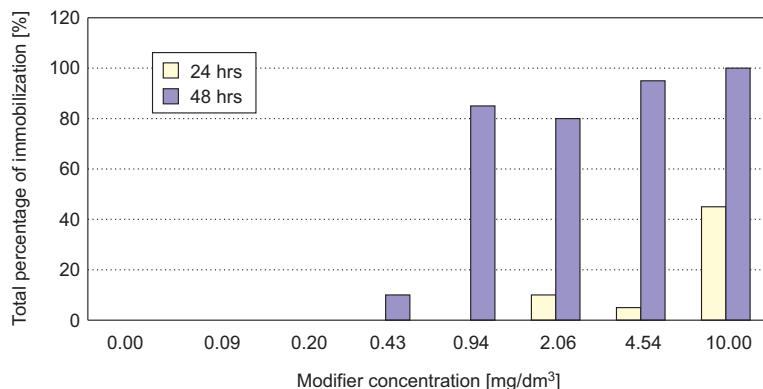


Fig. 1. Percentage of immobilization of *Daphnia magna* after 24 and 48 hrs of exposure to various concentrations of the iron-based modifier

In the second phase of the experiment, the ToxRat Professional 2.10 was used to calculate the mean effective concentrations, causing 100 % (EC_{100}), 50 % (EC_{50}), 20 % (EC_{20}), 10 % (EC_{10}) of immobilization, the highest concentration causing no immobilization, as well as the lowest concentration causing a statistically significant effect, as compared with the control (LOEC) and the highest test concentration at which no significant effect was observed in comparison with the control (NOEC) after 24 and 48 hrs of exposure. Final values based on nominal concentrations of the iron-based modifier and potassium dichromate (reference material) are shown in Table 5.

Table 5
Final values based on nominal concentrations of the iron-based modifier

Final values [mg/dm ³]	Time of exposure			
	Reference material		Iron-based modifier	
	24 hrs	48 hrs	24 hrs	48 hrs
EC_{50}	0.84	0.60	13.2	0.84
EC_{20}	0.69	0.55	5.38	0.44
EC_{10}	0.62	0.53	3.36	0.31
EC_0	0.32	0.32	0.94	0.20
EC_{100}	1.8	1.0	> 10.00	10.00
LOEC	1.00	1.00	10.00	0.94
NOEC	0.56	0.56	4.54	0.43

Assessment of risks to human health

Test for the acute irritation/corrosion of the eye in rabbit according to OECD Guideline No. 405

Ocular lesions were observed in the conjunctiva in rabbits after application of the test material (iron-based modifier), although such changes were not detected in the iris or cornea. Clinical observation 1 hr after the test substance application detected diffuse crimson redness in the conjunctiva in three rabbits, accompanied by congestion of the nictitating membrane and circumcorneal injection. Moreover, minor conjunctival swelling and swelling of the nictitating membrane was found in rabbits 2 and 3, while swelling of the nictitating membrane alone was observed in rabbit 1.

24 hours after the test substance application, rabbits 1 and 3 showed hyperaemia of some blood vessels and of the nictitating membrane, while rabbit 2 showed diffuse crimson redness, hyperaemia of the nictitating membrane and circumcorneal injection. In addition, swelling of the nictitating membrane was observed in rabbits 1 and 2.

Clinical observation 48 hours after the test substance application showed hyperaemia of some blood vessels and of the nictitating membrane in the conjunctiva in the three rabbits. 72 hours after the test substance application, no ocular lesions were found in the conjunctiva in rabbits 1 and 3 while only rabbit 2 continued to have hyperaemia of some of its blood vessels and of the nictitating membrane.

Table 6 shows grading of acute eye irritation/corrosion based on the grading scale referred to in Table 2, pursuant to OECD Guideline 405 (Method B.5). The effect of acute eye irritation/corrosion in rabbit was assessed based on average results observed after 24, 48 and 72 hours. Pursuant to the OECD Guideline, results observed 1 hour after the application of the iron-based modifier are omitted from such grading.

Grading of acute eye irritation/corrosion

Table 6

Rabbit	Eye part	After				Average results after 24, 48 and 72 hours
		1 hr	24 hrs	48 hrs	72 hrs	
1	Cornea	0	0	0	0	0.0
	Iris	0	0	0	0	0.0
	Conjunctiva – redness	2	1	1	0	0.7
	Conjunctiva – swelling	1	1	0	0	0.3
2	Cornea	0	0	0	0	0.0
	Iris	0	0	0	0	0.0
	Conjunctiva – redness	2	2	1	1	1,3
	Conjunctiva – swelling	1	1	0	0	0.3
3	Cornea	0	0	0	0	0.0
	Iris	0	0	0	0	0.0
	Conjunctiva – redness	2	1	1	0	0.7
	Conjunctiva – swelling	1	0	0	0	0.0

Skin sensitization test according to OECD Guideline No. 406

No sensitization skin reactions were observed in the exposed animals in the skin sensitization test (Table 7). No pathological changes were observed on the skin of the control animals.

Table 7

Skin sensitization test of the iron-based modifier

Designated parameter	Control group	Exposed group
Number of animals in the group	8	20
Number of animals subjected to final value assessment	8	20
Number of dead animals	0/8	0/20
Skin changes after challenge exposure in the region where the medium (peanut oil) was applied	no changes	no changes
Skin changes after challenge exposure in the region where the test material (iron-based modifier) was applied	no changes	no changes
Number of animals in which sensitization reaction was observed	0/8	0/20
% of sensitized animals	—	0 %

Using Equation 4, the intensity of skin sensitization of the iron-based modifier was then calculated; the result was 0 %.

The animals were examined for the occurrence of general clinical symptoms throughout the experiment and none were observed. After completion of the experiment, weight loss was observed in two animals in the exposed group whereas the other test or control animals were found to have gained on weight.

Analysis of the test results

Assessment of environmental risks

The respiration inhibition test according to OECD Guideline No. 209

The results of tests of the iron-based modifier indicate that, in experimental conditions in a test concentration range from 100 to 1000 mg/dm³, the test material shows an inhibitory effect on respiration of micro-organisms in activated sludge. The test material concentration for which 50 % respiration inhibition of micro-organisms in the activated sludge was observed (EC₅₀) is higher than 1000 mg/dm³.

The acute immobilization of *Daphnia magna* according to OECD Guideline 202

Based on the test results and nominal concentrations of the iron-based modifier, it was demonstrated that the mean concentration causing immobilization (inability to

swim) in 50 % animals after 48 hours of exposure was 0.84 mg/dm³. The value for the reference material was 0.6 mg/dm³.

The highest test concentration at which no significant effect, as compared with the control, was observed after 48 hours of exposure was 0.43 mg/dm³ (0.56 mg/dm³ for the reference material). The lowest concentration at which a significant effect, as compared with the control, was observed was 0.94 mg/dm³ (1.00 mg/dm³ for the reference material).

The test results for the reference material and for the test material (iron-based material) indicate sensitivity of *Daphnia magna* to the materials.

Assessment of risk to human health

Test for the acute irritation/corrosion of the eye in rabbit according to OECD Guideline No. 405

After application of the test material (iron-based modifier), no ocular lesions were detected in the cornea and iris while the conjunctiva of the test animals showed only temporary lesions. Average results after 24, 48 and 72 h for the conjunctiva in three rabbits were 0.9 for the redness and 0.2 for the swelling.

Based on the above results, it was found, pursuant to Annex to the Regulation of the Minister of Health of 10 August 2012 on the criteria and methods for the classification of chemical substances and mixtures, that the iron-based modifier for liquid fuels had no irritant effect on the eye in rabbit [21].

Moreover, pursuant to Regulation of the European Parliament and of the Council (CE) No. 1272/2008 of 16 December 2008 on the Classification, Labelling and Packaging of Substances and Mixtures (CLP), the iron-based modifier for liquid fuels is not categorized at all which means that it is not a hazardous substance and poses no risk to human health [22].

Skin sensitization test according to OECD Guideline No. 406

No sensitization changes were observed after application of the test material to the skin of the exposed animals. Based on the test results and classification of the intensity of sensitization according to Magnusson and Kligman, the iron-based modifier is classified as a material with no sensitizing effect.

Conclusions

This paper presents the results of toxicological and ecotoxicological tests based on four selected examples. The toxicological results are discussed using the example of the acute eye irritation/corrosion test in rabbit and the skin sensitization test. No ocular lesions were observed in the cornea or in the iris while only temporary lesions were detected in the conjunctiva. The skin sensitization test has shown that the iron-based modifier is safe to the human health and causes no sensitization effect.

Ecotoxicological tests have been discussed on the examples of the percentage of respiration inhibition in activated sludge and acute immobilization of *Daphnia magna*. The test results indicate that the modifier's concentration causing 50 % respiration inhibition in the activated sludge micro-organisms is higher than 1000 mg/dm³. As regards the test for acute immobilization of *Daphnia magna*, the mean concentration causing immobilization (inability to swim) in 50 % animals after 48 hours of exposure was found to be 0.84 mg/dm³. As a reference, for potassium dichromate, which is a known sensitizer in the case of *Daphnia magna*, the value was 0.6 mg/dm³. The animals are sensitive also to the test material (iron-based modifier), although the values are in the range reported in literature [23], therefore, the result is regarded as satisfactory.

Other results of toxicological and ecotoxicological tests indicate that the test iron-based modifier for liquid fuels is a safe substance, posing no risk to human health or the environment. Although not discussed in this paper, such tests are going to be used for preparing the product registration documents under REACH.

Based on the test results and pursuant to the Annex to the Regulation of the Minister of Health of 10 August 2012 on the criteria and methods for the classification of chemical substances and mixtures [21], the iron-based modifier for liquid fuels is found to be a safe substance, posing no risk to human health or to the land and air and the aquatic environment.

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WŁASNOŚCI TOKSYKOLOGICZNE I EKOTOKSYKOLOGICZNE MODYFIKATORA ŻELAZOWEGO SPALANIA PALIW CIEKŁYCH

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Abstrakt: W celu ograniczenia emisji szkodliwych produktów spalania paliw ciekłych do atmosfery występuje konieczność stosowania specyficznych modyfikatorów. Jednym z rodzajów są rozpuszczalne w paliwie organiczne sole metali, które w warunkach procesu spalania tworzą tlenki metali poprawiające własności utleniające paliw. Opisany modyfikator zastosowany został w testach spalania paliwa ciekłego, gdzie wykazał korzystny wpływ w ograniczeniu emisji węglowodorów, CO i NO_x.

Dopuszczenie do stosowania wyżej wymienionych modyfikatorów wymaga określenia ich własności fizykochemicznych, toksykologicznych i ekotoksykologicznych w ramach rozporządzenia REACH. Najważniejszym celem tego rozporządzenia jest zapewnienie właściwej ochrony zdrowia ludzkiego i środowiska, przy jednoczesnym dążeniu do zachowania konkurencyjności europejskich przedsiębiorstw na światowym rynku.

W pracy przedstawiono wyniki wybranych badań wpływu na zdrowie człowieka oraz na środowisko wodne, lądowe i powietrzne modyfikatora żelazowego do paliw ciekłych. Badania fizykochemiczne, toksykologiczne i ekotoksykologiczne tego dodatku wykonane zostały zgodnie z dobrą praktyką laboratoryjną oraz wytycznymi OECD.

Na podstawie uzyskanych wyników badań stwierdzono, iż badany modyfikator jest substancją bezpieczną i niestanowiącą zagrożenia dla zdrowia człowieka oraz środowiska.

Słowa kluczowe: modyfikatory paliw, toksykologia, ekotoksykologia, spalanie, paliwa ciekłe

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ASSESSMENT OF THE MICROBIOLOGICAL CONTAMINATION OF AIR IN A MUNICIPAL SOLID WASTE TREATMENT COMPANY

OCENA ZANIECZYSZCZENIA MIKROBIOLOGICZNEGO POWIETRZA NA TERENIE ZAKŁADU PRZETWARZANIA ODPADÓW KOMUNALNYCH

Abstract: The subject of the study is the analysis of the number of microorganisms forming a microbiological aerosol in a municipal solid waste treatment plant in Krakow. The storage time of mixed municipal solid waste in the plant hall is 6–48 hours and the storage time of the produced alternative fuel is within the range of 12–96 hours. 18 employees work in the three shift system in the sorting facility. The air for research was sampled using a MAS-100 impactor (Merck, Switzerland) in three locations within the plant, four times within a year to assess the effect of meteorological conditions (temperature, humidity and dustiness) on the number of selected groups of microorganisms. It was found that the number of microorganisms changes with seasons and depends on the meteorological conditions as well as the air sampling location. Since the border values of bioaerosol concentration were exceeded, further research is required to assess the changes in the number of microorganisms with potential negative impact on human health.

Keywords: microbiological aerosol, air, municipal solid waste

Waste management is a civilizational problem. The human residence and business activity results in the creation of waste [1]. Due to the possibility of penalties incurred by Poland for large scale storage of biodegradable waste, mechanical, biological and thermal treatment and processing of municipal waste in special installations has become more popular in recent years. Bis at al [1] as well as Przybulewska at al [2] claim that the direct surroundings of this type of facilities may be contaminated microbiologically, with bacteria, viruses and fungi. This is the reason why every environmentally noxious

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facility of this type should be monitored, provided with a safety zone and recultivated after it is no longer in use. The problem of the microbiological contamination of air within and in proximity of waste storage and treatment facilities is growing [3]. More attention is given to the hygiene and sanitary work conditions for persons employed in waste segregation and treatment. Sufficient insulation of staff and office facilities from the working area in order to minimise the propagation of organic dust containing potentially pathogenic microorganisms is an important issue in waste sorting facilities. Unfortunately, there is little testing provided in Poland in the area of the microbiological analysis of air inhaled by the employees of such facilities. Due to occupational safety and health regulations, the dust content levels and noise emission levels are monitored in waste treatment plants. Analyses are also provided regarding the exposure to harmful factors, including microbiological aerosol in work areas. Possible health effects are also monitored, since work in the waste treatment industry involves the risk of allergies, upper respiratory tract infections, skin and mucous membranes conditions as well as immunotoxic and infectious diseases [4–9]. The degree of microbiological contamination of air in waste management facilities varies and depends on multiple parameters, such as: number of employees, room construction and ventilation, storage conditions, operation system, air temperature, humidity and dust content level [3, 10, 11]. This is the reason why, due to sanitary reasons, observing safe work principles should be promoted among employees [10]. Employees of waste management facilities are under the risk of exposure to aerosol-forming microbiological factors, such as: viruses, prions, bacteria, fungi, biological allergens and endotoxins [12–14]. Krajewski et al [15] studied the number and species composition of microorganisms present in the breathing areas available to employees working with the collection and treatment of municipal solid waste. Air samples were collected in selected work areas, *i.e.* waste collection point, reloading, sorting facilities and composting plant. In all analysed locations, the number of mesophilic bacteria and filamentous fungi exceeded $10^4 \text{ cfu} \cdot \text{m}^{-3}$ of air, an increased number of actinobacteria was found. A number of microorganisms potentially dangerous to human health was found, such as: *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium* as well as *Pseudomonas* bacteria.

Despite the fact that the air is not a natural habitat for microorganisms, as it does not favour their growth and divisions, it may form a temporary environment for them and enables their long distance transport. The time for which the microorganisms can survive in these conditions depends on their resistance to drying and availability of nutrients. They can be transported for long distances from their original habitat with wind [16]. Microorganisms are most frequently found in air in the form of endospores, spores, mycelium fragments as well as vegetative forms (bacteria, viruses), while still maintaining their potential harmfulness to the health of humans, animals and plants [10].

This is the reason for the assessment of the microbiological contamination of air in selected locations of a municipal solid waste treatment company in Krakow. Furthermore, the purpose of the analyses completed four times within a year was the determination of the impact of seasonal changes, including temperature, humidity and dust content to the number of the selected groups of microorganisms.

Materials and methods

Microbiological testing of air was completed using the MAS-100 impactor (Merck, Switzerland), in compliance with the guidelines of Polish Standards: PN-Z-04008-08, PN-89/Z-04111/02 and PN-89/Z-04111/03 [17–19]. The analysis consisted of collecting air samples in three locations within MIKI Recykling Sp. z o.o. in Krakow – a company producing alternative fuel from municipal waste and other types of waste. The air was sampled in two locations within the hall used for storage of waste intended for processing (point A) and alternative fuel – processing remnants (point B) as well as in one location outside the hall (point C). The waste sorting hall has 3 entry gates (Fig. 1), gravitational and forced ventilation systems. Inside the production hall, there are 13 belt conveyors and 5 machines marked on Fig. 1 with the following numbers:

- 1 – two preliminary shredders with magnetic separators,
- 2 – drum sieve (downstream of a magnetic separator),
- 3 – air separator (under the belt conveyor),
- 4 – final shredder,
- 5 – sorting chamber for recycling material separation from municipal waste.

Samples were collected using microbiological growth media for: bacteria (bacteriological agar, BTL), fungi (glucose-potato agar PDA, BTL), actinobacteria (Pochon agar, BTL) and staphylococci (Chapman agar, BTL), *Escherichia coli* (Endo agar, TBX agar, BTL), *Salmonella* spp. and *Shigella* spp. (SS agar, BTL), *Enterococcus faecalis* (Slanetz Bartley growth medium, BTL), *Pseudomonas fluorescens* (King B agar, BTL). Air temperature and humidity were also measured (HT-9213 thermohygrometer, ATM,

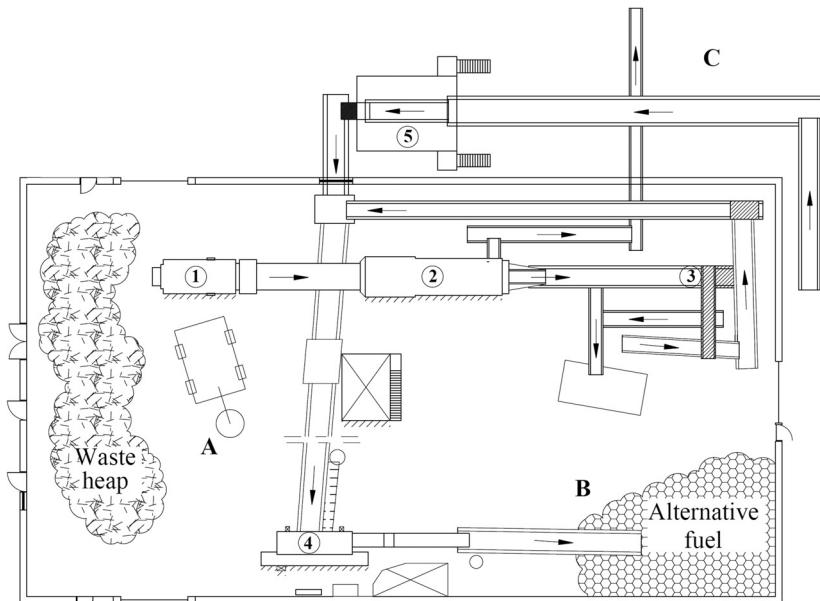


Fig. 1. Simplified diagram of the MIKI Recykling Sp. z o.o. alternative fuel production installation and microbiological analysis sampling points (A, B, C)

China). Wind speed was determined using the information retrieved from the Internet weather archive [20], while the atmospheric pressure and suspended dust content (PM10 and PM2.5) were determined using the data provided by the Environment Protection Inspectorate in Krakow (PIOS) [21]. Samples were collected in three instances, four times a year (April, July, September and December of 2014) in order to account for the seasonal changes. Sample collection lasted for 1 minute, the amount of airflow through the impactor was 100 litres, the device was placed 1.5 m over ground level. Immediately after collection, the dishes were transported into the laboratory and placed in thermostats with temperatures appropriate for the growth of specific microorganisms (bacteria 37 °C for 24–48 h; fungi 28 °C for 3–5 days and actinobacteria 28 °C for 5–7 days). After the incubation period, the grown colonies were counted and the collected isolates were identified using diagnostic keys [22–25]. The results were presented as cfu (colony forming units) in m³ of collected air (cfu · m⁻³).

A statistical analysis was made in order to calculate the average number of microorganisms in the tested air. A variance analysis (ANOVA) was performed in order to verify the significance of temporal and spatial differences in bioaerosol concentrations. The statistical analysis was performed using the Statistica v. 10 software (StatSoft).

Results and discussion

The results of the analysis of the amount of microbiological aerosol within the municipal waste processing and treatment company from April to December 2014 are presented in Tables 1–5 below and in Fig. 2. Explanations: A, B – waste storage and processing hall, C – open area, outside the hall; * mean air contamination, ** heavy air contamination according to Polish Standards [17, 18].

Table 1
Mean number of bacteria [cfu · m⁻³] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	290	1980*	2890*	980
B	342	2210*	2920*	1050*
C	120	280	303	350

Table 2
Mean number of fungi [cfu · m⁻³] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	283	1013	2590	3520*
B	733	1316	2970	2350
C	996	823	943	720

Table 3

Mean number of actinobacteria [cfu · m⁻³] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	26*	593**	493**	150**
B	23*	390**	593**	110**
C	16*	43*	76*	30*

Table 4

Mean number of staphylococci [cfu · m⁻³] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	66	516	2890	2120
B	46	390	2760	550
C	10	86	150	290

Table 5

Mean number of *E. faecalis* [cfu · m⁻³] in selected locations

Sampling point	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
A	50	126	83	200
B	43	146	113	110
C	0	0	0	0

The number of bacteria exceeded the values recommended by the Polish Standard [17] in points A and B in July and September and in point B in December. Fungi were abundant in the analysed air and in December, their number in point A exceeded the values recommended by the Polish Standard [18]. The number of actinobacteria deserves special attention. The concentration of actinobacteria in analysed air exceeded the limit values in all measurement points in every season of the year. Based on the determined number of actinobacteria, the air was classified as averagely contaminated (spring – points A, B and C; summer, autumn and winter – point C) or heavily contaminated (summer, autumn and winter – points A and B) [17]. The presence of staphylococci in analysed air is concerning – the results from autumn show 2890 cfu · m⁻³ in point A. Furthermore, the presence of faecal bacteria *E. faecalis* in the analysed air was found in all seasons in points A and B. The samples did not show the presence of *E. coli* and *P. fluorescens* bacteria or *Salmonella* and *Shigella* bacteria. Furthermore, the highest concentration of microorganisms was found in the waste storage and processing hall (points A and B). The air samples from point C (located outdoors, outside the hall) show significantly smaller bioaerosol concentrations. Only in

the case of fungi, during the spring collection, their number was significantly smaller in point A, compared to point C.

Table 6 below presents selected weather parameters, *ie* wind speed, atmospheric pressure, humidity, suspended dust levels and air temperature during sample collection.

Table 6
Meteorological conditions during sample collection

Parameter \ Sampling time	Spring 18.04.14	Summer 21.07.14	Autumn 9.09.14	Winter 15.12.14
Wind speed [km/h]	4.75	8.25	3	8
Atmospheric pressure [hPa]	987	987	993	994
Humidity [%]	61	58	77	82
Suspended dust PM2.5 [$\mu\text{g} \cdot \text{m}^{-3}$]	31*	13	18	116*
Suspended dust PM10 [$\mu\text{g} \cdot \text{m}^{-3}$]	47	23	28	166*
Temperature [$^{\circ}\text{C}$] in point A	17.5	25.5	22.4	2.3
Temperature [$^{\circ}\text{C}$] in point B	17.3	25.7	23.7	2.5
Temperature [$^{\circ}\text{C}$] in point C	18.1	26.7	21.8	2.1

* Value exceeds the limit specified in the Regulation of the Minister of Environment of 24 August 2012 on the levels of selected substances in air (Dz.U. of 2012, item 1031).

Air humidity and temperature are important factors for the seasonal changes in microorganisms concentrations. It is to be noted that air humidity was high during sample collections (58–82 %). Average temperature for the three collection points differed depending on the season from 2.3 °C to 26 °C. The obtained results validate the proposition of a correlation between the season of the year and the presence of microbiological aerosol. The concentration of the bioaerosol was at its lowest point in spring and grew to its maximum value in autumn. During the winter, the number of most microorganisms, apart from the fungi and *E. faecalis*, declined again (Fig. 2).

Suspended dust levels were compared to the limit values specified in the Regulation of the Minister of Environment of 24 August 2012 on the levels of selected substances

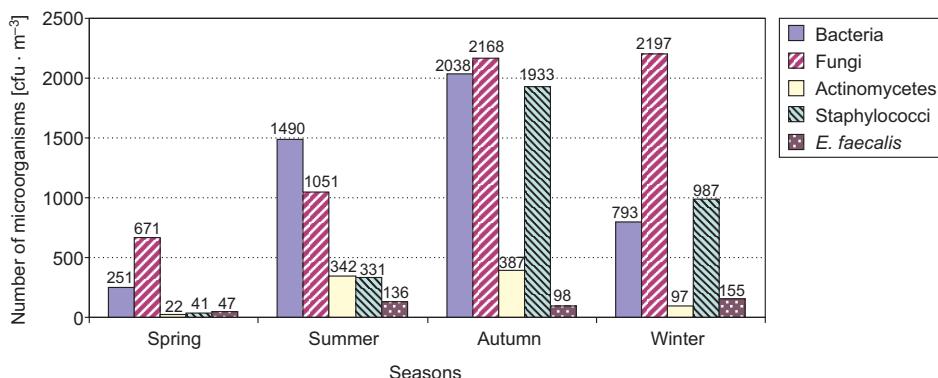


Fig. 2. Seasonal changes in bioaerosol concentrations – mean values from all analysed locations

in air [26]. It was found that the limits for PM10 ($50 \mu\text{g} \cdot \text{m}^{-3}$), and PM2.5 ($25 \mu\text{g} \cdot \text{m}^{-3}$) were exceeded in winter and for PM2.5 in spring. It was noted that the number of microorganisms as well as the concentration of suspended dust in the air were very high in winter. Fraczek and Grzyb [27] suspect a correlation between the presence of suspended dusts and microbiological contamination of air.

Own research shows that the highest concentration of fungi can be observed in December. This tendency looks very different in the studies by [28–31] they claim that the highest concentrations of fungi in atmospheric air can be found in late summer and early autumn, while in winter, their number declines. The study found high concentration of fungi in air in autumn and the coming of winter did not reduce their number. It is to be noted, however, that air temperatures in December were above 0 °C, no recorded frosts. Fraczek and Grzyb [27] analysed the propagation of the fungi aerosol in Krakow and found a significant increase in winter. Similar results were presented by Lin and Li [32], who found a correlation between the concentration of fungi spores in the air and meteorological conditions. In summer, the number of fungi decreased with higher temperatures and in winter increased with higher temperatures.

Aspergillus, *Penicillium*, *Mucor*, *Cladosporium*, *Rhizopus* and *Alternaria* were dominant among the fungi found in the samples. Apart from the previously mentioned pathogenic bacteria, *Bacillus* spp. and *Streptobacillus* spp. spores as well as *Micrococcus* spp., *Diplococcus* spp., *Sarcina* spp. and *Streptococcus* spp. Among the actinobacteria the presence of *Streptomyces* was found. The identified microorganisms form a typical microflora of air, only the presence of toxin-forming fungi, i.e. *Aspergillus*, *Penicillium* and *Cladosporium*, may be harmful to the health of the employees, since they produce mycotoxins and allergic reactions [33].

Based on the statistical analysis of the results, it was found that the differences of bioaerosol concentrations between the air sampling locations are statistically significant (Table 7). The differences in the number of found microorganisms during the year are statistically significant in the case of fungi, actinobacteria and staphylococci (Table 7).

Table 7

Variance analysis results for the temporal and spatial differences in bioaerosol concentrations

Microorganism	F factor value (collection point)	F factor value (season of the year)
Bacteria	20*	1.8
Fungi	4.4*	8.7*
Actinobacteria	6.8*	9.9*
Staphylococci	5.4*	9.8*
<i>E. faecalis</i>	28*	2.3

* Values are significant with $p < 0.05$.

Conclusions

1. The number of microorganisms found in the microbiological aerosol depends on the season of the year, meteorological conditions and air sampling location.

2. The most contaminated air (in terms of number of microorganisms) was found in the two points inside the waste storage and processing hall.

3. At some points in time and in some locations, the air may negatively impact the human health, because the limit values for the concentrations of bacteria, fungi and actinobacteria were exceeded.

4. The presence of pathogenic bacteria, *ie E. faecalis, Staphylococcus* spp., and toxin-forming fungi was found in collected air samples.

5. It is necessary to continue the research of air in order to assess the changes of microbiological aerosol amounts with potential detrimental effects to human health.

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OCENA ZANIECZYSZCZENIA MIKROBIOLOGICZNEGO POWIETRZA NA TERENIE ZAKŁADU PRZETWARZANIA ODPADÓW KOMUNALNYCH

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Abstrakt: Celem pracy była analiza liczebności drobnoustrojów stanowiących biaerozol mikrobiologiczny na terenie zakładu zajmującego się przetwarzaniem odpadów komunalnych w Krakowie. Czas magazynowania zmieszanych odpadów komunalnych w hali zakładu wynosi 6–48 godzin, natomiast czas magazynowania wytworzonego z nich paliwa alternatywnego zawiera się w przedziale 12–96 godzin. W sortowni pracuje 18 osób na 3 zmianach. Powietrze do badań pobierano z użyciem impaktora MAS-100 (Merck, Szwajcaria) w trzech punktach zlokalizowanych na terenie przedsiębiorstwa, cztery razy w roku, aby ocenić wpływ warunków meteorologicznych, tj. temperatura, wilgotność oraz zapylenie na liczebność wybranych grup drobnoustrojów. Stwierdzono, że liczebność badanych mikroorganizmów podlega zmianom sezonowym i jest uzależniona od warunków meteorologicznych oraz punktu poboru powietrza. Ponieważ wartości graniczne dotyczące stężenia bioaerozolu zostały przekroczone, konieczne jest prowadzenie dalszych badań, których celem będzie ocena zmian liczebności drobnoustrojów mogących negatywnie oddziaływać na zdrowie ludzi.

Słowa kluczowe: aerozol mikrobiologiczny, powietrze, odpady komunalne

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THE ABILITY OF SELECTED BACTERIA TO GROW IN THE PRESENCE OF GLYPHOSATE

ZDOLNOŚĆ WYBRANYCH BAKTERII DO WZROSTU W OBECNOŚCI GLIFOZATU

Abstract: Glyphosate is an active substance in the Roundup herbicide. The key process affecting its decomposition in the soil is microbial biodegradation. Bacteria that are able to break the C-P bond use this substance as a source of phosphorus. The aim of the study was to investigate the ability of thirty strains of soil bacteria to grow in the presence of glyphosate which was the sole source of phosphorus. Morphologically and physiologically varied soil bacteria strains were the subject of the study. Their ability to grow in the presence of glyphosate being the only phosphorus source was examined using a modified Dworkin-Foster growth medium. The modification itself consisted in introducing to the medium $0.5 \text{ mM} \cdot \text{dm}^{-3}$ of glyphosate which was to serve as an alternative source of phosphorus. The control sample in the study was the bacterial growth in two Dworkin-Foster growth media: a complete one (unmodified) and a phosphorus-free one. The growth intensity of the analyzed strains was assessed by means of spectrophotometry ($\lambda = 490 \text{ nm}$). Substantial differences in the growth intensity of the analyzed bacterial strains were observed in the presence of glyphosate, which was the sole source of phosphorus. Only eight out of the analyzed strains showed growth similar to what was observed in the case of the unmodified Dworkin-Foster medium, whereas all the remaining ones grew at a much slower rate.

Keywords: indigenous soil bacteria, glyphosate, growth kinetics

Introduction

Plant protection products, and especially herbicides, are commonly used in modern agriculture. Herbicides compounds are highly toxic to living organisms and pose a potential threat to humans, animals and the environment [1, 2].

In spite of close supervision in the use of pesticides there is a serious risk that these agents are able to spread into the environment and contaminate water, soil, food, and feedstuffs. Recently, more and more studies have been focused on understanding the toxic mechanisms of herbicides actions.

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One of the most popular and commonly used herbicides that eradicate unwanted weed is Roundup introduced to agriculture in 1971 by a company called Monsanto. The herbicidal properties consist in inhibiting the biosynthesis of aromatic amino acids whereby the shikimic acid pathway in plants is blocked [3]. The inhibitor of 5-enol-pyruvylshikimate-3-phosphate synthase (one of the enzymes of shikimic acid) occurring in plants and microorganisms is glyphosate (N-(phosphonomethyl)glycine) [4].

Glyphosate, the active substance in the Roundup herbicide, belongs to organo-phosphorus compounds that contain in their structure a strong C-P bond that cannot be easily broken by physicochemical actions. Roundup is adsorbed and retained in the sorption complex soil and this affects the development of the environmental conditions of the soil which determines the development and activity of microorganisms. The biodegradation of glyphosate takes place mainly due to specialized strains of fungi and bacteria having the ability to break C-P bonds and use the resulting products mostly as a source of phosphorus, and, to a lesser extent, as sources of nitrogen and carbon [5–9]. The following genera of bacteria are known to possess the ability to break organo-phosphorus compounds: *Escherichia*, *Klebsiella*, *Shigella*, *Bacillus*, *Enterobacter*, *Pseudomonas Serratia*, *Pseudomonas Arthrobacter*, *Rhizobium* or *Streptomyces* [5–8, 10, 11]. Only a few strains have ability to use glyphosate as the sole source of nitrogen [6–9, 11, 12].

Degradation of N-(phosphonomethyl)glycine (PMG) proceeds primarily involving specialized bacterial and fungal strains [8, 9, 13]. This ability results directly from the activity of adaptive and constitutive enzymes whose synthesis is induced in the presence of a xenobiotic. As a result of glyphosate biodegradation, sarcosine may be produced due to the activity of C-P – lyase or aminomethylphosphonic acid (AMPA), which is the product of glyphosate oxidoreductase (GOX) [14]. Rueppel et al [15] and Araujo et al [16] proved that AMPA could be either accumulated or biodegraded in the soil, albeit at a slower rate than glyphosate.

The time it takes glyphosate to decompose in the soil varies from several days to several years, depending on the physicochemical properties of the soil and the number of microbes. Depending on the strain, the C-P bonds may be broken into inorganic phosphorus and acetaldehyde [17] or methane [18] as the final products.

The specifics of glyphosate decomposition, as carried out by microbes and enzymes, are peculiar as it depends on the strain properties of the autochthonous microflora. In the case of microorganisms relying on the activity of glyphosate oxidoreductase (GOX), glyphosate-tolerance genes were detected, which allowed one to create Roundup Ready Crops (genetically engineered crops) [19].

The aim of the study was to investigate the ability of thirty strains of soil bacteria to grow in the presence of glyphosate, which was the sole source of phosphorus.

Materials and methods

The subject of the analysis was thirty bacterial strains isolated from the soil by means of microbial culture on Dworkin-Foster growth medium [20]. The study was conducted in July, after the process of rapeseed desiccation.

The predominant and macroscopically varied bacterial colonies underwent biochemical identification with the use of the API system: API 20 NE and ID32GN – Gram-negative bacilli, API 50 CHB – Gram-positive bacilli and ID32 STAPH – Gram-positive cocci.

The growth kinetics of the analyzed strains was examined in 250 cm³ Erlenmeyer flasks containing 100 cm³ of Dworkin-Foster (D) culture medium with 0.5 mM · dm⁻³ of glyphosate (PMG) as the only source of phosphorus (D-P + PMG).

Glyphosate, (N-phosphonomethylglycine) used in this study was obtained from commercial formulation Roundup® (Monsanto).

The cultures were injected with inoculum of density $\zeta = 2$ at wavelength $\lambda = 460$ nm and incubated for 72 hours at a temperature of 25 °C. The relative control sample was the complete (unmodified) Dworkin-Foster (D) culture medium on which the strains grew. The absolute control sample was the phosphorus-free Dworkin-Foster culture medium (D-P).

The growth intensity of the analyzed strains was assessed on the basis of the extent to which the cultures became turbid after 4, 8, 24, 48 and 72 hours of incubation with the use of spectrometry ($\lambda = 490$ nm).

Results and discussion

After the process of rapeseed desiccation with the Roundup herbicide, the most frequently isolated bacterial genera from the soil were: *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Corynebacterium*, *Micrococcus*, *Proteus* and *Sarcina* (Table 1).

Table 1
The bacterial strains isolated from the soil exposed to the Roundup herbicide
on the complete Dworkin-Foster culture medium

Gram-positive	Gram-negative	
Bacilli	Rods	
<i>Bacillus subtilis</i> DII 2	<i>Pseudomonas Ps13</i>	<i>Alcalibacter denitrificans</i>
<i>Bacillus cereus</i> DII 4	<i>Pseudomonas fluorescens</i>	<i>Arthrobacter atrocyaneus</i>
<i>Bacillus</i> DII 5	<i>Pseudomonas stutzeri</i>	<i>Proteus mirabilis</i>
<i>Bacillus</i> DI 3	<i>Pseudomonas putida</i>	<i>Serratia odorifera</i>
<i>Bacillus</i> DIII	<i>Commomonas testosteroni</i>	<i>Serratia fonticola</i>
Coccus	<i>Sphingomonas paucimobilis</i>	<i>Entrobacter agglomerans</i>
	<i>Burkholderia cepacia</i>	<i>Entrobacter aerogenes</i>
<i>Micrococcus lyliae</i>	<i>Chryseomonas luteola</i>	<i>Ochrobactrum anthropi</i>
<i>Staphylococcus warneri</i>	<i>Stenotrophomonas maltophilia</i>	<i>Aeromonas caviae</i>
<i>Staphylococcus xylosus</i>	<i>Flavobacterium sp.</i>	<i>Ralstonia pickettii</i>
<i>Sarcina</i> sp.	<i>Acinetobacter baumani/ Iwoffii</i>	<i>Moraxella spp.</i>

Clear differences in the growth intensity of the analyzed strains were observed in the presence of glyphosate as the sole source of phosphorus. In the case of twenty-two analyzed strains, growth intensity comparable to the absolute control sample was

observed, *i.e.* similar to the growth in the phosphorus-free culture medium. None of the strains of *Bacillus* isolated from this environment were able to grow in the presence of glyphosate, albeit in many studies it was proven that these bacteria could use glyphosate as the source of both phosphorus and nitrogen [21].

However, the growth of the remaining eight bacterial strains differed in terms of the adaptation period and growth tendencies. The length of lag phase growth by bacteria strains was varied according to the species.

Out of these eight strains five showed growth that was inductive in nature, this having been proven by the presence of the adaptation phase. An eight-hour adaptation phase was present in the case of *Staphylococcus warneri*, *Proteus mirabilis* and *Serratia odorifera* (Fig. 1–3), whereas a twenty-four-hour phase in the case of *Ralstonia pickettii*

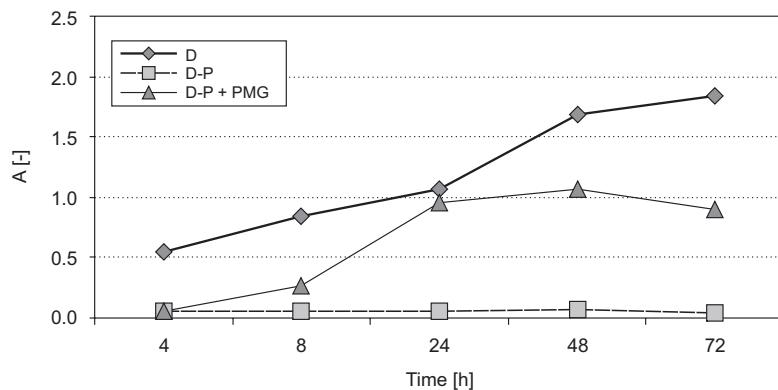


Fig. 1. Growth kinetics of *Staphylococcus warneri* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

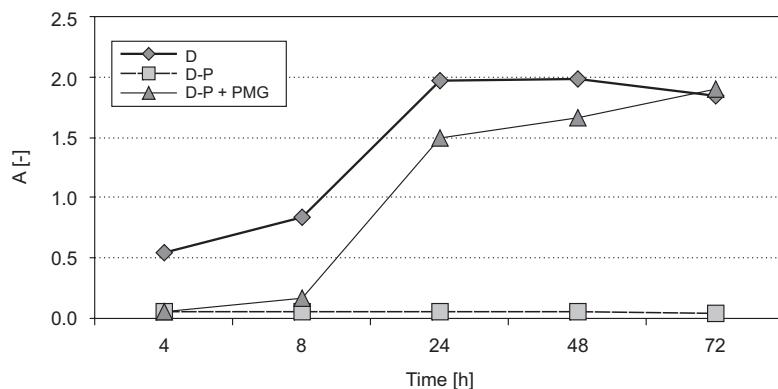


Fig. 2. Growth kinetics of *Proteus mirabilis* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

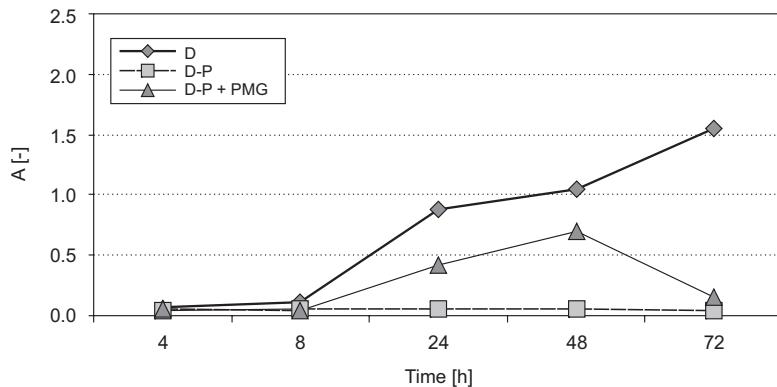


Fig. 3. Growth kinetics of *Serratia odorifera* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

(Fig. 4). Differences between bacteria being able to use glyphosate were also observed by Dick and Quin as well as Araujo et al [16, 22].

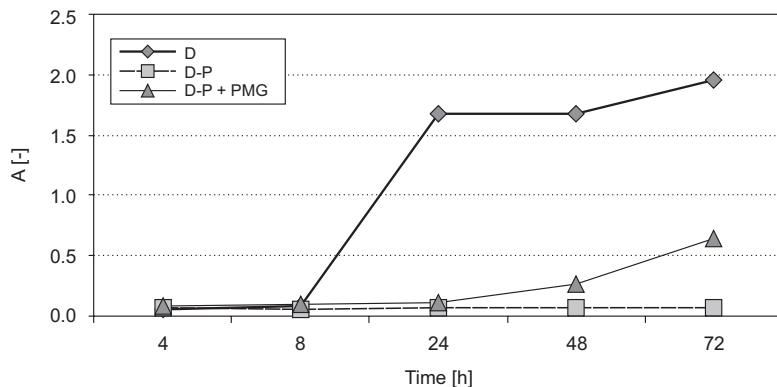


Fig. 4. Growth kinetics of *Ralstonia picketii* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

In contrast, in the case of *Sphingomonas paucimobilis* and *Pseudomonas fluorescens* strains, an intensive growth was observed without the adaptation phase (Fig. 5 and 6) which indicated that these Gram-negative bacilli were able to directly break the C-P bond. Other researchers, eg Gimsing, had obtained similar results [23].

The majority of the microbial strains isolated from the soil, under laboratory conditions, were able to use glyphosate as the source of phosphorus by breaking the C-P bond with the use of the C-P – lyase [14]. Gimsing et al [23] claim that the Gram-positive bacteria conduct a biological decomposition of glyphosate by breaking

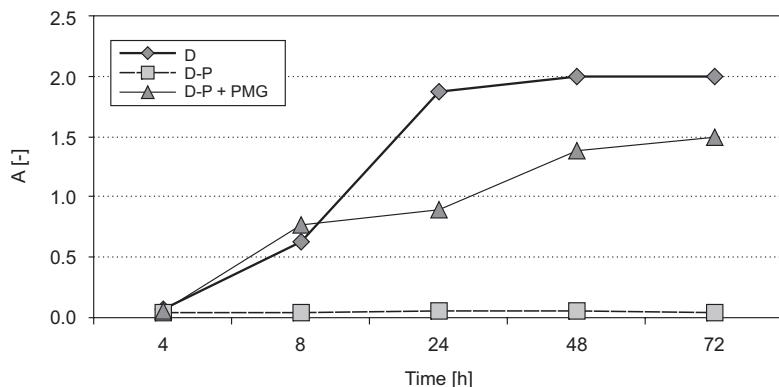


Fig. 5. Growth kinetics of *Sphingomonas paucimobilis* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

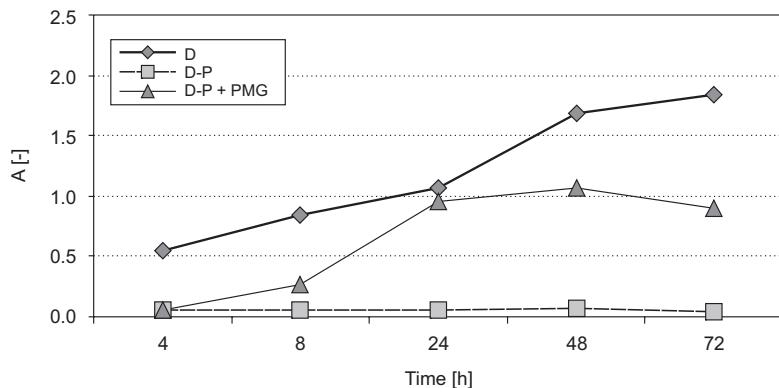


Fig. 6. Growth kinetics of *Pseudomonas fluorescens* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

away phosphorus from the organophosphorus compounds, whereas the Gram-negative ones rely on breaking the C-P bond.

It is very likely that many bacterial enzymes involved in cleavage of the C-P bond are of the “C-P lyase” type; however, it has now been established that at least three other, Pi-insensitive, C-P cleavage enzymes do exist within microorganisms [17].

Some bacteria strains may rely on still other biochemical ways of glyphosate decomposition. *Arthrobacter sp.* GLP-1 uses glyphosate as the source of nitrogen [25], while strains of *Streptomyces* break glyphosate in the same way, using it as the source of either phosphorus or nitrogen or both nitrogen and phosphorus [7, 21]. Therefore, it may be assumed that bacterial activity in biodegradation of glyphosate is a property

related to particular strains [16, 24]. However, glyphosate degradation in soil is a co-metabolic process and decomposition rate depend on the general activity of soil bacterial and fungi, soil type and environmental conditions [25].

Conclusions

The effects of glyphosate degradation by microbes depended on the cell-substrate interaction. The influence of herbicides on the growth of microbes consisted in disrupting the cell's metabolism, albeit it was not always the case that the growth was inhibited. In the presence of glyphosate as the alternative source of phosphorus the bacterial growth depended on individual features. The inductive character of the growth was present in the case of four of the analysed bacterial strains – *Ralstonia pickettii*, *Serratia odorifera*, *Proteus mirabilis* and *Staphylococcus warneri*. Finally, *Sphingomonas paucimobilis* and *Pseudomonas fluorescens* grew without the adaptation phase.

All in all, when soil bacteria using glyphosate as the source of phosphorus are present in the soil, they will biodegrade this compound and, by implication, prevent it from accumulating in the environment.

Acknowledgments

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ZDOLNOŚĆ WYBRANYCH BAKTERII DO WZROSTU W OBECNOŚCI GLIFOZATU

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Abstrakt: Glifozat jest substancją czynną herbicydu Roundup. Kluczowym procesem wpływającym na jego rozkład w glebie jest degradacja mikrobiologiczna. Bakterie zdolne do rozkładu wiązania C-P wykorzystują go jako źródło fosforu. Celem badań była ocena zdolności do wzrostu trzydziestu szczeprów bakterii glebowych w obecności glifozatu jako jedynego źródła fosforu. Materiał badawczy stanowiły zróżnicowane morfologicznie i fizjologicznie szczepy bakterii glebowych. Zdolność bakterii do wzrostu w obecności glifozatu (FMG) jako jedynego źródła fosforu oceniano w zmodyfikowanym podłożu Dworkin-Fostera. Modyfikacja polegała na wprowadzeniu 0,5 mM FMG jako alternatywnego źródła fosforu. Kontrolę stanowił rozwój bakterii w pełnym i pozbawionym źródła fosforu podłożu Dworkin-Fostera. Intensywność rozwoju

testowanych szczepów oceniano metodą spektrofotometryczną ($\lambda = 490$ nm). Zaobserwowano znaczne różnice w intensywności wzrostu testowanych szczepów w obecności glifozatu jako jedynego źródła fosforu. Tylko osiem spośród nich rosło podobnie jak w pełnym podłożu Dworkin-Fostera, a rozwój pozostałych był zdecydowanie słabszy.

Słowa kluczowe: autochtoniczne bakterie glebowe, glifozat, kinetyka wzrostu

Dagna MACULEWICZ¹

BINUCLEATE *Rhizoctonia* spp. AS A BIOCONTROL AGENTS AGAINST PLANT PATHOGENS

MOŻLIWOŚCI ZASTOSOWANIA DWUJĄDROWYCH IZOLATÓW *Rhizoctonia* spp. W BIOLOGICZNEJ OCHRONIE ROŚLIN

Abstract: *Rhizoctonia* spp. is a large, diverse, ubiquitous soil inhabiting group of fungi. They are generally regarded as plant pathogens causing root rot and other plant diseases which results in crop losses of many economically important plant species, but they can also enter into symbiotic associations with orchids.

Binucleate species commonly cause root, stem and foliar diseases, but due to its wide range of host plants and frequent causing losses in important agricultural crops, probably the most studied *Rhizoctonia* species is multinucleate *Rhizoctonia solani* Kühn.

Crops, including fruits and vegetables, are exposed to diseases caused by microorganisms, especially pathogenic fungi, during growth and after harvest. The occurrence of plant diseases results in lower harvest, in worsens their quality and raises production costs. The use of chemical plant protection results in the contamination of soil and increase pathogen resistance to commonly used fungicides. Biological control is an alternative and safe for the environment method of plant protection.

Hypovirulent BNR isolates from different anastomosing groups can be successfully used as biocontrol agents in plant protection against pathogenic *Rhizoctonia* and fungi from other species. They may occupy the same ecological niches as pathogens, compete successfully for nutrients, induce plant resistance and promote plant growth.

Keywords: mechanism of biocontrol, binucleate *Rhizoctonia*

Fungal diseases are a numerous and, from an economic point of view, the most important group of plant diseases. Plant protection most often involves the use of fungicides, which represent 15 % of pesticide sales. Residues of chemicals are a threat to human health. Moreover, they cause reduction of beneficial organisms and pathogen resistance to chemicals. This is the reason for the increased interest in biological control agents (BCA). When conventional pesticides cannot be used eg in production of organic food, BCA can be used. However, biocontrol agents represent only 1 % of agricultural chemical sales [1].

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The “biological control” and its abbreviated synonym “biocontrol” regarding to plant protection means the use of one or more antagonistic microorganisms to suppress plant diseases. The organism that suppresses the pathogen is called the biological control agent. Biocontrol organisms belong to different taxonomic groups – viruses, bacteria, fungi or protozoa [2].

Usually their action involves the prevention of the infection, reduction in the colonization of host tissues or disruption of life and sporulation of the pathogen. Efficient prevention requires the presence of the antagonist on the plant surface before the infection [3].

Many of BCA exist naturally in the plant roots and leaves as a saprotrophs in a particular ecological niche. BCA should have high capacity to colonization, high growth rate and greater ability to survive and development in a wide range of pH, temperature and humidity than pathogen. It should be effective even in low concentrations and have wide spectrum of activity. It is particularly important not to produce metabolites toxic for humans and not to exert adverse effects on raw material [3].

Biocontrol agents generally protect against specific pathogens from infecting the target plant. Hypovirulent or avirulent strains can infect the host without causing disease symptoms. The protection of plants by BCA is the result of interaction of many different mechanisms. Protection may be direct (antagonism against the pathogen), or indirect (through competition for ecological niches, or enhance the host defence through induced resistance) [4, 5].

Direct antagonism by obligate parasites of a plant pathogen requires a high degree of selectivity for the pathogen, called hyperparasitism. The pathogen is directly attacked by a specific BCA that attack living hyphae or its propagules [3, 6].

Microbial predation is more general than hyperparasitism, pathogen is non-specific and provides less predictable levels of disease control. Sometimes BCAs exhibit predatory behavior under nutrient-limited conditions, such activity generally is not expressed under typical growing conditions. For example, some species of *Trichoderma* produce chitinase that are directed against cell walls of *R. solani* when concentration of readily available cellulose decreases [3].

Some binucleate nonpathogenic-*Rhizoctonia* isolates directly parasitized hyphae of *Pythium ultimum*, *P. oligandrum* and *P. ultimum* var. *sporangiferum* [7].

Indirect antagonism includes the production of enzymes or antibiotics, competition and induced-resistance mechanisms [3].

Many microorganisms have the ability to secrete enzymes which destroy cells walls of other organisms and use their protoplasmic material as a source of nutrients. These chemical substances are highly specific and have the ability to inhibit the growth and metabolism of selected microorganisms or kill them. Antimicrobial activity was exhibited eg by *Aspergillus* sp. against *Escherichia coli*, *Penicillium* sp. against *Bacillus* sp. and *Cladosporium* sp. against *Escherichia coli* [8].

Non-pathogenic *Rhizoctonia* (NpR) and pathogens can compete for nutrients, and this is a very important process for limiting disease incidence and severity. The most of them are generally thought to protect the plant by rapid colonization and exhausting the limited available nutrients so that none are available for pathogens to grow [3].

The colonization of the plant or substances secreted by avirulent isolate can activate plant resistance responses, which, in the case of a subsequent infection by pathogens, are faster and more efficient. The first of pathways is termed systemic acquired resistance (SAR), mediated by salicylic acid (SA). SA is produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins, which include a variety of enzymes. They may lyse pathogen cells, reinforce cell walls to resist infections, or induce cell death in the infection site. The second pathway is induced systemic resistance (ISR), mediated by jasmonic acid (JA) and/or ethylene which causes changes in cel wall composition, production of chitinases and glucanases and synthesis of phytoalexins. Once stimulated plant, exhibits an enhanced resistance upon inoculation with a pathogen [5, 6].

Binucleate *Rhizoctonia* spp. in plant protection

Rhizoctonia spp. is a group of soil fungi with diverse morphology and pathogenicity. This genus was established in 1815 by de Candolle [9]. *Rhizoctonia* spp. can be divided into two groups – binucleate (BNR) and multinucleate (MNR). Multinucleate isolates may display a large variation of number of nuclei in cells (more than 4) and belong to *Rhizoctonia solani* with *Thanatephorus cucumeris* teleomorph [10].

Binucleate *Rhizoctonia* spp. include various fungi which can be divided into groups with hyphal anastomosis. Ogoshi et al [11] described 13 anastomosis groups (from AG-A to AG-O). AG-B has been further divided into AG-Ba, AG-Bb and AG-Bo subgroups. Currently 19 AGs are described (from AG-A to AG-U). Perfect states of BNR correspond with *Ceratobasidium* sp. [12, 11].

Rhizoctonia spp. are fungi with a wide range of trophic strategies. They can be plant pathogens, saprotrophs or enter into symbiotic mycorrhizal associations with orchids [13].

Several genera of fungi, including binucleate *Rhizoctonia* [4, 14], *Trichoderma* [15,16], *Gliocladium* [16], or *Cladrrhinum* [17], can control the development of pathogenic isolates of *R. solani*. Binucleate *Rhizoctonia* spp. controlled *Rhizoctonia* diseases on creeping bentgrass [18], bean [4], potato [19], bedding plants [20], sugarbeet [21], cotton [22], cabbage [23], cucumber [24], and many more. BNR also have been shown to control *Pythium* and *Alternaria* diseases [25, 22].

Protective capacity in relation to plants exhibit representatives of many AGs (A, Ba, Bb, B(o), F, G, H, J, K, L, M, N, O, P, R and S). They can protect plants of various species against phytopathogenic fungi belonging to the genus *Rhizoctonia* and other species. The induction of host defenses may be associated with the appearance of small disease symptoms caused by the hypovirulent isolate and isolates causing minor damage to plants are potentially the best for biological plant protection. In addition to the inability to cause severe disease symptoms, hypovirulent isolates have, usually similar to those virulent, ability to colonize and occupy the same ecological niches in tissues and on the plant surface [26].

The authors of one of the first reports of the possibility of plant protection against pathogens by avirulent isolates of *Rhizoctonia* spp. are Burpee i Goult [18]. They

studied BNR effect on pathogenic isolates of *R. solani* on turfgrasses and in all three experiments they observed the suppression of brown patch.

Muslim et al [27] demonstrated that BNR causes a significant reduction in disease symptoms caused by *Fusarium oxysporum* on tomatoes, Cardoso and Echandi [4] observed significant protection by BNR against *R. solani* on bean, root exudates from bean seedlings colonized with a binucleate np-R inhibited virulent *R. solani* *in vitro*, and fewer infection cushions were formed by the pathogen on the np-R-colonized seedlings as compared to that on control seedlings [4, 26]. Olson and Benson [28] reported induction of resistance against *Botrytis cinerea* on geranium. Xue et al [24] observed increase of the activity of peroxidases in the presence of BNR in all bean tissues in the absence of disease symptoms.

Four isolates of hypovirulent binucleate *Rhizoctonia* were used for control of *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. BNR isolates could significantly reduce foliar symptoms and discoloration inside the stem [27].

Villajuan-Abgona et al [24] reported that three isolates of BNR (AG-A and AG-Ba) provided infection of 58 to 71 % against pathogenic *R. solani* AG2-2 on cucumber seedlings. BNR (*Ceratobasidium albatensis*) has been shown to control *R. solani* induced damping-off in *Pinus* spp. [29]. When BNR was inoculated onto soybean prior to *R. solani*, there was a significant reduction in disease severity, even when BNR had grown for only 24 h [30]. Sneh and Ichilevich-Auster [31] reported > 60 % protection of the cabbage and 73–95 % of the cucumber seedlings by BNR AG-Bo and AG-P against *R. solani* AG1-IA and AG4. Biocontrol of BNR fungi provided effective control of preemergence damping-off of impatiens caused by *R. solani*. Disease control of either BNR isolate was similar to the standard fungicide treatment of thiophanate-methyl and better than that with the commercial biocontrol product derived from *Gliocladium virens* [14].

Hypovirulent BNR may have a positive effect on plants also by improving their growth and development [32]. Some NpR show slower growth of mycelium compared to the pathogens. This problem can be eliminated by early inoculation of NpR mycelium *e.g.* on grains and their application near the sowing. The rich food base strengthens their growth, gives them the possibility of early colonization of plant tissues, which in turn gives them an advantage over pathogens occurring naturally in the environment. The earlier colonization by NpR gives better plant protection.

Np-R isolates can survive on the crop roots and after harvest, in plant debris, but it depends on the type of substrate, soil depth and humidity. Propagule density is increased on fresh organic substrates or when germinating seeds are available [26, 33].

Sneh et al [7] observed that there was no correlation between in vitro growth rate of hypovirulent isolates and their disease protection ability, but a higher growth rate would be advantageous for faster and more extensive colonisation of the infection sites on the host surface before the approaching hyphal tips of the pathogen.

BNR protective effects against *R. solani* are usually obtained following BNR pre-inoculation before *R. solani* attack, either after short periods of preinoculation *e.g.* 24 h or 48 h and up to 7 or more days of pre-inoculation [5]. Pre-incubation of BNR or

delayed inoculation of pathogen provided an increased protection on cucumber seedlings [24].

Poromarto et al [30] examined the interactions of intermingling hyphae of BNR AG-K and *R. solani* AG 4 in vitro and on the surface of the host. There was no evidence of lysis, mycoparasitism, inhibition of growth, or any other form of antagonism between hyphae. The results of these studies strongly suggest that induced resistance is the mechanism of biocontrol of *R. solani* on soybean by BNR. The inhibition of hyphal growth of *R. solani* on the surface of soybean tissue preinoculated with BNR appears to be a novel characteristic of induced resistance.

Xue et al [34] did not observe evidence of hyperparasitism or antibiosis between BNR and *R. solani* AG-4 or *Colleotrichum lindemuthianum* in vitro tests. Many researchers discounted that mechanisms as likely mechanisms of suppression of *R. solani* by BNR. They reported that BNR AG-G induced systemic resistance and protection of bean plants against *R. solani* AG-4 and *C. lindemuthianum*.

In the absence of pathogenic isolates an acceleration of growth of radish, lettuce, carrot, cotton, wheat, pepper and some ornamental plants were observed [26]. Muslim et al [27] observed a significant increase in fresh weight of stem and leaf of tomato in the presence of BNR.

BNR AG-K and AG-F can increase *Capsicum annuum* shoot mass and elongation and protect against weak pathogens [35]. Root colonisation with BNR increased cucumber seedlings tolerance to low soil moisture levels [31].

Xue et al [34] described the anatomical and cytological changes in tissues of bean seedlings protected with a nonpathogenic BNR isolate prior to their inoculation with a virulent isolate of *R. solani* which was associated with a deposition of cell wall material rich in lignin, suberin, and phenols. These compounds provide a physical or chemical barrier to the pathogen. NpR are inducers of peroxidases, 1,3 β -glucanases, and chitinases at the local and the systemic level in beans. It shows that induced resistance is multicomponent.

These studies demonstrate a significant protective role of binucleate *Rhizoctonia*, but only few of them specify their membership to anastomosis groups or subgroups. Khan et al [36] emphasize that BNR have the protective potential, but they require a more accurate testing, in particular in the field.

Isolates from orchids

Some *Ceratobasidium* species are mycorrhizal symbionts of orchids, whose seeds require an association with a fungus to obtain sufficient nutrition for germination. Orchid mycorrhizae are unlike other types of mycorrhizae in that the fungus probably receives little or nothing from the plant in most cases [2, 13, 37].

Mosquera-Espinosa et al [37] tested the biocontrol ability of BNR isolates from Colombian orchid roots. They caused low severity disease on rice and protected rice plants from pathogenic *R. solani* AG1-1A.

Disease protection by ectomycorrhizal fungi may involve multiple mechanisms including antibiosis, synthesis of fungistatic compounds by plant roots in response to mycorrhizal infection and a physical barrier of the fungal mantle around the plant root [2].

Microbes that contribute most to disease control are most likely those that could be classified as competitive saprophytes, facultative plant symbionts and facultative hyperparasites. These can generally survive on dead plant material, but they are able to colonize and express biocontrol activities while growing on plant tissues. A few, like avirulent *Fusarium oxysporum* and binucleate *Rhizoctonia*-like fungi, are phylogenetically very similar to plant pathogens but lack active virulence determinants for many of the plant hosts from which they can be recovered. Others, like *Pythium oligandrum* are currently classified as distinct species. However, most are phylogenetically distinct from pathogens and, most often they are subspecies variants of the same microbial groups [2].

Combination of biocontrol fungi and bacteria

Mixtures of some non-pathogenic bacteria and fungi may enhance the level of biocontrol of different plant pathogens [38]. Eken and Yuen [39] reported that BNR isolate BN-8 in combination with *Lysobacter enzymogenes* strain C3 reduced disease severity of wheat cultivars (Russ and Alsen) caused by *Bipolaris sorokiniana* and *Rhizoctonia solani* AG-4.

The cucumber seedlings grown on water agar and treated with mycelia disks of hypovirulent BNR isolate W7 two days prior to inoculation of the virulent *R. solani* isolate C4 showed a low disease severity, which is highly significant compared with hypocotyls of seedling inoculated with virulent *R. solani* without non-pathogenic BNR. The BNR isolate provided 63.2 % protection to the seedling against the pathogen. The cucumber seedling inoculated with the non-pathogenic BNR alone exhibited low disease severity which indicate faint lesion and slight browning of the root portion and can be considered as a non-pathogenic reaction [40].

Summary and conclusions

In recent years the interest in biological control of plant has been growing. Chemicals become insufficient, and their use carries the risk of environmental pollution, changes in global climate, the emergence of novel, more virulent pathogens and threat to human health. Fungi can be effectively and safely used as biocontrol agents. A great attention is paid to the fungi of the genus *Rhizoctonia*, especially binucleate isolates which are common in the plant rhizosphere. They are considered the microorganisms usable as an alternative to chemical pesticides. Many literature data confirm the use of these microorganisms to plant protection by competition for nutrients with pathogens and thus they inhibit their growth. BNR can promote plant growth and stimulate plant resistance against different phytopathogens.

Research conducted for about 25 years led to a commercial use of the registered biological agents for controlling plant diseases.

Further study of mechanisms of biocontrol and the ecology of the NpR should aid in the selection and development of the most effective biocontrol agents from within these fungi.

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MOŻLIWOŚCI ZASTOSOWANIA DWUJĄDROWYCH IZOLATÓW *Rhizoctonia* spp. W BIOLOGICZNEJ OCHRONIE ROŚLIN

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Abstrakt: *Rhizoctonia* spp. to duża, zróżnicowana i powszechnie występująca w glebie grupa grzybów. Zwykle są one patogenami wielu ważnych gospodarczo gatunków roślin. Powodują zgnilizny korzeni, są także często przyczyną chorób łodyg i liści. Mają szeroki zakres roślin żywicielskich. Obok możliwości oddziaływanego patogenicznego mogą one również wchodzić w związki symbiotyczne, np. ze storczykami. Gatunki dwujądrowe *Rhizoctonia* są dość często w środowisku i występują obok innego ważnego patogena roślin, którym jest wielojadrowy *Rhizoctonia solani* Kühn.

Występowanie chorób roślin skutkuje niższymi i o gorszej jakości plonami, a ochrona przed skutkami chorób roślin podnosi koszty produkcji. W celu ograniczenia strat możliwe jest stosowanie chemicznych środków ochrony roślin, to jednak powoduje skażenie gleby i przyczynia się do wyksztalcania u patogenów odporności na fungicydy. Alternatywą jest bezpieczna dla środowiska biologiczna ochrona roślin z wykorzystaniem hypovirulentnych izoltów dwujądrowych *Rhizoctonia* (BNR). W tym celu stosowane są izolaty z różnych grup anastomozowych. Mogą one być wykorzystywane jako czynniki biologiczne w ochronie roślin przeciw różnym grzybom chorobotwórczym, w tym także przeciw *Rhizoctonia solani*. Jest to możliwe, dlatego że zajmują one te same nisze ekologiczne, co patogeny. Dodatkowo izolaty BNR mają zdolność wzbudzania naturalnej odporności roślin i stymulowania ich wzrostu.

Słowa kluczowe: biologiczna ochrona, dwujądrowe *Rhizoctonia*

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**EVALUATION OF THE POSSIBILITY
TO USE ORGANIC ACIDS SOLUTIONS
FOR PRODUCTION OF FERTILIZERS BASED ON
HORTICULTURAL MINERAL WOOL WASTE**

**OCENA MOŻLIWOŚCI WYKORZYSTANIA
ROZTWORÓW KWASÓW ORGANICZNYCH
DO OTRZYMYWANIA PREPARATÓW NAWOZOWYCH
NA BAZIE ODPADOWEJ OGRODNICZEJ WEŁNY MINERALNEJ**

Abstract: The aim of the research was evaluation of the possibility to use organic acids solutions for production of fertilizers based on mineral wool waste. This substrate thanks to inert character and good air-water relationship is commonly used in greenhouse industry. Increased production of vegetable in soilless culture due to world's population growth. However mineral wool after full crop cycle is non-biodegradable – dangerous waste. Possibility of extraction nutrients from this kind of substrate could be solution of that important environmental problem. Before analyses mineral wool waste was grinded on 0.40 mm sieve. Then the extraction process was conducted using 2 % m/m and 10 % m/m citric acid, 2 % m/m and 10 % m/m formic acid, 0.2 % m/m and 2 % m/m acetic acid for 3 and 6 hours at temperature 25 °C. Solid phase before analyses was mineralized. Iron, phosphorus was analyzed using spectrophotometer methods. Iron analysis is based on formation of a coloured ferric ion complex with 2,2'-bipyridyl. Phosphorus analysis is based on formation of molybdenum blue complex. Calcium and magnesium was examined by complexometric titration with EDTA and using Eriochrome Black T as an indicator, while calcium ions concentration was analyzed using calconcarboxylic acid as an indicator. Content of nutrients after extraction from mineral wool waste allows to conclude that obtained liquid phase can be used as a fertilizer component while solid phase can be used in mixed substrate production.

Keywords: gardening mineral wool, waste, fertilizers, organic acids solutions

Introduction

Global population is constantly increasing. It is likely to achieve 9 billion by 2050. As an effect water consumption will be bigger and agricultural production should be

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doubled in order to provide enough food [1]. Solution of that problem might be hydroponics, which allow to produce crops in greenhouses, private gardens, on the roofs of the buildings or in places where traditional methods cannot be used. Hydroponics cultivation, where plants grow with their roots in an inert medium feed with soluble minerals and nutrients, has many advantages: it requires less water and has shorter harvest time than traditional one [2].

There are many kind of substrates used in hydroponics: organic like coconut fibre, peat and inorganic for example perlite or rock wool. The most commonly used substrate in European Union is mineral wool. It posses qualities like good air-water relationship, high water holding capacity and longevity, which characterize an effective substrate [3].

One of the leading rock wool producer in Poland is Rockwool Group. The first step of rock wool production is melting process in which raw materials (basalt rocks, chalk and coke) are melted at temperatures above 1500 °C. The melts goes onto the spinning machine, where the fibres are spun. Binding agents are added to make stable fibres. The last step is forming ready product by cutting into slabs, blocks or cubes and packed [4].

Rock wool slabs after full crop cycle turns into non-biodegradability waste. It is serious ecological problem due to big quantity (even 115 thousand tons in Europe), hygroscopic properties and big volume of used substrate. Rock wool slabs are very often accumulated on ‘wild dumps’, which negatively change landscape and can be dangerous for animals and people because of rock wool respirable fibres. Rational methods of reuse of that kind of waste must be found [5, 6].

Rock wool slabs used as a substrate in agriculture contain parts of plants like roots and nutrients from the fertilizers. Therefore extraction of that residues seem to be an interesting opportunity. Phases after extraction can be source of nitrogen, iron, phosphorus, calcium or magnesium, which are important fertilizers components. Mineral substances are essential for appropriate plant growth, they take part in many functions like transport of energy, they regulate enzymes activity and increase immunity from diseases. Production of fertilizers based on extracts containing nutrient for plants can be a solution of the ecological problem such as mineral wool waste [7–10].

Materials and methods

The aim of this research was evaluation of the possibility of nutrients extraction from mineral wool waste using organic acid solutions and reuse liquid and solid phases for fertilizers purposes. For analysis has been taken wool after one year of tomato cultivation, one year of cucumber cultivation and wool after two years of mixed cultivation.

Samples of post-mineral wool waste, before the extraction process were dried at 30 °C for one day. Dry and brittle material was grinded to a diameter of 0.40 mm.

Extraction process was conducted at 25 °C for two extraction time: 3 hours and 6 hours. Three kind of acids were used: 2 % m/m and 10 % m/m citric acid, 2 % m/m and

10 % m/m formic acid, 0.2 % m/m and 2 % m/m acetic acid. The mass ratio solid phase to liquid was $\varphi = 1:10$. Extraction was performed using Water Bath Shaker Type 357, ELPAN. Phases were separated using centrifuge MPW-360 for 10 minutes and speed 2500r/min. Liquid phase was diluted to volume of 250 cm³ with distilled water. The solid fracture was mineralized in 2:3 (by volume) mixture of nitric acid and sulphuric acid by heating at temperature of 250 °C for 30 minutes. After cooling, small amount of water was added and samples were heated again at temperature of 250 °C for 15 minutes. After process samples were diluted to the mark in volumetric flasks (250 cm³).

The content of phosphorus was determined by spectrophotometric method based on formation of a blue complex phospho-molybdic according to PN-88/C-87015. The absorbance was measured in a quartz cuvette with an absorption layer thickness of 1 cm at a wavelength of 690 nm using a spectrophotometer Jasco V-630 [11].

The content of iron was determined according to PN-85/C-84092. Ions of iron III are reduced to ions of iron II using hydroxylammonium chloride. The coloured complex of iron ions II with 2,2'-bipyridyl is created in solution at pH = 3.1. The absorbance was measured in a quartz cuvette with an absorption layer thickness of 1 cm at a wavelength of 520 nm using a spectrophotometer Jasco V-630 [12].

The content of calcium and magnesium was carried out by titration of samples using EDTA solution at a pH of 10 and Eriochrome Black T as an indicator. The content of calcium was determined using complexometric titration using also EDTA solution but at a pH of 13 and calconcarboxylic acid as an indicator.

Results and discussion

Disposal of mineral wool slabs is very difficult due to its non-biodegradability. However, post-production rockwool contains residues of important nutrients, which can be recovered and reused as a component for new fertilizer product.

Table 1 shows the results of phosphorus, iron, calcium and magnesium content in liquid phase after its extraction from mineral wool waste using for this purpose 2 % m/m and 10 % m/m citric acid, 2 % m/m and 10 % m/m formic acid, 0.2 % m/m and 2 % m/m acetic acid at temperature of 25 °C and for two extraction time: 3 and 6 hours.

The highest content of nutrients in liquid phase after extraction was obtained using 10 % (m/m) citric acid and 10 % (m/m) formic acid. Longer time of extraction affected in highest content of examined nutrient. Moreover higher concentration of extractant used in process leads to higher amount of iron, phosphorus, calcium and magnesium in analyzed liquid phase. The biggest amount of phosphorus, iron and calcium was achieved for 6-hour-long extraction using 10 % (m/m) citric acid. Content of mineral substances was the lowest when as an extractant acetic acid was used.

Table 2 shows the results of phosphorus, iron, calcium and magnesium content in solid phase after its extraction from mineral wool waste using for this purpose 2 % m/m and 10 % m/m citric acid, 2 % m/m and 10 % m/m formic acid, 0.2 % m/m and 2 % m/m acetic acid at temperature of 25 °C and for two extraction time: 3 and 6 hours.

Table 1

The mineral substances content in the liquid phases obtained by extraction of nutrients from mineral wool waste by using solutions of acetic, citric and formic acid

Extractant	Extractant's concentration [% m/m]	Extraction time [h]	PO ₄ ³⁻ content [% m/m]	Fe ²⁺ content [% m/m]	Ca content [% m/m]	Mg content [% m/m]
Acetic acid	0.2	3	0.30	0.06	0.40	0.06
	2	3	0.54	0.02	0.28	0.04
Citric acid	2	3	1.02	0.61	1.40	0.34
	10	3	1.60	2.27	1.92	0.19
Formic acid	2	3	0.01	0.48	1.73	0.02
	10	3	0.31	2.21	2.79	0.14
Acetic acid	0.2	6	0.32	0.02	0.35	0.22
	2	6	0.31	0.03	0.48	0.48
Citric acid	2	6	1.18	0.63	1.46	0.37
	10	6	1.85	2.62	5.22	1.23
Formic acid	2	6	0.02	0.54	1.69	0.77
	10	6	0.15	2.35	3.97	2.43

Table 2

The mineral substances content in the solid phases obtained by extraction of nutrients from mineral wool waste by using solutions of acetic, citric, formic acids

Extractant	Extractant's concentration [% m/m]	Extraction time [h]	PO ₄ ³⁻ content [% m/m]	Fe ²⁺ content [% m/m]	Ca content [% m/m]	Mg content [% m/m]
Acetic acid	0.2	3	2.58	1.89	9.74	0.18
	2	3	1.82	1.79	6.99	0.85
Citric acid	2	3	1.22	0.42	7.18	2.00
	10	3	1.02	0.51	5.82	1.55
Formic acid	2	3	2.19	1.70	5.37	2.00
	10	3	1.79	1.86	6.43	0.94
Acetic acid	0.2	6	2.77	2.39	13.52	0.05
	2	6	1.95	1.78	9.34	0.31
Citric acid	2	6	1.22	1.49	11.80	0.39
	10	6	0.97	0.04	6.64	1.04
Formic acid	2	6	2.18	0.69	6.40	0.26
	10	6	2.26	0.17	7.10	1.49

The highest content of phosphorus, iron, calcium and magnesium was obtained for extraction using acetic acid solutions. Magnesium content in solid phase after extraction was the lowest. Most of process using extractant of lower concentration and for longer time of extraction affected in biggest amount of examined mineral substances.

The highest content of nutrients was obtained for 6-hour-long extraction using 10 % (m/m) citric acid. The results are presented in Fig. 1. Citric acid is recommended in

Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilizers as an effective extractant of phosphorus, which is very important nutrient. Global consumption of phosphates which are main phosphorus source is rising. The result of that is phosphates price increase. Therefore recovery of phosphorus from mineral wool slabs is very important.

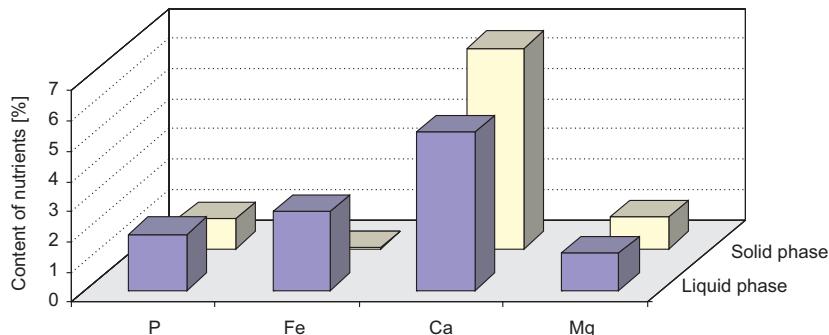


Fig. 1. Mineral substances content in the liquid and solid phases obtained from mineral wool by using 10 % (m/m) citric acid during 6 hours extraction

Figure 2 presents block diagram of receiving exemplary hydroponic solution for tomato cultivation. Liquid phase obtained from extraction process using 10 % (m/m)

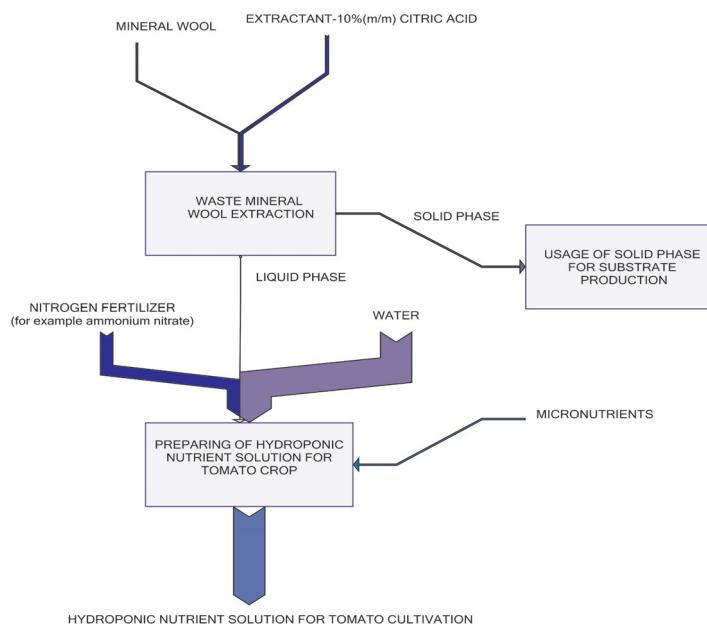


Fig. 2. Block diagram of receiving the hydroponic solution for tomato cultivation based on horticultural mineral wool waste

citric acid after dilution and necessary microelements supplementation can be used to create fertilizers. Nitrogen fertilizer for example ammonium nitrate is added to provide nitrogen in hydroponic solusion. It is possible to use also solid phase obtained after extraction using citric acid for substrate production in order to improve their air-water properties. Nutrients contained in solid phase is another advantage of this proposition of miner wool waste reuse.

Conclusions

Rockwool is commonly used substrate in horticulture. Big amount of mineral wool slabs after crop production negatively affects environment, therefore a rational method of reuse must be found. Extraction of nutrients residue from mineral wool waste may solve this problem. The extraction process was conducted using organic acid solutions as a extractant. The content of mineral substance in liquid and solid phase after extraction was determined by type of extractant and extraction time. The highest results was obtained for 6-hour-long extraction process using 10 % (m/m) citric acid. The results show that organic acid solution can be used to extract essential nutrients from horticultural mineral wool waste. Liquid phase which was obtained during extraction can be used to receive fertilizer products and solid phase for example as a supplement in substrates production in order to improve their air-water properties.

Acknowledgments

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**OCENA MOŻLIWOŚCI WYKORZYSTANIA ROZTWORÓW KWASÓW ORGANICZNYCH
DO OTRZYMYWANIA PREPARATÓW NAWOZOWYCH
NA BAZIE ODPADOWEJ OGRODNICZEJ WELNY MINERALNEJ**

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Abstrakt: Ciągłe wzrastająca liczba ludności na świecie jest przyczyną zwiększonego zapotrzebowania na żywność, a tym samym konieczne jest zwiększenie poziomu produkcji rolnej. Można osiągnąć większą wydajność zbiorów dzięki zastosowaniu upraw hydroponicznych, które są popularne w hodowli kwiatów ciętych oraz warzyw. Dominującym podłożem w tego typu uprawach jest wełna mineralna, która charakteryzuje się korzystnymi właściwościami powietrzno-wodnymi. Podłoż po uprawie stanowi uciążliwy, nieulegający biodegradacji odpad. Podejmowane są działania mające na celu rozwiązywanie tego ekologicznego problemu. Jednym z nich jest wykorzystanie składników mineralnych, takich jak żelazo, fosfor, wapń i magnez, pozostałych po uprawie w podłożu. Przeprowadzono badania mające na celu określić możliwość wykorzystania do ekstrakcji tych składników roztworów kwasów organicznych. Oznaczenie w poekstrakcyjnych fazach (ciekłej i stałej) zawartości badanych pierwiastków pozwoli na ustalenie optymalnych warunków pozwalających wykorzystać je do uzyskiwania preparatów nawozowych. W otrzymanych próbkach po procesie ekstrakcji trwającej 3 i 6 godzin przy użyciu roztworów kwasów octowego, cytrynowego i mrówkowego zbadano zawartość fosforu, żelaza, wapnia i magnezu. Próbki stałe poddano mineralizacji przed przeprowadzeniem analiz. Oznaczanie fosforu i żelaza wykonano, używając metod spektroskopowych. Badanie zawartości wapnia i magnezu wykonano za pomocą miareczkowania, używając jako titrania roztworu EDTA. Na podstawie uzyskanych wyników można stwierdzić, że poekstrakcyjne fazy ciekłe mogą być wykorzystane przy produkcji preparatów nawozowych, natomiast faza stała może stanowić dodatek poprawiający właściwości podłoży ogrodniczych.

Słowa kluczowe: wełna mineralna, odpad, preparaty nawozowe, roztwory kwasów organicznych

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**COMPARISON OF THE EFFECTIVENESS
OF DIFFERENT TYPES OF EXTRACTANTS
FOR RECOVERY OF IRON
FROM MINERAL WOOL WASTE**

**PORÓWNANIE SKUTECZNOŚCI WYKORZYSTANIA
RÓŻNEGO TYPU EKSTRAHENTÓW DO ODZYSKU ŻELAZA
Z ODPADOWEJ WEŁNY MINERALNEJ**

Abstract: The paper presents the results of iron extraction from wool waste. In studies as extractant was used: water, EDTA, acetic acid, formic acid, DTPA, ammonium lactate and calcium lactate. For analysis has been taken wool after one year cultivation of tomato and cucumber and wool after two years of mixed cultivation. The main aim of the analysis was to determine which extractant allows the greatest recovery of the iron from waste mineral wool. Current trends in the agriculture development and the fertilizer industry are aimed at maximizing the recovery of nutrients from waste for re-use. Demonstration of the effectiveness of the recycling of valuable trace elements contained in the mineral wool can make develop a method of wool utilization profitable. In addition, the high cost of fertilizer components makes a new type of medium containing nutrients obtained by extraction from mineral wool waste, can be an interesting option in the future. Implementation of this type of process is consistent with the objectives of Sustainable Development, as well as the environmental policy of the European Union. The proposed way to get iron from mineral waste wool consists of following steps: drying at 30 °C for 24 hours, grinding to particles with a sieve size of 0.40 nm, extraction and phase separation. The studies included the effect of time, temperature and type of extractants on the efficiency of the process. The iron content in the samples was determined by spectrophotometric method based on the ferric ion complex of 2,2-pyridyl in a solution at pH of 3.1.

Keywords: horticultural mineral wool, iron, fertilizers, re-use

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Introduction

Disposal of post-mineral wool is still unsolved problem. Until now, the only way to deal with this waste is storage in landfills.

Mineral wool is obtained from rock. The production process consists of two stages. The first stage involves the grinding of raw materials in ball mills and melted in a furnace. Melting point of such kind of rocks is about 1550 °C. In the second stage, the molten material is fiberized [1–4]. Process for the preparation of mineral wool is continuously refined to reduce its energy consumption and waste production. Mineral wool is important product for the infrastructure and economy [5].

The main market of mineral wool, besides the construction industry, is agriculture. From the economic and trade point of view the most profitable method of cultivation is hydroponics [4, 6]. Hydroponics has several advantages. It allows to overcoming the impact of pathogens and gives a high degree of control over the factors that stimulate growth [7]. In the soilless cultivations inert substrates such as described wool are used. Except wool inert substrates also include such materials as pumice and perlite. Farmers also often used organic substrate as peat, coconut wool or straw [8, 9]. The problems associated with disposal of mineral wool cause the higher consumption of organic substrates. Especially coconut fiber is highly appreciated. This phenomenon is also observed in the construction sector. Today a lot of research is carried out to increase the hydrophobicity requirements of natural materials (flax, hemp) and which are met by mineral wool [10].

Researchers of the disposal problem of mineral wool made a number of proposals for its reuse [11–16]. The main ideas relate to the use of wool waste as a raw material for reclamation and rehabilitation of degraded land [11, 12]. Some research which was carried out with a solution of DTPA demonstrated that it is possible to obtaining some of the microelements from the structure of the mineral wool [13]. In addition, mineral wool after the completion of the crop cycle contains a residue of fertilizer. This gave the opportunity to analyze the possibility of utilization of mineral wool with obtaining nutritional ingredients.

Generally wastes are used in the production fertilizers in small scale. The main raw materials are local waste. The purpose of this type of production is utilization of the wastes which are produced in neighborhood. Wastes which are generally used in the production of fertilizers derive from agri-food production as sugar plants, starch plants, distilleries but also industrial waste as leather waste from tanneries [17].

The idea of using waste in the production of fertilizers is consistent with the principles of Sustainable Development. Modern fertilizers should be produced according with the new trends. The current requirements are not only the economic but also environmental and social [17]. The environmental aspect during launching of new products on the market is very important. Before placing a product on the market, it is important to determine its impact on the environment. For this purpose a Life Cycle Assessment (LCA) is used [18]. According to the LCA analysis liquid fertilizers are safer for the environment. They also have other advantages such as ease of application, efficacy, long duration of action and many others.

The demonstration of the possibility of recovery of essential nutrients contained in the mineral wool waste may allow for obtain cost-effective liquid fertilizer. The main aspect of the profitability of the process is the selection of the extracting agent [19]. This type of fertilizer may be a breakthrough product on the market.

Materials and methods

The aim of the study was to determine the possibility of obtaining liquid fertilizer with extract from mineral wool waste. In the study the following extractants were used: water, EDTA, acetic acid, formic acid, DTPA, ammonium lactate and calcium lactate. For analysis has been taken wool after one year of tomato cultivation, one year of cucumber cultivation and wool after two years of mixed cultivation.

Before appropriately extraction process wool waste has been subjected to drying process. The dried material has a sufficient brittleness to be able to comminute it on a sieve of mesh size 0.40 mm. The milled wool with a mass of 5 g was subjected to extraction process in flasks with a capacity of 250 cm³. The mass ratio of solid to liquid phase was 1 : 10. This step was carried out at different temperature (25, 30, 50, 70 °C) time – 1, 3, 6, 15 hours. Process of extraction was carried out on a shaker ELPAN company equipped with a thermostat type 357 Water Bath Shaker. The phase separation was carried out on a laboratory centrifuge MPW-360.

Samples of raw mineral wool waste, and a liquid phase after the extraction process, were mineralized in a mixture of nitric acid (20 cm³) and sulfuric acid (30 cm³). Samples were heated for 30 min after the start of boiling. After this time the sample were taken from the heater, cooled to room temperature and poured into the 100 cm³ of distilled water.

Analysis of the iron content was carried out according to standard PN-85/C-84092 [20]. The iron content was analyzed by spectrophotometric method based on formation of a colored ferric ion complex of 2,2-pyridyl solution at pH = 3.1. The absorbance was measured in a quartz cuvette with an absorption layer thickness of 1 cm at a wavelength of 520 nm using an spectrophotometer Jasco V-630.

Results and discussion

The Table 1 shows the results of iron content in the liquid phase obtained after its extraction from wool after one year of tomato cultivation and using water as extractant depending on temperature.

The Table 2 shows the results of iron content in the liquid phase obtained after its extraction from wool after one year of cucumber cultivation and using water as extractant depending on temperature.

Table 1

The iron content in the liquid phase obtained from mineral wool after one year cultivation of tomato and using water as extractant depending on process temperature

The temperature of the extraction process	Time [h]	Content [% mas. Fe]
25 °C	1	0.0028
	3	0.0019
	6	0.0029
	15	0.0395
30 °C	1	0.0002
	3	0.0039
	6	0.0031
	15	0.0406
50 °C	1	0.0080
	3	0.0033
	6	0.0013
	15	0.1206
70 °C	1	0.0035
	3	0.0060
	6	0.0047
	15	0.0558

Table 2

The iron content in the liquid phase obtained from mineral wool after one year cultivation of cucumber and using water as extractant depending on process temperature

The temperature of the extraction process	Time [h]	Content [% mas. Fe]
25 °C	1	0.0018
	3	0.0006
	6	0.0008
	15	0.0379
30 °C	1	0.0003
	3	0.0008
	6	0.0005
	15	0.0525
50 °C	1	0.0021
	3	0.0051
	6	0.0027
	15	0.0377
70 °C	1	0.0076
	3	0.0022
	6	0.0010
	15	0.0358

On the basis of the results it can be seen that for both types of wool, the iron content in the extract is comparable. Figures 1 and 2 showed that in both cases of extraction process the range of favorable efficiency can be obtained even at temperature of 25 °C. Increasing the temperature to 70 °C allowed to achieve similar results as in the process carried out at a temperature of 25 °C or led to a slight increase in efficiency of the process. Process at 70 °C requires a considerable amount of energy, so more preferred will be conduct process at lower temperatures. It seems to be advantageous to lowering the temperature of the process even up to 20 °C and use another, more effective extractant to improve iron extraction efficiency like for example chelating agents.

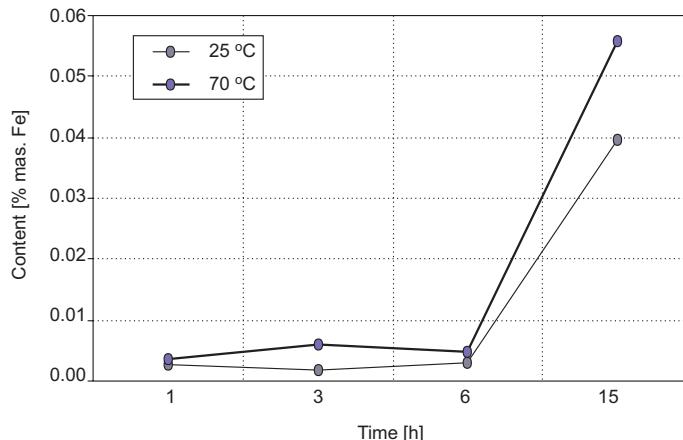


Fig. 1. The dependence of the iron content according to the temperature in the liquid phase obtained from wool after the cultivation of tomato and using water as extractant

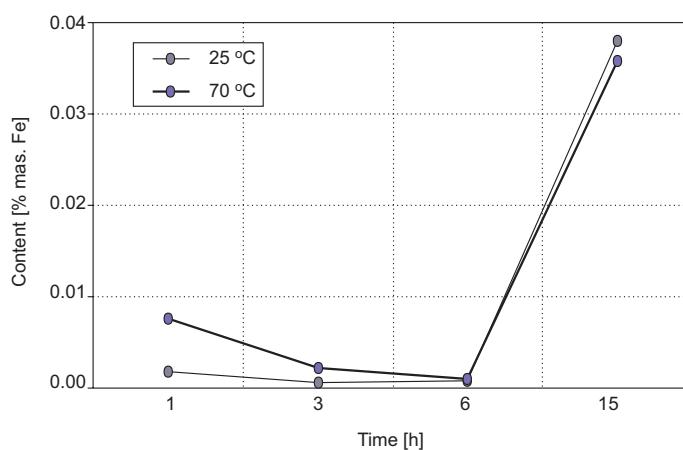


Fig. 2. The dependence of the iron content according to the temperature in the liquid phase obtained from wool after the cultivation of cucumber and using water as extractant

The Table 3 shows the results of iron content in the liquid phase obtained after its extraction from wool after one year of tomato cultivation and using 0.1 M EDTA as extractant depending on process temperature.

Table 3

The iron content in the liquid phase obtained from mineral wool after one year cultivation of tomato and using 0.1 M EDTA as extractant depending on process temperature

The temperature of the extraction process	Time [h]	Content [% mas. Fe]
25 °C	1	0.1634
	3	0.1238
	6	0.1415
	15	0.1292
30 °C	1	0.1418
	3	0.1529
	6	0.1394
	15	0.0870
50 °C	1	0.1591
	3	0.1436
	6	0.1274
	15	0.1118
70 °C	1	0.1341
	3	0.1416
	6	0.1333
	15	0.1071

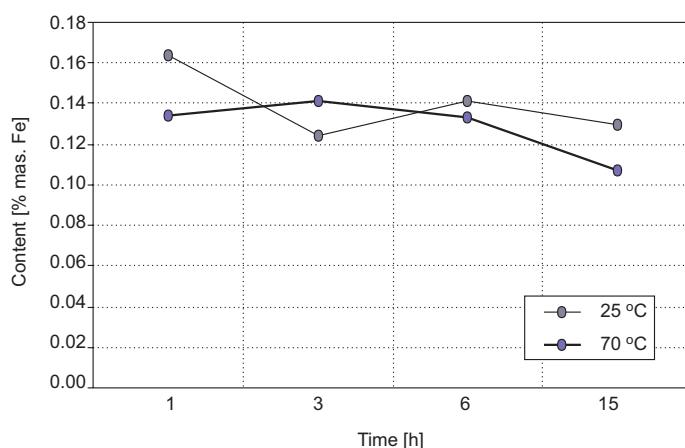


Fig. 3. The dependence of the iron content according to the temperature in the liquid phase obtained from wool after the cultivation of tomato and using 0.1 M EDTA as extractant

The Table 4 shows the results of iron content in the liquid phase obtained after its extraction from wool after one year of cucumber cultivation and using 0.1 M EDTA as extractant depending on process temperature.

Table 4

The iron content in the liquid phase obtained from mineral wool after one year cultivation of cucumber and using 0.1 M EDTA as extractant depending on process temperature

The temperature of the extraction process	Time [h]	Content [% mas. Fe]
25 °C	1	0.0609
	3	0.0614
	6	0.0767
	15	0.0888
30 °C	1	0.0882
	3	0.0793
	6	0.0988
	15	0.1080
50 °C	1	0.0651
	3	0.0946
	6	0.0992
	15	0.0950
70 °C	1	0.0842
	3	0.1075
	6	0.1121
	15	0.1086

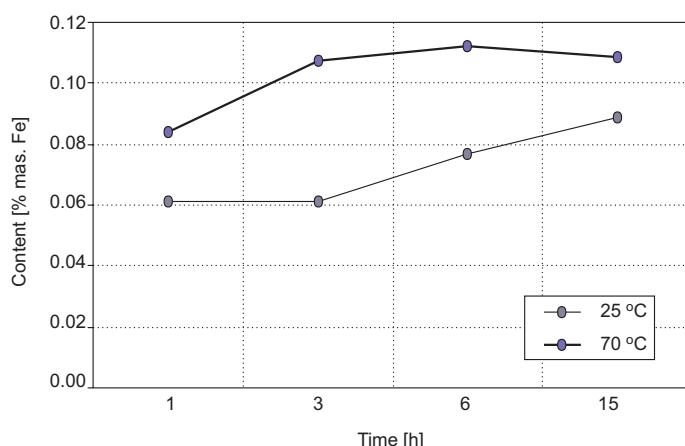


Fig. 4. The dependence of the iron content according to the temperature in the liquid phase obtained from wool after the cultivation of cucumber and using 0.1 M EDTA as extractant

In the presented preliminary studies as a nutrient extractant water was used. The content of iron obtained from the mineral wool waste, under the conditions of the experiments, are relatively low. In the liquid phase obtained by using a solution of EDTA can be notice that iron content is increased. This observation has forced the need to verify a similar increase in the efficiency of using another extractant and taking into account the optimal technological conditions. Figures 5 and 6 show the results of iron content in the liquid phase obtained after its extraction from wool respectively after one year of tomato cultivation and after two years of mixed cultivation and using different extractants which have similar properties as EDTA. These processes were conducted at 25 °C and for 1, 3 and 6 hours

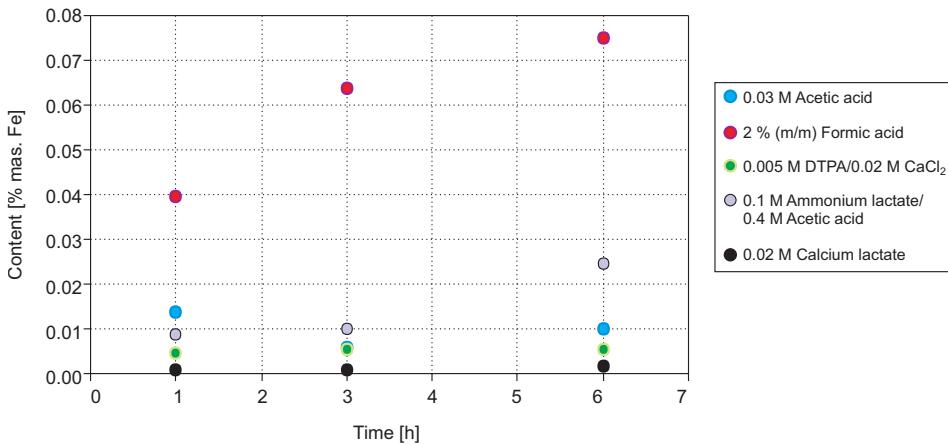


Fig. 5. Comparison of the iron content in the liquid phase obtained from mineral wool after one year cultivation of tomato depending on extractant type

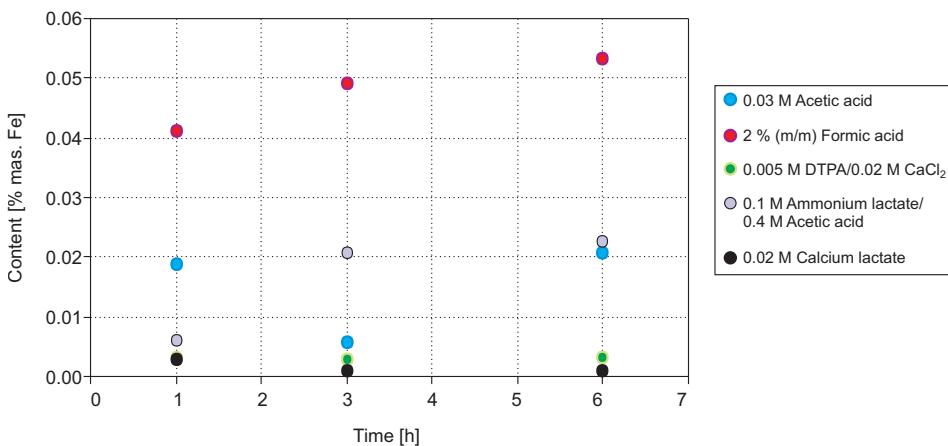


Fig. 6. Comparison of the iron content in the liquid phase obtained from mineral wool after two years of mixed cultivation depending on extractant type

Comparing the obtained results it can be concluded that the least efficient extractant of all included in the study in respect of iron extraction is 0.02 M solution of calcium lactate. Formic acid solution was characterized by the relatively high degree of extraction. However, the content of iron in this case was lower than with using 0.1 M EDTA solution.

Moreover studies have shown that iron extraction from wool after two cycles of cultivation (several processes of fertilization) not always give better results of iron content in extract than in this obtained from wool after one year of cultivation.

Conclusions

The extraction process was a key element of the proposed process of the re-use of mineral wool. Selecting the appropriate parameters of the process is cost-determining factor for the possible implementation of the process. This operation should allow to achieve a relatively high performance extraction of nutrients at a relatively low cost.

The highest recovery of iron from mineral wool waste is possible thanks to use solution of EDTA as extractant. The most important from a technological point of view is that it is possible for low-temperature and short time of extraction. Studies have shown that wool after two cycles of cultivation (several processes of fertilization) does not give better results of iron content in extract than in this obtained from wool after one year of cultivation. Favorably results of iron content were also obtained for process with using acetic acid solution.

The studies make it possible to analyze the practical use of mineral wool waste in the production of fertilizers.

Acknowledgement

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PORÓWNANIE SKUTEZNOŚCI WYKORZYSTANIA RÓŻNEGO TYPU EKSTRAHENENTÓW DO ODZYSKU ŻELAZA Z ODPADOWEJ WEŁNY MINERALNEJ

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Abstrakt: W pracy zostały przedstawione wyniki ekstrakcji żelaza z odpadowej wełny mineralnej. W badaniach jako ekstrahenty wykorzystano: wodę, roztwór EDTA, kwas octowy, kwas mrówkowy, DTPA, mleczan amonu i mleczan wapnia. Analizie została poddana wełna jednoroczna po uprawie pomidora i ogórków, a także dwuletnia po uprawie mieszanej. Głównym celem analizy było określenie, który ekstrahent umożliwia największy odzysk żelaza z poprodukcyjnej wełny ogrodniczej. Obecne tendencje rozwoju rolnictwa, jak i przemysłu nawozowego są skierowane na maksymalizowanie odzysku składników odżywczych z odpadów, w celu ich powtórnego wykorzystania. Wykazanie skuteczności recyklingu cennych mikroelementów zawartych w wełnie mineralnej może pozwolić na opracowanie opłacalnej metody utylizacji tego odpadu. Ponadto wysoki koszt komponentów nawozowych sprawia, że nowy typ pożywki zawierający składniki pokarmowe pozyskane na drodze ekstrakcji z odpadowej wełny mineralnej może być w przyszłości interesującym rozwiązaniem. Wdrożenie tego typu procesów jest zgodne z założeniami Zrównoważonego Rozwoju, a także polityką ochrony środowiska Unii Europejskiej. Mikroelementowe żelazo pozyskiwane było z odpadowej wełny na drodze: suszenia w temperaturze 30 °C przez 24 h, rozdrabniania na siecie na cząsteczki o wymiarach 0,40 nm, ekstrakcji i rozdzielenia faz. W prowadzonych badaniach analizie poddano wpływ czasu ekstrakcji, temperatury, a także rodzaju stosowanego ekstrahenta. Zawartość żelaza w próbках oznaczano metodą fotokolorimetryczną z wytworzeniem kompleksu jonów żelazowych z 2,2-pirydylem w roztworze o pH wynoszącym 3,1.

Słowa kluczowe: ogrodnicza wełna mineralna, żelazo, nawozy, wtórne wykorzystanie

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Krystyna HOFFMANN^{1*} and Józef HOFFMANN¹

INFLUENCE REACTION TIME TO DEGREE OF COMPLEXATION ZINC IONS BY FERTILIZERS CHELATING SUBSTANCES

WPŁYW CZASU REAKCJI JONÓW CYNKU Z NAWOZOWYMI SUBSTANCIAMI CHELATUJĄCYMI NA STOPIEŃ SKOMPLEKSOWANIA

Abstract: In recent years, a big problem for agriculture is the shortage of available micronutrients in the soil, thus reducing yields. This deficiency is due to the intensification of agriculture, cultivation of new species of plants require fertilization and a significant share of soils with low content of available micronutrients, which is why in recent years looking for the appropriate forms in which they can be applied to not have a negative impact on the environment. Of particular importance in the field of micronutrient fertilizers gained chelates. These compounds are characterized by stability. Chelating agents stabilize the cations of microelements in a wide pH range. Their application optimizes supplement micronutrient deficiency. Adaptation of nutrients to the needs of the plants can reduce the negative impact on the environment, without causing soil salinity, as in the case of salt micronutrients. Can be applied to both soil and foliar application. They are produced in the form of liquid or fine-crystalline. In the European Union are set chelating agents used as additives for liquid fertilizers. These are synthetic compounds belonging to the aminepolycarboxylic compounds (APCAs) which form stable complexes which are soluble in water. APCAs form chelates of trace elements in a molar ratio 1:1. The most commonly used component is the disodium salt of ethylenediaminetetraacetic acid (EDTA). The Regulation of the European Parliament and of the Council EC No 2003/2003 of 13 October 2003 includes the requirements for chelates used in agriculture. In accordance with these requirements complex the trace element level should be at least 80 % of the declared water-soluble, the total metal content. Download time micronutrient by the plant in the soil environment should correspond to the period degradability. Too low biodegradability may adversely affect the environment, causing mobilization heavy metals from bottom sediments and lead to phytotoxic complexes. Formal requirements resulted in the continued search for new chelating agents, and improving the technology used. The aim of the study was to determine the degree of complexation of the selected ions of micronutrients with chelating substance at different reaction times. The molar ratio of metal-ligand equal to 1:1. The degree of complexation was determined by differential pulse voltammetry. Was selected micronutrient zinc. Comparison was made to a compound belonging to the group of derivatives of aminepolycarboxylic compounds and for substances of natural origin – amino acid. These

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studies will help determine the optimal response time complexation of micronutrients by chelating agents commonly used in the fertilizer industry.

Keywords: chelate, micronutrients, liquid fertilizers

Introduction

Plants need for proper development of both macro and micronutrients. These components cannot be replaced by another element, as they play an important role in the metabolism of plants and plant without them cannot go full cycle of vegetation. Macronutrients are acting as building blocks and are taken from the soil in large quantities. For micronutrients include about thirty elements. For micronutrients used as fertilizer formulations include eight of them, iron, manganese, zinc, copper, boron, molybdenum, nickel and chlorine. They participate in biochemical processes, mainly as an essential element for the proper functioning of the enzymes. Also have a stimulating effect on the efficiency of macronutrients. The need for these elements is much smaller than in the case of macronutrients (several to several hundred grams per 1 ha of cultivated area) [1].

The demand for plant micronutrients depends on many factors such as soil pH, content of clay and humus in the soil, microbial activity of the substrate, on the type, variety and age of the plant. Micronutrient fertilization should ensure maximum uptake of fertilizer with high efficiency, the diverse elements depending on the demand and enable the use of plant fertilizer for intervention. This has led to the development of micronutrient fertilizer industry. Such fertilizers are produced in order to ensure optimum and effective nutrition while minimizing adverse impacts on the environment. Excess micronutrients in the soil solution can lead to eutrophication, heavy metals mobilization from the bottom sediments and rivers and, consequently, to introduce them into the food chain. Microelement fertilizers are produced in the form of liquid or fine-crystalline. Liquid fertilizers are characterized by an effective and fast response and high efficiency.

Microelements are applied in various forms: the macronutrient fertilizers containing trace elements, in the form of inorganic salts, in the form of glazes fertilizer, in the form of liquid fertilizers containing micronutrients and in the form of chelates. In recent years the most commonly used are trace elements chelates. These compounds are resistant to external factors, are characterized by high durability, ensure a high degree of bioavailability of micronutrients in plants. There is a low probability of phytotoxicity. Fertilizer chelates are readily soluble in water. Thanks to the structure of the chelate can be acquired in whole or may be subject to a slow dissociation so that the ions are released gradually, allowing for optimal supplement the shortage without any negative impact on the environment. Micronutrient fertilizer chelates are 2 to 5 times more effective than the sulphate salts [2–5].

Selected for use as fertilizers are substances that allow a sustainable manner micronutrient cations stabilize a wide range of pH and in the presence of other nutrient. Particular stability characterized by compounds belonging to the aminepolycarboxylic compounds (APCAs). These substances form a chelate with the trace element in a molar

ratio metal to ligand equal to 1:1. Such compounds are widely used in the manufacture of detergents, fertilizers, textiles and paper. The fertilizer industry uses ligands like in Table 1. It can be acid or sodium, potassium or ammonium salts.

Table 1
Fertilizers chelating agents [4]

No.	Designation	Alternative designation	Chemical formula
1	ethylenediaminetetraacetic acid	EDTA	C ₁₀ H ₁₆ O ₈ N ₂
2	2-hydroxyethyl ethylenediaminetriacetic acid	HEEDTA	C ₁₀ H ₁₈ O ₇ N ₂
3	ethylenetriaminepentaacetic acid	DTPA	C ₁₄ H ₂₅ O ₁₀ N ₅
4	ethylenediamine- <i>N,N'</i> -di[(<i>ortho</i> -hydroxyphenyl)acetic acid]	[<i>o,o</i>] EDDHA	C ₁₈ H ₂₀ O ₆ N ₂
5	ethylenediamine- <i>N</i> -[(<i>ortho</i> -hydroxyphenyl)acetic acid]- <i>N'</i> -[<i>(para</i> -hydroxyphenyl)acetic acid]	[<i>o,p</i>] EDDHA	C ₁₈ H ₂₀ O ₆ N ₂
6	ethylenediamine- <i>N,N'</i> -di[<i>(ortho</i> -hydroxy-methylphenyl)acetic acid]	[<i>o,o</i>] EDDHMA	C ₂₀ H ₂₄ O ₆ N ₂
7	ethylenediamine- <i>N</i> -[<i>(ortho</i> -hydroxy-methylphenyl)acetic acid]- <i>N'</i> -[<i>(para</i> -hydroxy-methylphenyl)acetic acid]	[<i>o,p</i>] EDDHMA	C ₂₀ H ₂₄ O ₆ N ₂
8	ethylenediamine- <i>N,N'</i> -di[(5-carboxy-2-hydroxy-phenyl)acetic acid]	EDDCHA	C ₂₀ H ₂₀ O ₁₀ N ₂
9	ethylenediamine- <i>N,N'</i> -di[(2-hydroxy-5-sulfo-phenyl)acetic acid] and its condensation products	EDDHSA	C ₁₈ H ₂₀ O ₁₂ N ₂ S ₂ + + n · (C ₁₂ H ₁₄ O ₈ N ₂ S)
10	iminodisuccinic acid	IDHA	C ₈ H ₁₁ O ₈ N
11	<i>N,N'</i> -di(2-hydroxybenzyl)ethylenediamine- <i>N,N'</i> -di-acetic acid	HBED	C ₂₀ H ₂₄ N ₂ O ₆

Currently, the most widely used chelating agents is the disodium salt of ethylenediaminetetraacetic acid (EDTA). However, there is a high probability that this substance does not have sufficient biodegradability. The compound may be present in the soil for up to 15 years [6–8].

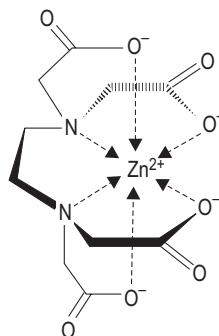


Fig. 1. EDTA complex with zinc ions

Due to the existing problem of environmental pollution by biodegradable chelates constantly looking for new substances, which may in future become a replacement for existing substances [9–10]. In recent years, the market has been introduced foliar amino acid complexes. Amino acids are the second type of natural compounds that plants produce to solubilise and translocation minerals. Digestibility of amino acid chelates in foliar is close to 100 %, they are less phototoxic to plants. Their application allows having a positive impact on the biochemical processes (precursors for the synthesis of plant hormones) and metabolic products. Plants manufacture these compounds to make minerals biologically available in the cell. As with organic acids, amino acid compounds also play a role in mineral uptake into plant tissue, because of the increase permeability effect of the amino acid on the cuticle. As in the case of feed additives used bioactive amino acids derived from the hydrolysis of proteins (*L*-form). Hydrolysis of protein is expensive to produce. This problem caused that amino acid compounds are application only in rare type of plants. Amino acid ligands are most often used: tryptophan, methionine, glycine, glutamic acid, proline and lysine.

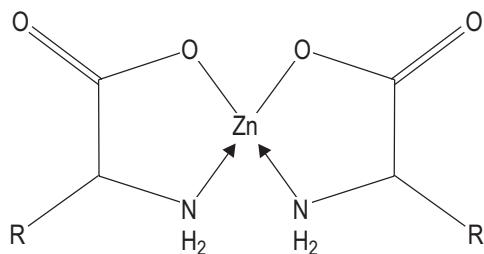


Fig. 2. Amino acid complex with zinc ions

These compounds are called stimulants or anti-stress agents. In Poland such formulations are applied after the winter, drought or floods in order to reduce the effects of stress plants. In Western European countries are used in the normal cycle of crops [11–13].

Materials and methods

For the determination of the reaction time to degree of complexation, were done a series of experiments in different molar ratios of metal-ligand. In experiments was used zinc. The micronutrient was introduced into the system in the salt form – heptahydrate zinc sulphate(VI) prod. POCh. Used ligands were synthetic derivative which belonging to aminepolycarboxylates group and natural ligand. Synthetic ligand introduced into the system in the form of disodium salt of ethylenediaminetetraacetic acid (EDTA) prod. POCh Gliwice and natural ligand, *L*-lysine, prod. Sigma-Aldrich

The effect of time on the reaction metal-ligand of complexation degree checked using differential pulse voltammetry.

The degree of zinc complexation was determined by differential pulse voltammetry. This method consists in measuring the current flowing from the sample during electrolysis depending on the linearly changing potential. Measurements were made of

Eco Chemie camera: AUTOLAB PGSTST 12 GPES software. Mercury electrode was used 663 VA Stand Methrom company working in SMDE mode (Static Mercury Drop Electrode). The reference electrode was silver chloride electrode, auxiliary electrode and a glassy carbon electrode. The experiments were repeated three times, and the results of arithmetic mean.

Table 2

The value of the voltammetric analysis used when the content of zinc ions

Parameters	The value of a given parameters
Size of mercury drop	0.25 mm ²
The value of the step potential	0.00495 V
Modulation amplitude	0.00255 V
Modulation time	0.05 s
Equilibration period	5 s
The value of the deposition potential	1.3 V
Time of deposition	60 s
The scope of the potential for Zn ²⁺	-1.2 V to -0.7 V

The study was conducted in an aqueous medium in the presence of basic electrolytes. Measurements of the degree of complexation of zinc ions are carried out in the presence of 0.1 mol/dm³ KCl at pH 7. The used ligands were: synthetic ligand EDTA and the natural ligand *L*-lysine. Using different reaction time 300, 600, 900 and 1200 s.

The test sample with a capacity of 25 cm³ containing zinc ions and a chelating ligand provided in the supporting electrolyte and the measurement cell was subjected to stirring for 300, 600, 900 and 1200 s and deoxygenating for 300 s. From the obtained voltammogramm read potential reduction of metal ions [V] and the signal height. Micronutrient concentration was determined on the basis of the signal current. The resulting signals are proportional to the concentration of ions in solution. Ion complexed trace element is electrically neutral. The degree of complexation was calculated from the difference in concentration before and after the chelation reaction.

The degree of complexation was calculated from the following formula:

$$X = \left(\frac{C_0 - C}{C_0} \right) \cdot 100 \%$$

where: X – degree of zinc complexation [%],

C_0 – zinc ion concentration in the nil sample [mol/dm³],

C – concentration of unbound zinc ions [mol/dm³].

Results and discussion

Figure 3 shows a voltamperogramm of zinc ions complexed by *L*-lysine at various reaction times. Used 4 different times: 300, 600, 900 and 1200 s. On the basis of the

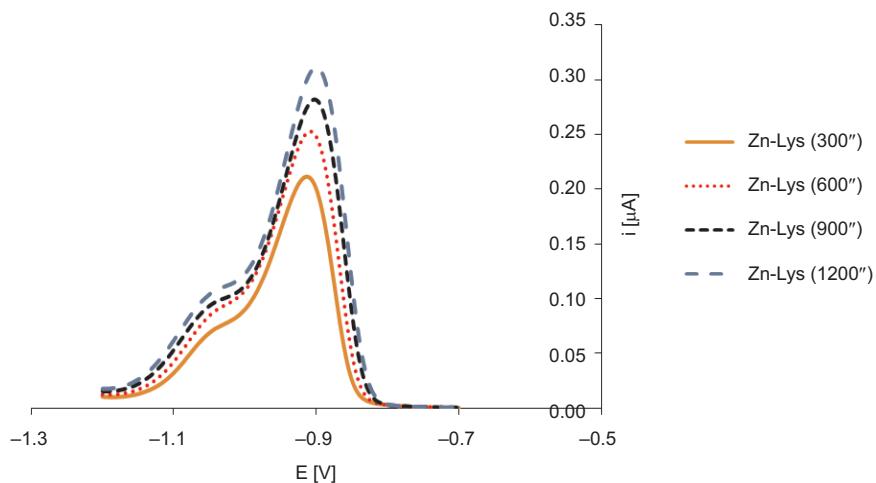


Fig. 3. Voltamperogramm of zinc and *L*-lysine complex in different reaction time

signal, concentration of zinc ions in each of the systems, and the calculated degree of complexation.

Experiments were performed under the same conditions for the complexation of substances belonging to the aminepolycarboxylic compounds: disodium salt of ethylene-diaminetetraacetic acid. Figure 4 shows a voltamperogramm of zinc ions complexed by EDTA at various reaction times.

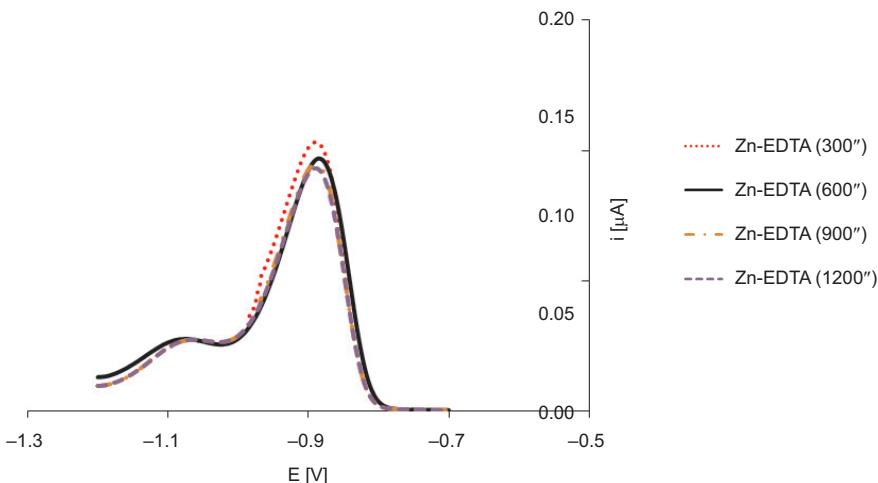


Fig. 4. Voltamperogramm of zinc and *EDTA* complex in different reaction time

The degree of complexation was calculated on the basis of the above voltamperograms. Comparison of the results for both of complexing agents is given in Table 3.

Table 3

Effect of reaction time the degree of complexation of zinc ions by *L*-lysine and EDTA

Time [s]	Degree of zinc ions complexation [%]	
	Zn-Lys	Zn-EDTA
300	33.33	98.51
600	30.92	99.23
900	29.05	~100.00
1200	21.71	~100.00

Conclusions

On the basis of the interpretation of the obtained voltamperogramms can determine the degree of complexation of zinc ions. In the case of synthetic substances the conditions laid down in Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003. These requirements have been met with a molar ratio of metal to ligand equal to 1:1. In the case of a natural substance, these requirements were not met. Probably other reaction conditions will influence the formation of stable complex which is characterized by a greater degree of complexation.

The obtained results indicate that increasing the zinc reaction time of the disodium salt of ethylenediaminetetraacetic acid will increase the degree of complexation slightly. In the case of a system comprising zinc ions and amino acids to increase the reaction time causes a slight decrease in the degree of complexation.

Research has shown that the reaction time of fertilizer zinc ion chelating substance to a minor extent, affects the degree of complexation.

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Wpływ czasu reakcji jonów cynku z nawozowymi substancjami chelatującymi na stopień skompleksowania

Instytut Technologii Nieorganicznej i Nawozów Mineralnych, Wydział Chemiczny
Politechnika Wrocławска

Abstrakt: W ostatnich latach dużym problemem dla rolnictwa jest niedobór przyswajalnych mikroelementów w glebie, co powoduje zmniejszenie plonów. Niedobór ten spowodowany jest intensyfikacją rolnictwa, uprawą nowych gatunków roślin wymagających nawożenia oraz znacznym udziałem gleb o niskiej zawartości przyswajalnych mikroskładników, dlatego w ostatnich latach poszukuje się odpowiednich form, w jakich, można je aplikować, by nie miały negatywnego wpływu na środowisko.

Szczególne znaczenie w branży nawozów mikroelementowych zyskały chelaty. Związki te dzięki swojej kleszczowej strukturze cechują się trwałością oraz swoją inertnością w roztworze glebowym. Chelatory stabilizują kation mikroelementu w szeroki zakresie pH. Ich stosowanie pozwala w sposób optymalny uzupełnić niedobór mikroelementów. Dostosowanie zawartości składników pokarmowych do potrzeb roślin pozwala ograniczyć negatywny wpływ na środowisko, nie powodując zasolenia gleb, jak w przypadku stosowania soli mikroelementów. Mogą być aplikowane zarówno do glebowo, jak i doliście. Produkowane są w formie płynnej lub drobnokrystalicznej. W krajach Unii Europejskiej określone są substancje chelatujące stosowane jako dodatki do nawozów płynnych. Są to związki syntetyczne należące do grupy aminopolikarboksylowych (APCAs), które tworzą trwale kompleksy rozpuszczalne w wodzie. APCAs tworzą chelaty z mikroelementami w stosunku molowym 1:1. Najczęściej stosowanym komponentem jest sól disodowa kwasu etylenodiaminotetraoctowego (EDTA). W Rozporządzeniu Parlamentu Europejskiego i Rady WE nr 2003/2003 z dnia 13 października 2003 r. znajdują się wymogi dotyczące chelatów stosowanych w rolnictwie. Zgodnie z tymi wymogami stopień skompleksowania mikroelementu powinien wynosić przynajmniej 80 % deklarowanej, rozpuszczalnej w wodzie, całkowitej zawartości metalu. Czas pobierania mikroelementu przez roślinę w środowisku glebowym powinien odpowiadać okresowi degradacyjności. Zbyt niski stopień biodegradacji może negatywnie wpływać na środowisko, powodując remobilizację metali ciężkich z osadów dennych i prowadzić do powstania fitotoksycznych kompleksów. Wymogi formalne spowodowały, że nadal poszukuje się nowych substancji chelatujących oraz ulepsza się stosowane technologie.

Celem badań było określenie stopnia skompleksowania wybranego jonu mikroelementu przy różnym czasie reakcji z substancją chelatującą. Zastosowano stosunek molowy metal-ligand równy 1:1. Stopień skompleksowania wyznaczono z wykorzystaniem woltamperometrii plusowej różnicowej. Wybranym mikroelementem był cynk. Porównanie wykonano dla związku należącego do grupy pochodnych aminopolikarboksylowych oraz dla substancji pochodzenia naturalnego – aminokwasu. Badania te pozwolą określić optymalny czas reakcji kompleksowania mikroelementów przez substancje chelatujące powszechnie stosowane w branży nawozowej.

Słowa kluczowe: chelaty, mikroelementy, nawozy płynne

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REACTIVITY OF CONCENTRATED AMMONIUM NITRATE SOLUTIONS IN THE PRESENCE OF ORGANIC IMPURITIES

REAKTYWNOŚĆ STĘŻONYCH ROZTWORÓW AZOTANU AMONU W OBECNOŚCI ZANIECZYSZCZEŃ ORGANICZNYCH

Abstract: Assessment of mixtures reactivity under certain conditions is essential for safe and conscious designing and improving manufacturing processes. One commonly used compound, the reactivity of which is very important, is ammonium nitrate and its solutions. The biggest influence on the reactivity of ammonium nitrate has the temperature and presence of other compounds, including impurities, that catalyze reactions leading to degradation. One of such impurities are organic compounds which impact on the ammonium nitrate has not yet been clearly described.

The aim of the research was to investigate the influence of selected organic impurities on the reactivity of aqueous solutions containing up to 50 wt % ammonium nitrate, buffered with 15 wt % diammonium phosphate. The measurements were carried out using differential scanning calorimetry. Solution without organic compounds showed no reactivity up to 240 °C. The addition of 8 wt % ammonium oxalate did not increase the reactivity of ammonium nitrate. It was observed an endothermic decomposition reaction of oxalic acid, beginning at 185 °C. During the measurement of a solution containing 6 wt % phenidone, it was registered an exothermic complex reaction, beginning at 189 °C and showing relatively small thermal effect. Addition of 6 wt % pyrogallol caused a significant increase in reactivity of ammonium nitrate solution. Strongly exothermic reaction beginning at 192 °C was observed. Spectrophotometric measurement showed a clear nitrate concentration loss in the sample after calorimetric measurement. The results show that the nitrate ion selectively oxidizes certain functional groups in organic compounds.

Keywords: ammonium nitrate, organic impurities, reactivity, calorimetry

Introduction

Chemical and technical safety is absolutely essential in industrial production. It extends to the apparatus and appliances design stage, as well as their proper operation,

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by means of complex control systems. The need to ensure an adequate level of safety of people and the environment is regulated by a number of legal acts. The most important of these is the European Parliament and Council Directive 96/82/EC (Seveso II) of 9 December 1996 on the control of major-accident hazards involving dangerous substances, amended by Directive 2003/105/EC of 16 December 2003. Its two main principles are the prevention of major-accident hazards involving dangerous substances, and in case of an accident – reduction of its negative consequences for man and the environment. The Directive covers not only the industrial production but also the storage of dangerous chemical substances [1]. The doctrine of Sustainable Development is also gaining an increasing importance in industrial production. It states that the needs of humanity should be met at the least possible negative impact on the environment. According to this principle, it is necessary to better understand the production processes, for enhancing the safety of people and the environment [2].

The preparation of the majority of the legally required reports and analyses is connected with the necessity of investigations and simulations, allowing for reliable and unambiguous opinion on the safety of the installation. Very often it comes down to the assessment of reactivity of given system and the effects associated with it. Learning them reduces the risk of losing control over the production process and the occurrence of adverse reactions leading to deterioration of the product [3].

One commonly used in the inorganic technology chemical compound susceptible to rapid thermal decomposition is ammonium nitrate and its solutions. It was the cause of many accidents in the industry and in spite of many years of research, the threat has not been completely eliminated [4, 5]. Pure ammonium nitrate is considered to be relatively safe because of its high stability at low temperature. At the stage of its manufacture, storage, transport and use, ammonium nitrate and its solutions often get contaminated by such compounds as inorganic acids, organic oils and others [6]. These pollutants have a significant impact on the reactivity of ammonium nitrate, catalyzing reactions leading to degradation and thermal instability. Therefore, learning about its interactions with other compounds is essential for the safe storage and handling of materials containing ammonium nitrate [7–9].

Trace amounts of chlorine or chlorides may result in an increased explosiveness of ammonium nitrate and reduction of the decomposition temperature by as much as 80 °C. Particularly much attention is paid to the products of stainless steel corrosion. They contain chromium compounds, which destabilizing effect on the ammonium nitrate is comparable with that of chlorides [5, 10]. Organic substances with confirmed negative impact on the safety of ammonium nitrate include: dinitrotoluene, nitro-naphthalene and similar nitro compounds, aliphatic alkylamines and their salts, ethyleneamine dinitrate, aliphatic alcohols and glycerine esters [11–14]. Effect of many organic compounds on the reactivity of ammonium nitrate, however, is not clearly described so far. Furthermore, Oxley et al noted that not only the type of the organic compound, but also the degree of its dispersion, has an influence on the reactivity of the mixture. According to these studies, emulsions are more resistant to thermal decomposition than less dispersed systems. It was demonstrated that this is not due to the presence of water in the system, by repeating the experience for the anhydrous emulsion

and obtaining the same results as for the emulsion with water. Probably, in a highly dispersed system, dissociation of ammonium nitrate is limited, which results in a higher thermal decomposition temperature [15].

For aforementioned reasons, the ability to assess the reactivity of the various systems under certain conditions is essential to safe and conscious designing and improving manufacturing processes. One of the techniques which enable testing the reactivity of the compounds and their mixtures is differential scanning calorimetry [16].

Materials and methods

The dominant component of the test solutions was ammonium nitrate, the nominal content of which was 50 wt %. Diammonium phosphate in an amount of 15 wt % served as a buffering and stabilizing compound. Selected organic substances, such as ammonium oxalate, phenidone and pyrogallol in an amount of 6–8 wt %, were added to the solutions. The nominal content of ammonium nitrate was properly reduced to obtain the same vapor pressure above the solution in each sample.

Reactivity of solutions was evaluated by measuring the thermal effect of the reaction in a differential scanning calorimeter of Calvet type, C80D Setaram. The solutions were placed in a glass tube with a capacity of 2 cm³, and the sample mass was 1 g. As a reference sample, it was used a solution of potassium sulfate at such concentration that the vapor pressure was similar to that in the measuring vessel. The vessel was equipped with a pressure sensor that allows its continuous recording. The concentration of nitrate ions (NO₃⁻) was determined in solutions after the calorimetric measurement. The measurements were made using a spectrophotometer Varian Cary 50 Bio UV/Vis. Nitrate concentration in the solution was calculated from the maximum value of absorbance at a wavelength of 303 nm.

Results and discussion

A solution of the nominal content of ammonium nitrate and diammonium phosphate, without the addition of organic compounds, showed no reactivity during the calorimetric measurement up to 240 °C. It is evidenced by the lack of significant heat effects during the calorimetric measurement and constant pressure value recorded at a constant temperature. Spectrophotometric measurement also confirmed no loss of nitrates in the sample. Overpressure registered in the measuring vessel was used to calculate the saturated vapor pressure above the solution (Fig. 1) which was then expressed in the form of Antoine equation (1):

$$\log p_{\text{H}_2\text{O}} = 9.21041 - \frac{7355.39}{430.514 + T} \quad (1)$$

Pressure of the gaseous products formed during the subsequent calorimetric measurements was determined by subtracting from the total registered overpressure the

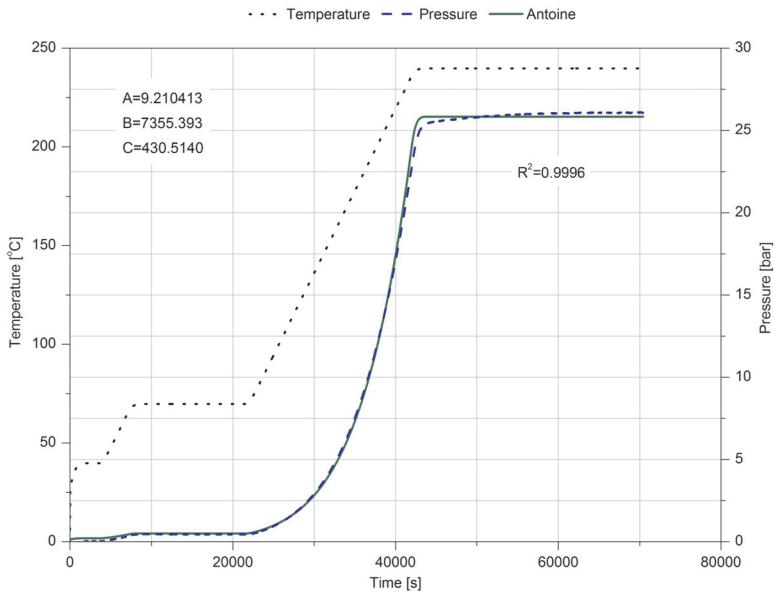


Fig. 1. The pressure of saturated water vapor calculated from calorimetric measurement of solution containing: 50 wt % ammonium nitrate, 15 wt % diammonium phosphate, 35 wt % water, and pressure expressed with Antoine equation

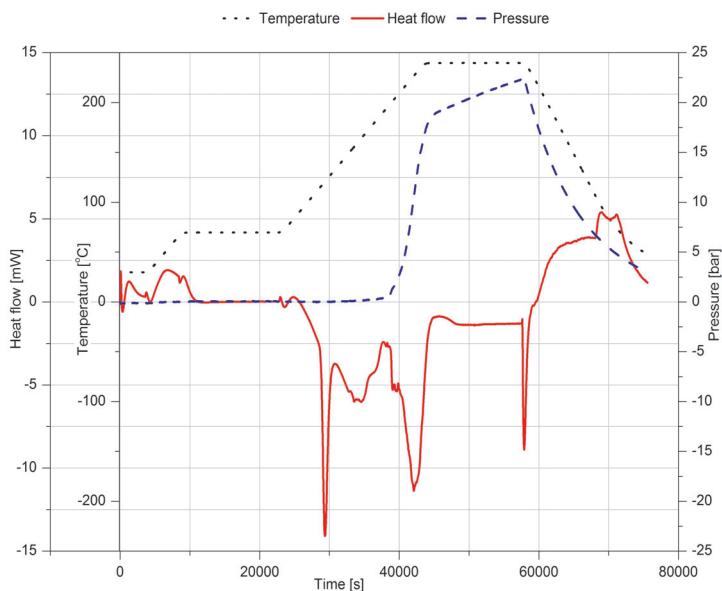


Fig. 2. The result of calorimetric measurement of solution containing: 8 wt % ammonium oxalate, 42 wt % ammonium nitrate, 15 wt % diammonium phosphate, 35 wt % water

air pressure in the measuring vessel and saturated vapor pressure above the solution, calculated according to the Antoine equation (1).

The addition of 8 wt % ammonium oxalate did not increase the reactivity of ammonium nitrate in the solution. During the measurement, it was recorded an endothermic reaction starting at 185 °C, the source of which could be the decomposition of the organic compound (Fig. 2). A spectrophotometric measurement of the nitrates concentration in the sample after the calorimetric measurement shows that the ammonium nitrate did not decompose. The maximum pressure of the gaseous products formed during the measurement was 22.3 bar and probably was generated as a result of decomposition of ammonium oxalate.

During calorimetric measurement of a solution containing 6 wt % phenidone, an exothermic reaction starting at 189°C was registered (Fig. 3). Its thermal effect is however slight, and the course is complex and difficult to interpret. Possibly it is the result of multi-stage transformation and decomposition of the organic compound. Generated pressure of gaseous products is low and reached 9.8 bar. Variability of the inclination angle of pressure increase curve also confirms the complexity of the registered process. The obtained results suggest that the addition of phenidone may slightly increase the reactivity of the ammonium nitrate in the solution.

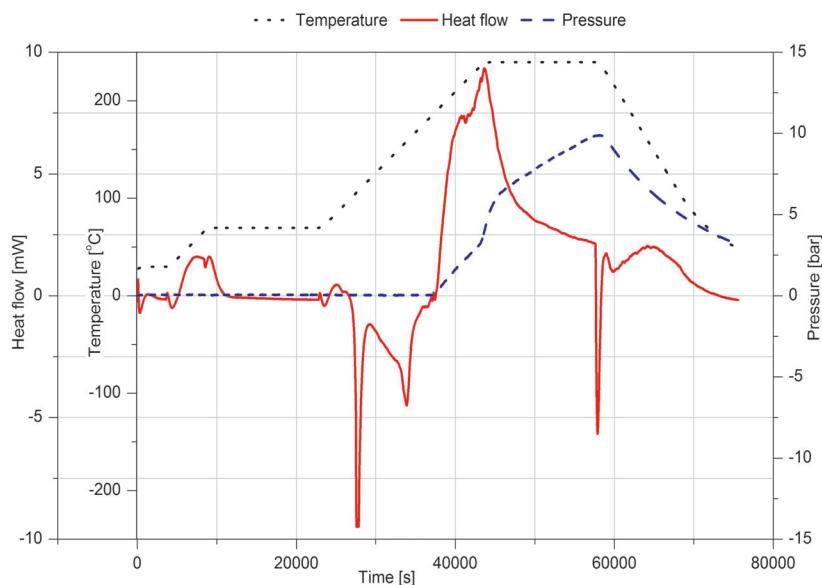


Fig. 3. The result of calorimetric measurement of solution containing: 6 wt % phenidone, 44 wt % ammonium nitrate, 15 wt % diammonium phosphate, 35 wt % water

Addition of 6 wt % pyrogallol significantly increased the reactivity of ammonium nitrate solution. Highly exothermic reaction beginning at 192°C was observed (Fig. 4). Spectrophotometric measurement showed a significant decrease in the concentration of

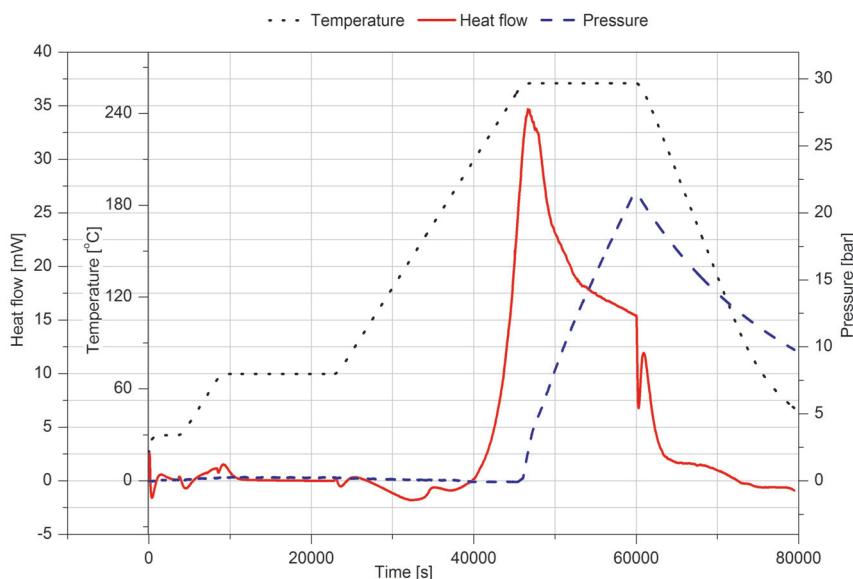


Fig. 4. The result of calorimetric measurement of solution containing: 6 wt % pyrogallol, 44 wt % ammonium nitrate, 15 wt % diammonium phosphate, 35 wt % water

nitrates in the sample after calorimetric measurement. Maximum pressure of formed gaseous products was 21.6 bar. Possibly nitrate ion NO_3^- was reduced to NO_2^- and then to NO, simultaneously oxidizing the hydroxyl groups of pyrogallol. According to the mechanism proposed by Dima et al, formed nitric oxide dimer after reduction to N_2O can decompose to nitrogen gas and other products of disproportionation, generating registered pressure [17].

Based on the results of the analyses summarized in Table 1, it can be seen that only some of the tested organic compounds exhibited the ability to increase the reactivity of ammonium nitrate in buffered solutions. These differences may be related to the type of substituents present in organic compounds. Probably nitrate ion has the ability to selective oxidation of some functional groups in organic compounds, thereby leading to increased reactivity of the ammonium nitrate and its degradation.

Table 1

Results of calorimetric measurements presenting the temperature of the beginning of reaction (T_{ONSET}) and the maximum pressure of the gaseous products (p_{max}) for each of tested organic compounds

Organic additive	Ammonium oxalate	Phenidone	Pyrogallol
Thermal effect	endothermic	exothermic	exothermic
T_{ONSET} [°C]	185	189	192
p_{max} [bar]	22.3	9.8	21.6
Functional groups	2 carboxylic	carbonyl phenyl	3 hydroxyl

Conclusions

Studies conducted using differential scanning calorimetry allowed to assess the influence of selected organic compounds on the reactivity of buffered aqueous solutions of ammonium nitrate. Addition of 8 wt % ammonium oxalate did not affect the reactivity of the test solution. This compound has probably undergone an independent endothermic decomposition at 185 °C. Interaction between 6 wt % phenidone and ammonium nitrate is not straightforward, due to the complexity of the observed changes. At 189 °C it was registered the beginning of an exothermic reaction, accompanied by a small thermal effect. It is conceivable that phenidone slightly increases the reactivity of ammonium nitrate in the solution. Pyrogallol added to the solution in an amount of 6 wt % significantly increased its reactivity. The beginning of strongly exothermic reaction was recorded at 192 °C, while the solution without organic compounds showed no reactivity up to 240 °C. Spectrophotometric measurements showed that in solution with pyrogallol a significant amount of ammonium nitrate reacted. Varied impact of organic compounds on the reactivity of the solution can be associated with the type of substituents present in them. Confirmation of the ability of nitrate ion to the selective oxidation of chosen functional groups requires further investigation.

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REAKTYWNOŚĆ STEŻONYCH ROZTWORÓW AZOTANU AMONU W OBECNOŚCI ZANIECZYSZCZEŃ ORGANICZNYCH

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Abstrakt: Ocena reaktywności mieszanin w określonych warunkach jest niezbędna, aby móc w sposób bezpieczny i świadomy projektować oraz usprawniać procesy technologiczne. Jednym z powszechnie stosowanych związków, którego reaktywność jest niezwykle ważna, jest azotan amonu i jego roztwory. Największy wpływ na reaktywność azotanu amonu ma temperatura oraz obecność innych związków, w tym zanieczyszczeń, katalizujących reakcje rozkładu. Jednym z takich zanieczyszczeń są związki organiczne, których wpływ na azotan amonu nie został do tej pory jednoznacznie opisany.

Celem pracy było określenie wpływu zanieczyszczeń organicznych na reaktywność wodnych roztworów zawierających do 50 % wag. azotanu(V) amonu, buforowanych wodorofosforanem(V) amonu w ilości 15 % wag. Pomary wykonano z wykorzystaniem skaningowej kalorymetrii różnicowej. Roztwór pozabawiony zanieczyszczeń organicznych nie wykazał reaktywności do 240 °C. Dodatek 8 % wag. szczawianu amonu nie zwiększył reaktywności azotanu amonu. Zaobserwowano endotermiczną reakcję rozkładu kwasu szczawiowego, rozpoczynającą się w 185 °C. Podczas pomiaru roztworu zawierającego 6 % wag. fenidonu, zarejestrowano w 189 °C egzotermiczną reakcję o złożonym przebiegu, której efekt cieplny jest niewielki. Dodatek 6 % wag. pirogalolu spowodował znaczny wzrost reaktywności roztworu azotanu amonu. Zaobserwowano silnie egzotermiczną reakcję rozpoczynającą się w 192 °C. Pomiar spektrofotometryczny wykazał wyraźny ubytek stężenia azotanów w próbce po pomiarze kalorymetrycznym. Wyniki badań wskazują na to, że jon azotanowy selektywnie utleniania wybrane grupy funkcyjne w związkach organicznych.

Słowa kluczowe: azotan amonu, zanieczyszczenia organiczne, reaktywność, kalorymetria

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THE IMPACT OF AGRONOMIC FACTORS ON THE CONTENT OF SELECTED MICROELEMENTS IN NAKED OAT (*Avena sativa* var. *nuda*) GRAIN

WPŁYW ZABIEGÓW AGROTECHNICZNYCH NA ZAWARTOŚĆ WYBRANYCH MIKROPIERWIASTKÓW W ZIARNIE OWSA NAGOZIARNISTEGO (*Avena sativa* ver. *nuda*)

Abstract: The components crucial for fulfilling plant nutrient needs are, beside macroelements, also microelements. Microelement content in plant raw materials is often modified by various agronomic measures, therefore it does not always meet the requirements for this element. The research was conducted to determine the effect of agronomic factors on the content of zinc, copper, iron, cobalt and manganese in oat grain. Three field experiments were set up acc. to fractional factorial designs (2^{5-1} and 3^{4-1}) in two localities (Wierzbica and Prusy) in 2003. Akt cv. revealed a lower content of zinc, copper, iron and manganese in comparison with STH 7000 and STH 4770 strains. Microelement concentrations in oat grain were determined by the selection of oat cultivar/strain and growth regulator dose. Zinc content in oat grain fulfilled the requirements for plants designed for human consumption and animal feeds. Optimal iron content but deficient contents of manganese, copper and cobalt were registered in oat grain treated as fodder.

Keywords: naked oat, microelements, fertilization, plant growth regulator

Introduction

Ensuring proper metabolism in human organism is conditioned by supplying not only main nutrients but also microelements. Most agricultural systems do not guarantee the content of these components in agricultural products on proper levels [1]. Researchers must more precisely characterize whole grains and analyze the role played by whole

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grains in the protection against diseases [2]. According to Butt et al [3] oat is the cereal species with a unique chemical composition. Oat grain constitutes a good source of vitamins from B group, crucial amino acids, unsaturated fatty acids and microelements.

Agronomic factors applied within the agricultural system modify the content and uptake of microelements by plants and in result determine their content in plant raw materials. The proper level of mineral and also foliar fertilization, seems to have a particularly significant effect ensuring proper plant supply in macro- and micro-elements [4]. The content of microelements in plants is diversified and depends on the habitat, genotype or absorption method [5–10]. Due to a considerable share of cereals in a daily food ration of people and potential excess of their permissible content in plant raw material, this group of plant should be particularly intensively researched in view of its chemical composition, including microelement concentrations. Oat reveals a considerable changeability of its chemical composition depending on agronomic factors and its being a plant commonly used in nutrition. It justifies the necessity to conduct such research [11, 12].

Microelement content in oat grain not always corresponds to consumer requirement for these components. Therefore, in the light of very few research reports on this issue, identification of the impact of various factors, including also agronomic ones, on zinc, copper, iron, cobalt and manganese content in oat grain, seems to be important.

Materials and methods

Field experiments were conducted in 2003 on two sites: Prusy ($50^{\circ}07'N$, $20^{\circ}04'E$) (one experiment) and in Wierzbica ($50^{\circ}29'N$, $19^{\circ}45'E$) (two experiments). Agricultural practices were carried out in accordance with the principles of oat cultivation.

The experiment in Prusy was set up according to fractional factorial design 3^{4-1} in two replications. The factors and their levels were presented in Table 1.

Table 1
Agronomic factors and their levels in experiments conducted in Prusy

Agronomical factor	Factor level		
	1 [low]	2 [average]	3 [high]
Genotype	Strain STH 7000	Cultivar Akt	Strain STH 4770
PK fertilization	$0 \text{ kg} \cdot \text{ha}^{-1}$ PK	$72 \text{ kg} \cdot \text{ha}^{-1}$ P	$256 \text{ kg} \cdot \text{ha}^{-1}$ PK
Foliar nitrogen application	$0 \text{ kg} \cdot \text{ha}^{-1}$ N	$9 \text{ kg} \cdot \text{ha}^{-1}$ N	$18 \text{ kg} \cdot \text{ha}^{-1}$ N
Plant growth regulator Moddus	$0 \text{ dm}^3 \cdot \text{ha}^{-1}$	$0.4 \text{ dm}^3 \cdot \text{ha}^{-1}$	$0.6 \text{ dm}^3 \cdot \text{ha}^{-1}$

The size of plots for harvest was 10 m^2 . Sowing density was 500 germinating seeds $\cdot \text{m}^{-2}$ of genotypes *Avena sativa* ver. *nuda*. The soil of the experimental field was degraded chernozem formed from loess with pH 6.8. In Wierzbica, field experiments were set up according to fractional factorial design 2^{5-1} in two replications. The plot area was 6 m^2 , but the yield and its components were estimated on the basis of sample area of 1 m^2 . Sowing density was also 500 germinating seeds $\cdot \text{m}^{-2}$ of genotypes *Avena sativa* ver. *nuda*. Experimental factors and their levels were presented in Table 2.

Table 2

Agronomic factors and their levels in experiments conducted in Wierzbica

Agronomical factor	Factor levels	
	1 [low]	2 [high]
Genotype [experiment I]	Strain STH 4770	Cultivar Akt
Genotype [experiment II]	Strain STH 7000	Cultivar Akt
PK fertilization	0 kg · ha ⁻¹ PK	226 kg · ha ⁻¹ PK
Foliar nitrogen application	0 kg · ha ⁻¹ N	17 kg · ha ⁻¹ N
Plant growth regulator Moddus	0 dm ³ · ha ⁻¹	0.4 dm ³ · ha ⁻¹
Plant growth regulator Promalin	0 dm ³ · ha ⁻¹	0.15 dm ³ · ha ⁻¹

The experiments in Wierzbica were conducted on typical brown soil with pH 5.9. The contents of bioavailable P, K and Mg forms were from low to average level.

The strains were chosen for the experiments on the basis of their particular characteristics: 1. increased 1000 grain weight (STH 4770) and 2. short culm (STH 7000).

Papers on field experiments conducted using fractional designs have recently started to appear in the literature of the subject. At this point papers by Witkowicz and Antonkiewicz [13] and Witkowicz [14] should be mentioned or the work by Fabjan et al [15]. However, it should be pointed out that the method used by Fabjan et al [15] to present his results is quite different, since it does not use the standard regression coefficients, which would allow for an additional comparative assessment of the studied sources of variability.

In oat grain from each replication, dry-combustion in a muffle furnace at 550 °C for eight hours, after the ash dissolution in 20 % nitric (V) acid, the contents of copper, zinc, iron, manganese and cobalt were assessed using ICP-AES method [16].

The results obtained were subjected to statistical analysis using analysis of variance procedure. For the experiment conducted in Prusy, where each factor occurred on three levels, factors variability was divided into linear and square factors. Zero working hypotheses:

$$H_0: \sum_{i=1}^k k_i^2 = 0$$

were verified on the basis of F-Fisher test. Before the analyses of variance were conducted, the goodness of fit of features distribution with normal distribution was tested using Kołmogorow-Smirnow test, and the assumption of variance of error homogeneity by means of Bartlett's chi-square test. A multiple regression analysis was also conducted using analyses of variance models. For better comparison of the influence of individual factors, also standardized regression coefficients, whose statistical significance confirms statistically significantly effect of the appropriate source of variability, were compiled in the tables. For the factors occurring on three levels, the square coefficients of effects were also determined.

Results and discussion

Microelement content in oat grain was determined by all sources of variability that were analyzed. The contents of microelements ranged from 49.5 to 64.3 mgFe; 37.9–43.8 mgMn; 25.3–57.2 mgZn; 2.2–3.6 mgCu; 0.021–0.034 mgCo · kg⁻¹ d.m. (Tables 3, 4).

Table 3

Content of microelements in oat grain [mg · kg⁻¹ d.m.] from experiments situated in Wierzbica

Factor	Experiment I			Experiment II		
	The factor levels and standardized regression coefficient					
	1	2	Coefficient	1	2	Coefficient
Zn						
Genotype	53.8	44.8	-0.567***	57.2	44.8	-0.780***
PK	51.1	47.5	-0.224	50.5	51.6	0.066
N	46.9	51.7	0.299*	50.2	51.8	0.099
Moddus	48.7	49.9	0.077	48.9	53.2	0.270
Promalin	48.5	50.2	0.109	50.5	51.5	0.060
Cu						
Genotype	2.56	2.47	-0.026	3.50	2.47	-0.294
PK	2.65	2.37	-0.079	3.22	2.75	-0.134
N	2.40	2.62	0.062	2.84	3.13	0.084
Moddus	2.80	2.21	-0.166	3.36	2.61	-0.213
Promalin	2.56	2.46	-0.027	3.10	2.86	-0.068
Fe						
Genotype	61.6	60.0	-0.097	64.4	60.0	-0.261
PK	60.0	61.7	0.105	61.6	62.8	0.076
N	59.6	62.0	0.147	62.4	62.0	-0.024
Moddus	61.6	60.0	-0.098	60.4	64.0	0.221
Promalin	62.3	59.4	-0.177	63.4	61.0	-0.146
Co						
Genotype	0.021	0.031	0.351	0.029	0.031	0.070
PK	0.028	0.024	-0.164	0.032	0.027	-0.164
N	0.024	0.027	0.117	0.027	0.032	0.211
Moddus	0.031	0.021	-0.398*	0.033	0.026	-0.258
Promalin	0.028	0.023	-0.164	0.030	0.029	-0.023
Mn						
Genotype	40.6	38.0	-0.274*	43.8	38.0	-0.610**
PK	38.5	40.0	0.159	39.5	42.3	0.292
N	38.8	39.7	0.094	41.3	40.4	-0.092
Moddus	38.4	40.2	0.188	39.5	42.3	0.294
Promalin	38.7	39.8	0.113	40.7	41.1	0.039

* 0.01 < α < 0.05; ** 0.001 < α < 0.01; *** α < 0.001.

Also Kashin and Ubugunov [8] also observed this diminishing sequence and approximate microelement content in oat grain. The experiments revealed that, irrespective of habitat conditions, Act cv. was characterized by a lower content of zinc, copper, iron and manganese in comparison with STH 4770 and STH 7000 strains.

Table 4

Content of microelements in oat grain [$\text{mg} \cdot \text{kg}^{-1}$ d.m.] from the experiment situated in Prusy

Factor	The factor levels			Standardized regression coefficient of effects	
	1	2	3	linear	square
Zn					
Genotype	28.57	25.39	30.79	0.201*	0.488***
PK	28.07	27.17	26.85	-0.110	0.082
N	26.81	27.87	26.34	-0.042	-0.079
Moddus	25.38	28.29	26.72	0.150	-0.174*
Cu					
Genotype	2.80	3.29	3.66	0.204	0.028
PK	3.24	3.15	3.55	0.035	0.073
N	3.29	3.44	2.78	-0.145	-0.050
Moddus	3.07	3.27	3.42	0.261	-0.238
Fe					
Genotype	56.26	51.31	54.02	-0.210	0.332*
PK	49.51	53.48	55.34	0.067	0.143
N	53.72	51.83	55.27	0.053	0.152
Moddus	52.94	53.27	52.47	0.009	0.028
Co					
Genotype	0.030	0.028	0.023	-0.172	0.014
PK	0.034	0.024	0.028	-0.150	0.253
N	0.027	0.029	0.022	-0.129	-0.072
Moddus	0.031	0.026	0.027	-0.150	0.123
Mn					
Genotype	42.22	39.63	42.99	0.084	0.437***
PK	41.13	40.18	42.70	0.170	0.286**
N	39.41	41.83	40.29	0.096	-0.167
Moddus	39.30	41.63	40.91	0.164	-0.111

* $0.01 < \alpha < 0.05$; ** $0.001 < \alpha < 0.01$; *** $\alpha < 0.001$.

The effect of factors on the content of selected microelements in naked oat was discussed, according to their sequence, in Tables 3 and 4. A statistically significant (by 0.299 of standard deviation unit) increase in oat grain zinc concentration in effect of foliar nitrogen application was observed in the experiment I conducted in Wierzbica.

The same dependence was registered in experiment II, but it was not statistically confirmed. In Prusy, copper content in grain was close to statistical modification only by application Moddus growth regulator (tendency). No statistically significant effect of agronomic factors on iron concentrations in grain was noted in either of the two experiments conducted in Wierzbica or in Prusy. In Wierzbica, cobalt concentrations in grain in first experiment (I) declined by 0.398 of standard deviation unit under the influence of applied Moddus growth regulator. On the other hand, the genotype affected this microelement content with the strength close to statistical significance (0.351), which means that Act cv. contained by $0.01 \text{ mg} \cdot \text{kg}^{-1}$ more of cobalt than strains. In Prusy, cobalt content in grain was close to statistical modification owing to phosphorus-potassium fertilization. Application of phosphorus fertilization only caused a decrease in this microelement content in comparison both with the control and full fertilization. In Wierzbica, manganese concentrations in oat grain were shaped only by the genotype. On the other hand, in Prusy manganese content was changing according to square effects for the genotype and phosphorus-potassium fertilization.

The analyses of variance that were performed on the data from experiments confirmed also statistically significant influence of some interactions of the studied factors on microelement concentrations in oat grain (only in Wierzbica). The most often observed and statistically significant was the interaction of foliar nitrogen application with Moddus growth regulator, which also shaped the content of iron and cobalt. In both experiments with no nitrogen foliar fertilization applied, the use of Moddus growth regulator caused an increase in iron content. Application of foliar nitrogen fertilization generally caused increase in iron content but in the presence of the regulator, a decline in this microelement content was noted in oat grain (in comparison with combination without nitrogen fertilization) (Fig. 1, 2). Iron content was also shaped by the interaction of the genotype with Moddus growth regulator (experiment I). Application of the regulator caused a decrease in case of STH4770 strain and in Akt c.v. increase in iron content in grain (Fig. 3). However, interaction of the genotype with phosphorus and potassium fertilization bears on the content of zinc in experiment II (Fig. 4). In case of STH 4770 strain application of phosphorus-potassium fertilization led to increase in this

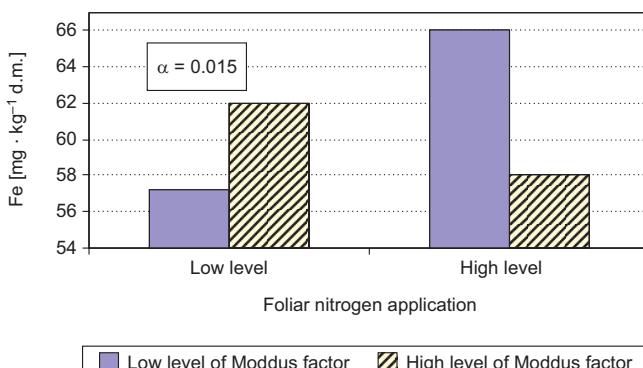


Fig. 1. Concentration of Fe in oat grain under the influence of interaction between urea foliar application and Moddus plant grow regulator (experiment I)

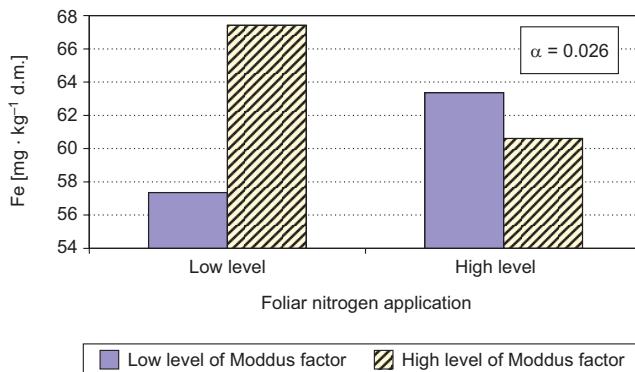


Fig. 2. Concentration of Fe in oat grain under the influence of interaction between urea foliar application and Moddus plant grow regulator (experiment I)

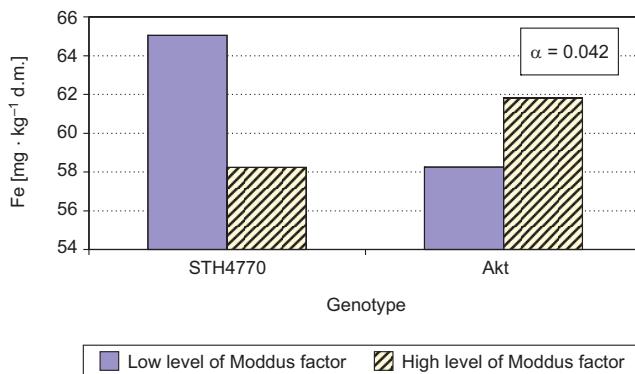


Fig. 3. Concentration of Fe in oat grain under the influence of interaction between genotype and Moddus plant grow regulator (experiment I)

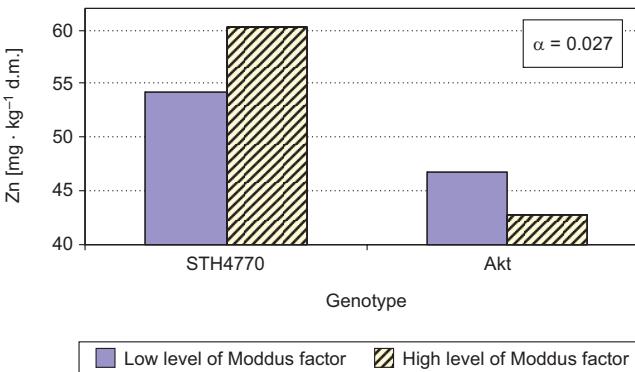


Fig. 4. Concentration of Zn in oat grain under the influence of interaction between genotype and PK fertilization (experiment II)

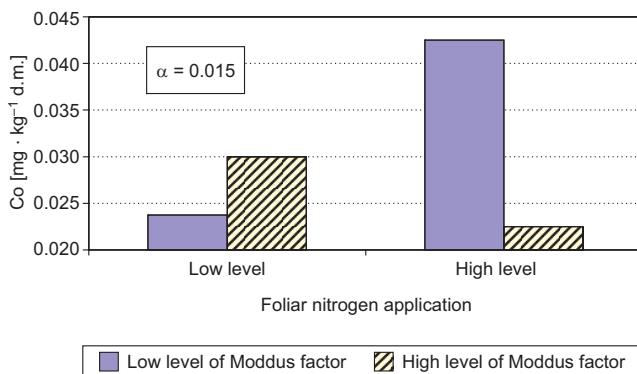


Fig. 5. Concentration of Co in oat grain under the influence of interaction between genotype and PK fertilization (experiment II)

element content, but in case of Akt c.v. led to a slight decline. Decreasing zinc content in Akt cv. grain may be connected with this metal precipitation in the insoluble form (zinc phosphates), *i.e.* unavailable to plants [17, 18]. The last interaction which has a statistically proven effect on cobalt concentrations in grain (experiment II) was the interaction of nitrogen foliar fertilization and Moddus growth regulator (Fig. 5). At the lack of nitrogen fertilization, application of Moddus growth regulator caused increased content of this element in grain, whereas a decline in the content was noted when foliar fertilization was applied.

According to the IUNG-PIB guidelines [19] the critical contents of zinc and copper in plant material destined for consumption are respectively: $50 \text{ mgZn} \cdot \text{kg}^{-1}$ and $20 \text{ mgCu} \cdot \text{kg}^{-1} \text{ d.m.}$. In the experiment situated in Prusy no effect was registered of either genotype or fertilization level and growth regulator on exceeding the permissible zinc concentration in oat grain. On the other hand, in the experiment in Wierzbica a slightly exceeded critical content of zinc was observed in STH 4770 and STH 7000 strains. Copper content in oat grain was on a very low level and from the perspective of farm animals needs it was an insufficient amount, because requirements of most animal groups are on the level of $20\text{--}30 \text{ mgCu} \cdot \text{kg}^{-1} \text{ d.m.}$ [20]. Optimum contents of iron and manganese in plants destined for fodders are $40\text{--}100 \text{ mgFe} \cdot \text{kg}^{-1} \text{ d.m.}$ and $50\text{--}60 \text{ mgMn} \cdot \text{kg}^{-1} \text{ d.m.}$ [21–23]. According to this criterion, iron concentrations in oat grain was on the optimal level, whereas manganese concentrations were below the optimal value. Mechanism of absorption and accumulation of zinc, copper and manganese allows to accumulate considerable amounts of these elements in grain and therefore may pose toxic hazard [8]. Microelement fertilization, which becomes more and more commonly applied particularly to plant raw materials, enhances the necessity of control and research on their content in plant raw materials. Blaziak [7] also confirmed in her research that under specific conditions of soil moisture the contents of these micro-elements in barley and oat grain were exceeded. The research on the effect of irrigation and NPK fertilization conducted by Koszanski et al [24] point to the effect of these factors on microelement content, but the authors did not confirm the fact by a statistical

analysis. Pisulewska et al [9] observed not only the effect of NPK fertilization on iron concentration in oat grain but also the lack of this factor effect on the contents of copper, manganese and zinc. Moreover, they did not notice any differences in these microelements concentrations between the traditional Dukat cv. and naked Akt cv.

Bergmann [25] divided cobalt content in plants designed for fodder into three classes: unsatisfactory – below 0.07 mg; average abundant – from 0.07 to 0.12 mg and sufficient over $0.12 \text{ mgCo} \cdot \text{kg}^{-1}$ d.m. Comparison of oat grain cobalt concentrations obtained in the experiments with German standards used for fodder value assessment shows that oat grain had insufficient content of this element.

Feed quality depends also on mutual quantitative relations between macro- and microelements. Stated value of the relations between elements has a cognitive importance (Tables 5, 6). A considerable range of proportion variability was found in the grain of studied genotypes, which evidences a big tolerance of oat to agronomic measures. Literature generally presents only selected proportions between elements, with reference to animal feed needs. Optimal iron to manganese ratio is 1.5–2.5:1 [20]. It is estimated that the ratio below 1.5 testifies a surplus of manganese and deficiency of iron, whereas when the iron to manganese ratio is over 2.5, a manganese deficiency and iron surplus occur in plants. The experiments showed that oat grain obtained in the experiment in Wierzbica revealed the optimal value of this ratio, whereas numerical value of this ratio in oat grain obtained in Prusy revealed a slight manganese surplus and iron deficiency.

Table 5

Relations between elements in oat grain in Prusy

Factor	The factor level			The factor level		
	1	2	3	1	2	3
	Relation Fe : Mn			Relation Fe : Co		
Cultivar/Strain	1.3	1.3	1,3	1875	1832	2349
PK	1.2	1.3	1,3	1456	2228	1976
N	1.4	1.2	1,4	1990	1787	2512
Moddus	1.3	1.3	1,3	1708	2049	1943
	Relation Mn : Co			Relation P : Zn		
Cultivar/Strain	1407	1415	1869	139.3	161.5	139.3
PK	1210	1674	1525	143.6	150.5	159.0
N	1460	1442	1831	154.8	148.9	152.6
Moddus	1268	1601	1515	159.6	147.0	153.4

It is considered that the limit value of phosphorus to zinc ratio should be 400:1 [20]. At the same time it has been emphasized that the value of this ratio may be often a better indicator of plant supply in zinc than this microelement content in plants. This ratio in sick plants, underfed with zinc is wider than in healthy plants. The research

shows that the oat grain revealed an optimal content of zinc and the limit value of phosphorus-zinc ratio was not exceeded.

Table 6

Relations between elements in oat grain in Wierzbica

Factor	Experiment I		Experiment II		Experiment I		Experiment II	
	The factor levels				The factor levels			
	1	2	1	2	1	2	1	2
	Relation Fe : Mn				Relation Fe : Co			
Cultivar/Strain	1.5	1.6	1.5	1.6	2935	1936	2219	1936
PK	1.6	1.5	1.6	1.5	2142	2571	1924	2327
N	1.5	1.6	1.5	1.5	2484	2298	2311	1937
Moddus	1.6	1.5	1.5	1.5	1988	2858	1829	2462
Promalin	1.6	1.5	1.6	1.5	2225	2581	2113	2103
	Relation Mn : Co				Relation P : Zn			
Cultivar/Strain	1933	1225	1511	1224	68.9	82.6	68.5	82.6
PK	1376	1668	1234	1566	71.2	79.1	75.2	73.9
N	1618	1471	1531	1264	78.8	71.5	76.0	73.1
Moddus	1238	1913	1196	1627	73.3	76.7	75.1	74.1
Promalin	1384	1731	1357	1416	78.2	72.1	77.5	71.6

The ratios of iron to cobalt and manganese to cobalt were diversified in oat grain and depended on the level of agronomic measures, including mineral fertilization. Introducing foliar application of nitrogen definitely declined numerical values of these ratios, whereas the application of phosphorus and potassium fertilization and growth regulators led to widening of these ratios. The research demonstrated that proportions between elements in oat grain were modified by agronomic factors. Spann et al [26] also observed disturbed chemical balance in plants under the influence of fertilization. As different whole grains have different composition, technologies should be developed to allow the use of versatile grain raw material, also multigrain products and knew product concepts [27].

Conclusions

1. It was observed, that irrespective of site conditions in which experiments were conducted, Akt c.v. generally revealed lower concentrations of zinc, copper, iron and manganese in comparison with STH4770 and STH 7000 strains.
2. Assessing zinc, copper and cobalt concentrations in oat grain from the angle of standardized regression coefficients values, it should be stated that generally their content to the greatest extent depended on Moddus growth regulator application.
3. The agronomic factors taken into consideration did not lead to exceeding the permissible content of zinc in oat grain destined for consumption.

4. Oat grain obtained in the experiments, perceived as fodder grain revealed the optimal contents of iron but deficiency of manganese, copper and cobalt.

5. Proportions among the elements in oat grain were modified by agronomic factors.

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**WPIĘW ZABIEGÓW AGROTECHNICZNYCH
NA ZAWARTOŚĆ WYBRANYCH MIKROPIERWIASTKÓW
W ZIARNIE OWSA NAGOZIARNISTEGO (*Avena sativa var. nuda*)**

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Abstrakt: Składnikami niezbędnymi do pokrycia potrzeb pokarmowych roślin, obok makroskładników, są również mikroelementy. Zawartość mikroelementów w surowcach roślinach jest często modyfikowana różnymi zabiegami agrotechnicznymi, dlatego też nie zawsze odpowiada zapotrzebowaniu na ten składnik. Celem przeprowadzonych badań było określenie wpływu czynników agrotechnicznych na zawartość cynku, miedzi, żelaza, kobaltu i manganu w ziarnie owsa. Trzy eksperymenty polowe założono według planów frakcyjnych (2^{5-1} i 3^{4-1}) w dwóch miejscowościach (Wierzbica i Prusy) w 2003 r. Odmiana Akt cechowała się niższą zawartością cynku, miedzi, żelaza i manganu w porównaniu do rodów STH 7000 i STH 4770. Zawartość mikropierwiastków w ziarnie owsa była determinowana doborem odmiany/rodu owsa oraz dawką regulatora wzrostu. Zawartość cynku w ziarnie owsa spełniała wymogi stawiane roślinom przeznaczonym na cele konsumpcyjne i paszowe. Stwierdzono optymalną zawartość żelaza, a niedoborową manganu, miedzi i kobaltu w ziarnie owsa traktowanego jako pasza.

Słowa kluczowe: owsie nagoziarniste, mikroelementy, nawożenie, regulator wzrostu

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MOLDING WATER CONTENT OF CLAY SOILS AND HYDRAULIC PROPERTIES OF MINERAL LINERS OF WASTE LANDFILLS

WILGOTNOŚĆ ZAGĘSZCZANIA MATERIAŁÓW ILASTYCH A WŁAŚCIWOŚCI HYDRAULICZNE PRZESŁON MINERALNYCH SKŁADOWISK ODPADÓW

Abstract: Municipal landfills as engineering constructions highly dangerous to the natural environment have to be isolated by liners in order to prevent the anthropogenic pollutants transport, together with *eg* landfill leachates. Mineral liners, properly prepared and compacted, sealing the bottom, sides and the top of the landfills are one of the most popular manners of their isolation. The mineral liners are usually constructed of compacted clay soils to obtain, the required by the Polish Decree of the Minister of Environment of 3rd April 2013 and the Council Directive 1999/31/EC of 26th April 1999 on the landfill of wastes, value of liner's saturated hydraulic conductivity lower than $1 \cdot 10^{-9} \text{ m} \cdot \text{s}^{-1}$. The value of hydraulic conductivity of saturated soils is directly affected by the conditions of soil compaction, especially a molding water content. This paper presents an attempt of the determination of the effects of the molding water content of a selected clay soil on its saturated hydraulic conductivity and hydraulic properties of the sealing liner, constructed according to the actual standards, of the compacted clay material. Range of our studies covered the *in situ* and laboratory measurements as well as numerical modeling. Saturated hydraulic conductivity under natural conditions was measured by BAT probe, (GeoNordic) while the hydraulic conductivity of the compacted clay soils was tested by Humboldt Mfg. Co. permeameters for compacted soils, in accordance with ASTM D5856. The assessment of hydraulic properties of a bottom liner made of the clay material under study was performed by the method of numerical modeling of infiltration process with the assumed value of groundwater head with an application of the FEFLOW, DHI-WASY modeling software. The lack of validation in our modeling attempt influences the fact that our studies should be treated as preliminary.

Keywords: clay materials, mineral lines, hydraulic conductivity, numerical modeling, waste landfill

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Introduction

Migration of numerous pollutants by air, surface runoff, and leachates resulting from landfilling of municipal wastes poses a considerable threat to the natural environment. The environmental impact of landfills is directly connected to the efficiency of restraining the pollution of air, water and soil by applied various techniques of sealing [1]. Leachate seepage and migration from landfill cells is prevented by barriers, known as liners, which utilize different technical solutions based on natural and geosynthetic materials.

Mineral clay liners that meet the requirements of local standards [2, 3] are one of several possible popular and durable solutions. These barriers are constructed of natural clays, the permeability of which is capable to secure the required value of hydraulic conductivity [4, 5]. In the European Union its value should be lower than $1 \cdot 10^{-9} \text{ m} \cdot \text{s}^{-1}$. The saturated hydraulic conductivity of clay soils may be higher under natural conditions than the required value [6–8] so the application of compaction may be necessary. The compaction changes bulk density and increases resistance of soil to water (or leachate) flow, as a result, saturated hydraulic conductivity is reduced [9]; however, the degree of reduction depends on the molding water content of the soil. Thus, the molding water content becomes one of the most important factors influencing hydraulic characteristics of liners consisting of compacted clay [10–14].

In addition to changes in bulk density and hydraulic conductivity, molding water content also affects the subsequent swelling and shrinking properties of clays influencing the sustainability of the liner [15–18]. Even highly compacted clays, are prone to shrinking when drying. The shrinking of clay materials is usually connected with their cracking [19]. Each drying and wetting cycle, combined with soil cracking, results in an increase of bulk density of soil, a decrease of its void ratio and an increase of hydraulic conductivity after resaturation. The aforementioned can be explained by the fact that each change of soil volume caused by wetting and drainage (or subsequent re-wetting) is related to changes in pore volume and distribution of pore sizes [19, 20]. It is therefore important to determine the influence of molding water content on hydraulic properties of clay material used in natural barriers construction in relation to its shrinking and swelling potential.

Additionally, molding water content of compacted clay materials – affecting their swelling-shrinkage properties – also influences the long-term stability of mineral liners [4]. However, in case of the bottom liner the eventual swelling may be treated as an advantageous feature, improving the sealing properties by increasing the water holding capacity of clays. The shrinkage of bottom liner covered by waste body and saturated by groundwater is less possible than the shrinkage of the top sealing clay liner, even with the additional cover by the recultivation layer.

This paper presents an attempt to assess the molding water content influence on saturated hydraulic conductivity as well as on bulk density of compacted clay soil and hydraulic properties of the bottom sealing liner, constructed according to the actual standards. Our studies were based on *in situ* and laboratory measurements, as well as on numerical modeling method.

Materials and methods

The mineral clay soil sampled in the open pit of a former brickyard in Łazek Garncarski, approx. 90 km south of Lublin, Poland was used in our studies. The particle size composition of the sampled soil and its basic characteristics such as bulk density, saturated hydraulic conductivity and water content under *in situ* conditions are presented in Table 1.

Table 1
Characteristics of the clay soil sampled in Łazek Garncarski, Poland, under *in situ* conditions

Particle fraction name	Sand [%]	4.5
	Silt [%]	51.0
	Clay [%]	44.5
Solid particle density [$\text{Mg} \cdot \text{m}^{-3}$]		2.614
Bulk density [$\text{Mg} \cdot \text{m}^{-3}$]		1.693
Gravimetric water content [%]		21.18
Total porosity [$\text{m}^3 \cdot \text{m}^{-3}$]		0.352
Saturated hydraulic conductivity [$\text{m} \cdot \text{s}^{-1}$]		$1.37 \cdot 10^{-10}$

Particle size distribution of the soil, presented in Table 1, was determined by the standard areometric method according to PN-B-04481: 1988 [21], solid particle density was measured in le Chatelier flask and gravimetric water content was obtained by the standard oven drying method according to ASTM C566-13 [22]. The saturated hydraulic conductivity of the tested soil under natural, undisturbed conditions was measured by a field permeameter for fine grained soils GeoN by Geo Nordic, Stockholm, Sweden, directly in the location of soil sampling in Łazek Garncarski.

The changes of the particle size distribution was additionally measured by the laser diffraction method. This method, based on measurement of light intensity scattered on investigated suspension, is wildly used in soil and sediment laboratories [23]. It is particularly useful when it comes to measuring the size distribution of very small volume and/or determination of the small differences in the content of each fraction [24].

The Mastersizer 2000 (Malvern, UK) with hydro MU was used as the laser diffractometer [25]. The following parameters and settings were used: speed of the pump and stirrer (in this dispersion unit both are integrated) – 2500 rpm., 4 min of ultrasounds with 35 W of the power, Mie theory with the refractive index equal to 1.52 and absorption index equal to 0.1. The use two different methods (sedimentation and laser diffraction method) for measurement of particle size distributions was dictated by the fact of significant differences in the results obtained by both [26]. Therefore the sedimentation method was used to determine the particle size distribution of investigated material (it allowed to classify it to the clays) and laser diffraction method allowed to show the subtle differences in the particle distribution (which is impossible in sedimentation methods).

Microscopic analyses of the tested soils structure were performed by scanning electron microscope Quanta SEM 200 FEG by FEI, USA.

Laboratory measurements of saturated conductivity of the tested clay material compacted at various water contents were performed in the permeameters for compacted soils by Humboldt Mfg. Co, USA. The H-4145 cylindrical compaction permeameters of mold's diameter equal to 101.6 mm and height of 116.4 mm and the falling water head method of measurements meeting requirements of ASTM D5856-95 [27] were applied to our studies. The soil was compacted, at different molding water contents, according to Polish standard PN-B-04481: 1988 [21]. The following values of molding water contents (by weight) were applied during our laboratory studies: 14 %, 17 %, 19 %, 21 %, 22 % and 23 %.

Two dimensional numerical modeling of hydraulic efficiency of a bottom mineral liner constructed of the compacted clay soil was performed by FEFLOW 6.0, WASY-DHI, a German modeling software. FEFLOW is a well-known and successfully verified numerical tool, based on the finite elements/volumes method allowing calculations of water and mass transport in saturated, unsaturated or variably saturated porous medium [28–32]. The developed two dimensional model represented a 1m wide mineral liner of 1m thickness, required by the actual Polish and European standards [2, 3]. The prepared model consisted of 2831 nodes and 5472 elements.

Numerical calculations of the two dimensional water flow in FEFLOW were based on standard forms of Darcy's and Richards' equations [33–36]:

$$\mathbf{q}_i = -\mathbf{K}_{ij} \frac{\partial h}{\partial x_j}$$

$$S_0 \frac{\partial h}{\partial t} = -\frac{\partial \mathbf{q}_i}{\partial x_i} + Q$$

where: \mathbf{q}_i – groundwater flux vector [$\text{m} \cdot \text{s}^{-1}$],

h – hydraulic pressure head [m],

t – time [s],

\mathbf{K}_{ij} – hydraulic conductivity tensor, $j = 1, 2$ [$\text{m} \cdot \text{s}^{-1}$],

Q – sink or source term [s^{-1}],

S_0 – specific storage compressibility [m^{-1}], $S_0 = 1 \cdot 10^{-4}$ [m^{-1}].

Mathematical description of water retention curve was presented by van Genuchten [36]:

$$\theta = \frac{\theta_s - \theta_r}{[1 + (\alpha h)^n]^m} + \theta_r$$

where: θ_s – saturated volumetric water content [$\text{m}^3 \cdot \text{m}^{-3}$],

θ_r – residual volumetric water content [$\text{m}^3 \cdot \text{m}^{-3}$], $\theta_r = 0$ [$\text{m}^3 \cdot \text{m}^{-3}$],

h – pressure head [m],

α – fitting parameter [m^{-1}],

n, m – fitting parameters, $m = 1 - n^{-1}$.

Hydraulic conductivity of unsaturated soils K was calculated in the presented model according to van Genuchten's formula [35, 36]:

$$K = K_s S_e^l \left[1 - (1 - S_e^{\frac{1}{m}})^m \right]^{-2}$$

where: K_s – saturated conductivity [$\text{m} \cdot \text{s}^{-1}$],

l – fitting parameter, $l = 0.5$ [36],

S_e – dimensionless effective saturation defined as:

$$S_e = \frac{\theta - \theta_r}{\theta_s - \theta_r}$$

The retention characteristics of the soil described by van Genuchten model [36] applied to numerical calculations are presented in Table 2. The isotropic soil was taken into consideration in our calculations due to the small scale model developed [32].

Table 2
Retentional characteristics of the clay soil applied to numerical calculations

Saturated water content by volume θ_s [$\text{m}^3 \cdot \text{m}^{-3}$]	Fitting parameter α [m^{-1}]	Fitting parameter n [-]
0.352	0.0269	1.354

The required input data for water retention characteristics were determined by laboratory measurements including a sand box in the range of $h < 0.1$ bar as well as pressure chambers with 1 bar, 2 bar, 5 bar and 15 bar ceramic plates, produced by Soil Moisture Equipment Corp, USA. Numerical modeling of two dimensional gravitation water flow through the mineral liner required assumption of the necessary initial and boundary conditions. The initial condition was assumed as a full liner's soil saturation, *ie* $S = 1.0$. The bottom boundary condition was assumed as the constant Dirichlet type condition in which the water head was equal to -5.0 m. The variable Dirichlet type top boundary condition represented by various values of water pressure head over the modeled liner was selected for our calculations. The applied values of assigned pressure head were assumed as 0.01 m, 0.5 m, 1 m and 5 m. The assumed time of simulation covered one hydrologic year, *ie* 365 days.

Results and discussion

The microscopy analyses performed for representative samples of the compacted clay material for all of the tested molding water contents showed visual differences in the spatial structure.

Figures 1 and 2 present the exemplary scanning pictures (magnification 100 and 1000 times) of soil samples surface after cracking of the prepared samples cut out from the mold showing differences in soil particles spatial arrangement. These figures reveal that the increase of water content during soil compaction results in a higher compaction

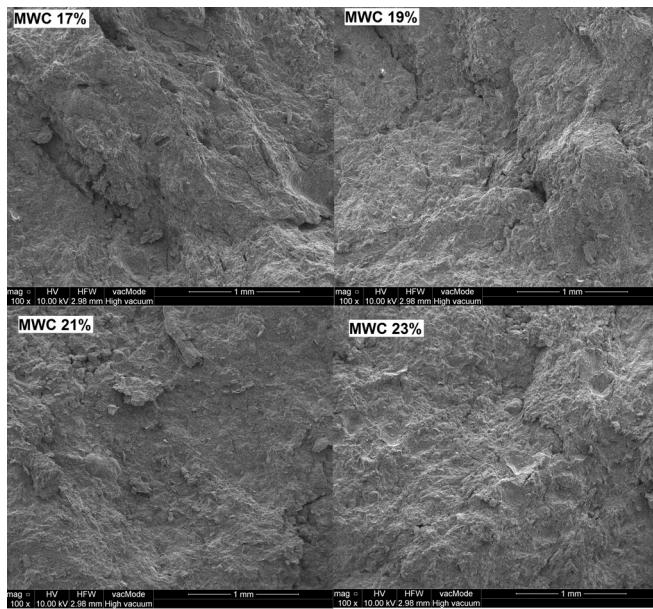


Fig 1. Scanning microscopy pictures of soil samples surface for selected molding water contents (MWC) applied in magnification of 100 times

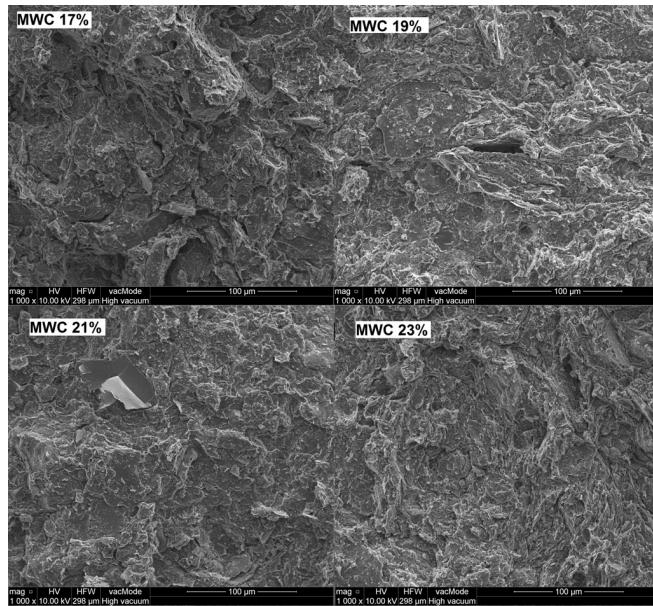


Fig 2. Scanning microscopy pictures of soil samples surface for selected molding water contents (MWC) applied in magnification of 1000 times

of soils particles. In the case of higher molding water content, the macropores ($> 75 \mu\text{m}$) and mesopores ($75\text{--}30 \mu\text{m}$) appear rarely, if ever observed. Fig. 2 presents the surface of soil samples without macropores and with limited mesopores, allowing for the assessment of the micropores influence on the total porosity of the presented samples.

The additional measurements of soil particle size distribution performed by laser light diffraction method enabled the assessment of the influence of temporal (approx. 30 days) saturation and the applied permeability tests on washing out of clay particles. After measuring the saturated hydraulic conductivity, a small decrease in the content of clay particles was observed for all tested samples. The range of the previously noted decrease was 1–9 % of clay content. The highest clay removal was observed for soil compacted with molding water content equal to 17 %. All values of clay fraction content decrease are presented in Fig. 3, showing clay content in compacted material related to its initial content in an undisturbed sample (100 %).

The applied method of laser diffraction used as an additional method of soil particle distribution measurement allowed us to assess the changes of particle distribution

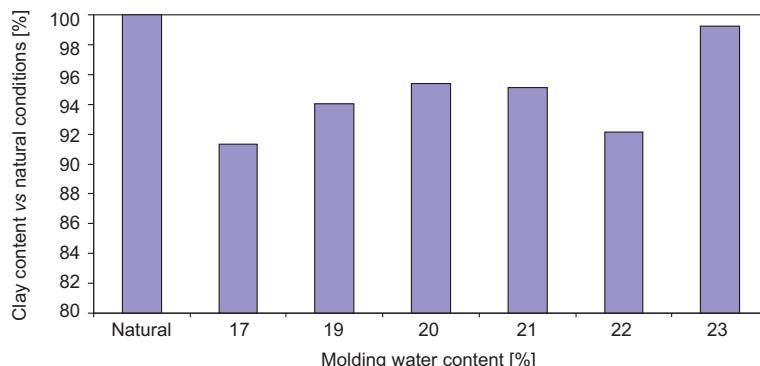


Fig. 3. Changes of clay fraction content in compacted soil related to its content in natural undisturbed sample (100 %)

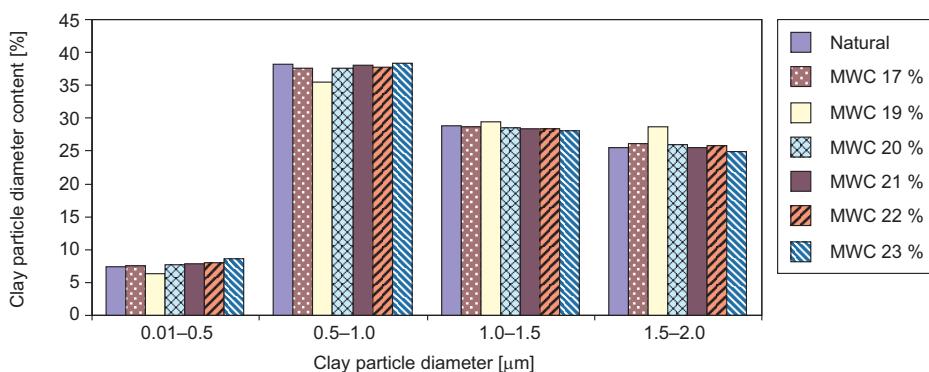


Fig. 4. Clay particles distribution in tested soil samples after permeability tests, 100 % = total clay content (MWC = molding water content)

belonging to selected intervals of clay material's diameters inside the 0.01–2.0 micrometers range – see Fig. 4. The following intervals were tested: 0.01–0.5, 0.5–1.0, 1.0–1.5 and finally 1.5–2.0 micrometers. Fig. 4. shows that despite the fact that the total clay content after permeability tests changed to a small extent (Fig. 3), the contents of particles in tested sub-fraction are comparable.

The results of saturated hydraulic conductivity measurements as well as bulk density and total porosity tests for the applied molding water contents are presented in Table 3 and in Fig. 5.

The data presented in Table 3 show a clear decrease in the saturated hydraulic conductivity with an increase of bulk density resulting from the increase of the molding water content. The results show that compaction was performed on both sides of the standard Proctor's curve. Additionally, it is visible that saturation of the compacted clay material, leading to its swelling of soil, affects its bulk density and total porosity.

Table 3

Saturated hydraulic conductivity, total porosity and bulk density of the soil dependent on molding water content

Tested parameter	Molding water content [% by weight]					
	14	17	19	21	22	23
Saturated hydraulic conductivity [$\text{m} \cdot \text{s}^{-1}$]	$3.936 \cdot 10^{-9}$	$1.000 \cdot 10^{-10}$	$7.325 \cdot 10^{-11}$	$3.694 \cdot 10^{-11}$	$3.280 \cdot 10^{-11}$	$3.210 \cdot 10^{-11}$
Soil bulk density after compaction [$\text{Mg} \cdot \text{m}^{-3}$]	1.604	1.659	1.702	1.707	1.707	1.669
Total porosity after compaction [$\text{m}^3 \cdot \text{m}^{-3}$]	0.386	0.365	0.349	0.347	0.347	0.361
Bulk density after swelling [$\text{Mg} \cdot \text{m}^{-3}$]	1.447	1.518	1.585	1.629	1.621	1.603
Total porosity after swelling [$\text{m}^3 \cdot \text{m}^{-3}$]	0.446	0.419	0.393	0.377	0.380	0.387

The decrease of hydraulic conductivity with the increase of molding water content, was very sharp ranging between 14 and 19 %, which is clearly visible in Fig. 5. Then, the value of saturated hydraulic conductivity decreased slightly, reaching an almost constant value for the highest molding water contents, between 21 and 23 %. The results of the laboratory measurement presented in Fig. 5 clearly show that increasing the molding water content for the tested clay soil from 14 to 23 % of gravimetric water content results in a decrease of saturated hydraulic conductivity by two orders of magnitude, *i.e* over 100 times.

The changes of bulk density and total porosity are also related to molding water content. The observed changes of soil bulk density and of its total porosity resulting

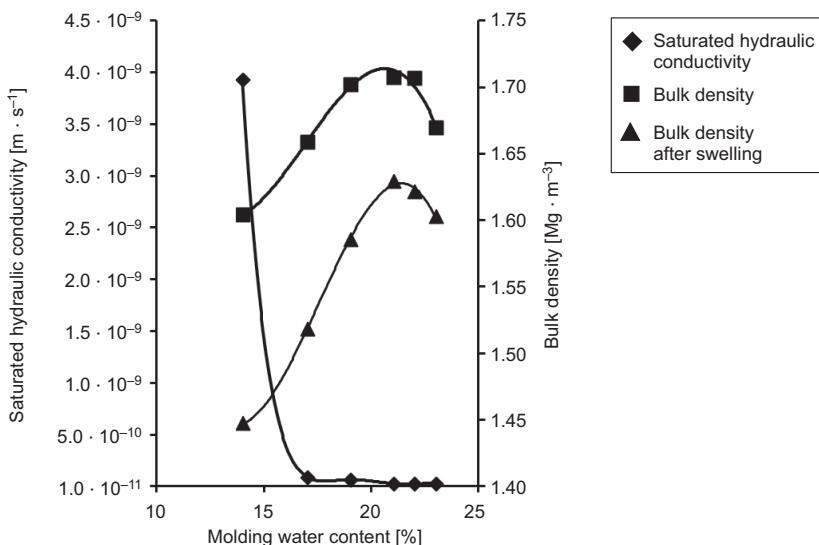


Fig. 5. Influence of molding water content by weight on saturated hydraulic conductivity, bulk density after Proctor test and bulk density after swelling for the tested clay material

from the increase of molding water content showed that for the tested clay soil sampled in Lazek Garnarski, the maximum value of soil bulk density and minimal value of total porosity were achieved for moisture content equal to 22 % by weight. The discussed changes in soil's bulk density induced changes of total porosity. The minimal observed porosity reached a value of 0.347 for the molding water content equal to 21 and 22 %. The highest degree of compaction results in the lowest porosity, limiting retention properties of soil.

Similar situations may be observed for changes of compacted soils bulk density after swelling. The maximum noted value of bulk density for swelled soil was observed at a molding water content of 21 %. Our studies showed the decrease in bulk density after swelling vs. bulk density of compacted soil in the range of 9.8 for 14 % of molding water content to 4.0 % for water contents of 21 and 23 %. The values of 13.5 to 6.6 % of compacted clay total porosity increase after swelling for molding water content of range 14–23 % were observed. These results support literature reports related to the increased water capacity of swelled clay materials.

The results of numerical calculations of water seepage (representing leachate infiltration) through a 1 m thick layer of the clayey material compacted with various molding water contents representing a bottom sealing liner of municipal waste landfill are presented in Fig. 6.

The results presented in Fig. 6 show that the hydraulic properties of the bottom mineral clay liner, which act as a barrier for pollutants propagation made of the compacted clay material directly depend on molding water content. The lower the molding water content, the higher saturated hydraulic conductivity and the higher infiltration rate for the same value of water head applied to the upper boundary of

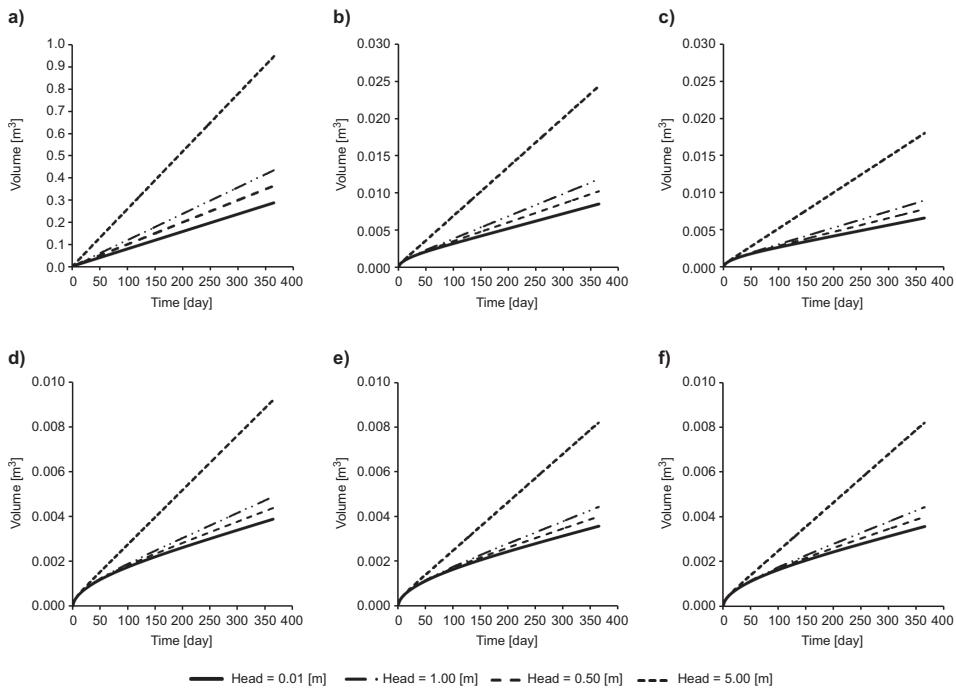


Fig. 6. Calculated cumulative volume of seepage through mineral bottom liner made of the clay soil compacted at different water contents: a) 14 %, b) 17 %, c) 19 %, d) 21 %, e) 22 %, f) 23 %

the bottom liner. Table 4 shows the observed mean values of daily seepage volume for all the applied values of water head and the molding water contents under consideration.

Table 4

Mean daily seepage for all the tested molding water contents
and the applied water pressure head values

Molding water content [% by weight]	Mean daily seepage [$\text{m}^3 \cdot \text{day}^{-1}$]			
	Water pressure head [m]			
	0.01	0.5	1.0	5.0
14	$0.792 \cdot 10^{-3}$	$0.999 \cdot 10^{-3}$	$1.191 \cdot 10^{-3}$	$2.597 \cdot 10^{-3}$
17	$0.023 \cdot 10^{-3}$	$0.028 \cdot 10^{-3}$	$0.032 \cdot 10^{-3}$	$0.067 \cdot 10^{-3}$
19	$0.018 \cdot 10^{-3}$	$0.021 \cdot 10^{-3}$	$0.024 \cdot 10^{-3}$	$0.049 \cdot 10^{-3}$
21	$0.011 \cdot 10^{-3}$	$0.012 \cdot 10^{-3}$	$0.013 \cdot 10^{-3}$	$0.025 \cdot 10^{-3}$
22	$0.010 \cdot 10^{-3}$	$0.011 \cdot 10^{-3}$	$0.012 \cdot 10^{-3}$	$0.023 \cdot 10^{-3}$
23	$0.010 \cdot 10^{-3}$	$0.011 \cdot 10^{-3}$	$0.012 \cdot 10^{-3}$	$0.022 \cdot 10^{-3}$

The results of the mean daily water seepage, related to the water pressure head values triggering infiltration flow, show that better sealing of landfill waste body by natural

bottom liner constructed of compacted clay material is obtained when the clay material is compacted at higher values of water content. This statement does not include the phenomenon of shrinkage potential which is much higher on the wetter branch of the Proctor curve and causes more intense cracking leading to preferential flow higher by several orders of magnitude [5].

Increasing molding soil water content from 14 to 23 % allowed for the reduction of the volume of seepage by two orders of magnitude for all the applied values of pressure head. However, the results of the calculations for the last three tested values of molding water content, *ie* 21 %, 22 % and 25 % show a minimal, insignificant decrease of daily seepage volume.

Conclusions

Our studies support literature reports proving that there is a direct relation between water content in a clay soil during compaction and its saturated water conductivity (inducing the modification of its general hydraulic characteristics). This relation creates more effective sealing properties, *ie* lower permeability of the compacted mineral liner when soil is compacted at higher values of water content. In our case, the increase of molding water content from 14 to 23 % resulted in a decrease of saturated hydraulic conductivity of the compacted soil from $3.936 \cdot 10^{-9}$ to $3.21 \cdot 10^{-11} \text{ m} \cdot \text{s}^{-1}$. Additionally, the performed numerical modeling of infiltration through the compacted clay liner showed that two orders of magnitude decrease daily infiltration rate through the 1.0 m thick clay liner, possible through the increase of molding water content by 9 % (from 14 to 23 %) for all the values of water pressure head under consideration (0.01–5 m). The above shows that selection of the proper molding water content during the construction of the municipal landfill cell liner of compacted clay material is crucial because it may significantly influence the effectiveness of the sealing, thus preventing migration of the pollutants into the natural environment. The possibility of soil cracking induced by dewatering reduces the sealing properties of the liner. This is significant in case of higher molding water contents, as it increases the shrinking potential of the soil. Our studies should be extended to include different types of clay soils and their shrinkage properties. The presented numerical assessment of water/leachate seepage through the modeled bottom liner in future should include various retention characteristics for each molding water content applied. The lack of validation in our simulation calculations influences the fact that our modeling studies should be treated as preliminary.

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WILGOTNOŚĆ ZAGĘSZCZANIA MATERIAŁÓW ILASTYCH A WŁAŚCIWOŚCI HYDRAULICZNE PRZESŁON MINERALNYCH SKŁADOWISK ODPADÓW

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Abstrakt: Składowiska odpadów jako szczególnie uciążliwe dla środowiska budowle inżynierskie muszą być izolowane przesłonami w celu zapobiegania rozprzestrzeniania się wraz z m.in. odciekami zanieczyszczeń antropogenicznych pochodzących ze składowiska. Jednym ze sposobów zapewniania izolacji składowisk są przesłony mineralne. odpowiednio przygotowane i zagęszczone, zabezpieczające dno, boki oraz powierzchnię składowiska. Przesłony mineralne są najczęściej wykonywane z odpowiednio zagęszczonych gruntów ilastych tak, aby zgodnie z Rozporządzeniem Ministra Środowiska z 30 kwietnia 2013 r. w sprawie składowisk odpadów oraz Council Directive 1999/31/EC z 26 kwietnia 1999 r. w sprawie składowania odpadów, przepuszczalność hydrauliczna przesłony była niższa niż $1 \cdot 10^{-9} \text{ m} \cdot \text{s}^{-1}$. Bezpośredni wpływ na wartość współczynnika przewodnictwa wodnego w stanie pełnego nasycenia mają warunki, w których prowadzone jest zagęszczanie gruntu, a dokładnie wilgotność ośrodka porowatego w czasie zagęszczania. Praca niniejsza przedstawia próbę określenia wpływu wilgotności zagęszczania wybranych gruntów ilastych na ich przepuszczalność w stanie pełnego nasycenia oraz właściwości hydrauliczne wykonanej z nich, zgodnie z obowiązującym statem prawnym, dolnej przesłony składowiska odpadów. Zakres pracy obejmował badania terenowe, laboratoryjne oraz modelowe. Przewodnictwo hydrauliczne gruntów w stanie naturalnym określono za pomocą polowej sondy BAT, GeoNordic, przewodnictwo zaś w stanie pełnego nasycenia po zagęszczaniu pomierzono za pomocą przepuszczalnościomierzy Humboldt Mfg. Co. do gruntów zagęszczonych wg ASTM D5856. Oceny właściwości hydraulicznych przesłon wykonanych z badanych materiałów ilastych zrealizowano poprzez modelowanie numeryczne procesu infiltracji przy zadanej wysokości naporu wód gruntowych zrealizowane za pomocą programu obliczeniowego FEFLOW, DHI-WASY. Ze względu na brak walidacji modelu otrzymane wyniki należy traktować jako wyniki badań wstępnych.

Słowa kluczowe: materiały ilaste. przesłony mineralne. przewodnictwo hydrauliczne. modelowanie numeryczne

Varia

INVITATION FOR ECOpole '15 CONFERENCE



CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 24th annual Central European Conference ECOpole '15, which will be held in 14–17.10.2015 (Wednesday–Saturday) in Hotel Ziemiowit in Jarnoltowek, PL.

The Conference Programme includes oral presentations and posters and will be divided into four sections:

- SI Chemical Pollution of Natural Environment and Its Monitoring
- SII Environment Friendly Production and Use of Energy
- SIII Forum of Young Scientists and Environmental Education in Chemistry
- SIV Impact of Environment Pollution on Food and Human Health

The Conference language is English.

Contributions to the Conference will be published as:

- abstracts on the CD-ROM (0.5 page of A4 paper sheet format),
- extended Abstracts (6–8 pages) in the semi-annual journal *Proceedings of ECOpole*,
- full papers will be published in successive issues of the *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (Ecol. Chem. Eng.) ser. A or S.

Additional information one could find on Conference website:

ecopole.uni.opole.pl

The deadline for sending the Abstracts is **15th July 2015** and for the Extended Abstracts: **1st October 2015**. The actualized list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one can find (starting from **31st July 2015**) on the Conference website.

The papers must be prepared according to the Guide for Authors on Submission of Manuscripts to the Journals.

The Conference fee is 400 € (covering hotel, meals and transportation during the Conference). It could be reduced (to 250 €) for young people actively participating in the Forum of Young Scientists. But the colleague has to deliver earlier the Extended Abstract (6-8 pages) of his/her contribution (deadline is on 15.08.2015), and a recom-

mendation of his/her Professor. Fees transferred after 1st September 2015 are 10 % higher. Please, fill in the **Registration Form** and send it via email or fax.

At the Reception Desk each participant will obtain abstracts of the Conference contributions as well as the Conference Programme recorded on electronic media (the Programme will be also published on the ECOpole '15 website).

After the ECOpole '15 Conference **it will be possible to publish electronic version of presented contributions** (oral presentations as well as posters) on this site.

Further information is available from:

Prof. dr hab. inż. Maria Waclawek

Chairperson of the Organising Committee
of ECOpole '15 Conference

University of Opole

email: Maria.Waclawek@o2.pl

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MEALS

Date	Breakfast	Lunch	Dinner
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**ZAPRASZAMY DO UDZIAŁU
W ŚRODKOWOEUROPEJSKIEJ KONFERENCJI ECOpole '15**



**SUBSTANCJE CHEMICZNE
W ŚRODOWISKU PRZYRODNICZYM
w dniach 14–17 X 2015 r. w hotelu Ziemowit w Jarnoltówku**

Będzie to **dwudziesta czwarta z rzędu** konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotyczącym różnych aspektów ochrony środowiska przyrodniczego.

Doroczne konferencje ECOpole mają charakter międzynarodowy i za takie są uznane przez Ministerstwo Nauki i Szkolnictwa Wyższego. Obrady konferencji ECOpole '15 będą zgrupowane w czterech Sekcjach:

- SI Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring
- SII Odnawialne źródła energii i jej oszczędne pozyskiwanie oraz użytkowanie
- SIII Forum Młodych (FM) i Edukacja prośrodowiskowa
- SIV Wpływ zanieczyszczeń środowiska oraz żywności na zdrowie ludzi

Materiały konferencyjne będą opublikowane w postaci:

- abstraktów (0,5 strony formatu A4) na CD-ROM-ie,
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- artykułów: w abstraktowanych czasopismach: *Ecological Chemistry and Engineering/ Chemia i Inżynieria Ekologiczna (Ecol. Chem. Eng.)* ser. A i S oraz w półroczniku *Chemistry – Didactics – Ecology – Metrology (Chemia – Dydaktyka – Ekologia – Metrologia)*.

Termin nadsyłania angielskiego i polskiego streszczenia o objętości 0,5–1,0 strony (wersja cyfrowa) planowanych wystąpień upływa w dniu 15 lipca 2015 r. Lista prac zakwalifikowanych przez Radę Naukową Konferencji do prezentacji będzie sukcesywnie publikowana od 31 lipca 2015 r. na stronie webowej konferencji. Aby praca (dotyczy to także streszczenia, które powinno mieć tytuł w języku polskim i angielskim, słowa kluczowe w obydwu językach) przedstawiona w czasie konferencji mogła być opublikowana, jej tekst winien być przygotowany zgodnie z wymaganiami stawianymi artykułu drukowanym w czasopismach *Ecological Chemistry and Engineering* ser. A

oraz S, które są dostępne w wielu bibliotekach naukowych w Polsce i za granicą. Zalecenia te są również umieszczone na stronie webowej Towarzystwa Chemii i Inżynierii Ekologicznej:

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Po konferencji zostaną wydane 6–8-stronicowe rozszerzone streszczenia wystąpień w półroczniku *Proceedings of ECOpole*. Artykuły te winny być przesypane do **1 października 2015 r.** Wszystkie nadsyłane prace podlegają zwykłej procedurze recenzyjnej.

Koszt uczestnictwa w całej konferencji wynosi 1700 zł (w tym 23% VAT) i pokrywa opłatę za udział, koszt noclegów i wyżywienia oraz materiały konferencji. Jest możliwość udziału tylko w jednym wybranym przez siebie dniu, wówczas opłata wyniesie 1200 zł i będzie upoważniała do uzyskania wszystkich materiałów konferencyjnych, jednego noclegu i trzech posiłków (śniadanie, obiad, kolacja). Opłata dla magistrantów i doktorantów oraz młodych doktorów biorących aktywny udział w Forum Młodych może być zmniejszona przez Radę Naukową konferencji do 1000 zł, przy zachowaniu takich samych świadczeń. Osoby te winny dodatkowo dostarczyć: rozszerzone streszczenia (6–8 stron) swoich wystąpień (do 15.08.2015 r.). Jest także wymagana opinia opiekuna naukowego. Prosimy o dokonywanie wpłat na konto Towarzystwa Chemii i Inżynierii Ekologicznej w Banku Śląskim:

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Po konferencji **będzie możliwość opublikowania elektronicznej wersji prezentowanego wystąpienia** (wykładu, a także posteru) na tej stronie.

Prof. dr hab. inż. Maria Waclawek
Przewodnicząca Komitetu Organizacyjnego
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Wszelkie uwagi i zapytania można kierować na adres:
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ZGŁASZAM UCZESTNICTWO W KONFERENCJI ECOpole '15

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- [2] Fasino CR, Carino M, Bombelli F. Oxidant profile of soy standardized extract. In: Rubin R, Stryger CS, editors. Joint Meeting 2001 – Book Abstracts '2001 Year of Natural Products Research'. New York: Harper and Row; 2001.
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- [7] Kowalski P. Statistical calibration of model solution of analytes. Ecol Chem Eng A. Forthcoming 2015.

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