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Tomasz CIESIELCZUK[1], Joanna POLUSZYŃSKA[2] and Monika SPOREK[3]

POTENTIAL USES FOR SOLID BIOFUELS FROM NON-FOOD CROPS

POTENCJALNE MOŻLIWOŚCI WYKORZYSTANIA STAŁYCH BIOPALIW Z ROŚLIN NIESPOŻYWCZYCH

Abstract: The Directive 2009/28/EC on the promotion of energy from renewable sources (RES), sets mandatory national targets so as to be able to achieve a 20% share of energy from renewable sources in gross final energy consumption in the Community in 2020, the aim of the Polish is to achieve by 2020 a 15% share of renewable energy in gross final energy consumption. Thus, use of fossil fuels for energy production should you gradually reduced in favor of renewable energy sources. Usually, however, the change in the method of heating or design of installations using renewable energy sources to incur significant capital costs. In addition, the economic balance during the operation also sometimes detrimental to the modern great human and environmental technologies. Therefore, you should look for low-cost renewable fuels that may be used in particular in those households where there is no possibility of the use of gas or heat delivered from sources of power plants. This paper describes the possibility of using untreated plant biofuels. After the species were taken into account: Canadian goldenrod (*Solidago canadensis* L.) and mugwort wormwood (*Artemisia absinthium* L.). These plants are considered weeds have many advantages enabling wider use for energy purposes.

Keywords: non-food plants, fuel, *Solidago, Artemisia*

Various initiatives around the world are made in order to counteract the observed climate changes. One of them is the promotion of renewable energy sources [1, 2]. Adopted in 2000 by the Council of Ministers, on energy development strategy it assumes that the share of renewable energy sources in Poland in 2020 in primary energy balance should be 15%.

Renewable energy sources that can be used to generate heat energy in diffuse sources constitute mainly of solar and biomass wood. Their annual resources in the world are estimated at about 420 billion Mg, which gives about 4500 EJ energy, with the potential of 100 EJ/a [3]. In the current practice, the extraction of biomass energy crops refers only to agricultural or forest crop. Many of these plants certainly have a high energy value. Biomass wood comes from wood plantations (willow, aspen, grey alder) and forests where it is obtained in the framework of pruning and cutting, and finally from wood processing (sawmills, carpenter). Agriculture provides mainly straw arising as waste in agricultural production [4] throughout the country, which reduces transportation costs. Nevertheless it requires significant volumes for storage and a special boiler adapted to its combustion. What is more, burned grains do not follow the normative regulations [5].

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[*] Contribution was presented during ECOpole'13 Conference, Jarnoltowek, 23-26.10.2013

A solution that could find wider interest is the acquisition of plants that do not compete with crops intended for human consumption or for animal feed. These are characterized by high efficiency, collecting the right amount of lignocelluloses, having little habitat requirements and requiring no tillage. Such plants include goldenrod (*Solidago* sp.) and mugwort (*Artemisia* sp.).

Material and methods

Botanical characteristics

Goldenrod (*Solidago* sp.) is a commonly found plant in Poland. Both the Canadian goldenrod (*S. canadensis* L.) and late goldenrod (*S. gigantea* Aiton) are classified as invasive plants [6]. In inhabited ecosystems they displace native flora species and become dominant. Goldenrod owes its success in mastering new areas to a great tolerance to habitat conditions, strong growth, the production of large quantities of seed, ease of vegetative propagation and mechanisms of allelopathy. Covering large areas with high density shoots it contributes to the homogeneity of the landscape and the loss of biodiversity [7]. Among the species representing the type of mugwort (*Artemisia* sp.) quite commonly found is mugwort wormwood (*A. absinthium* L.). As a folk tradition it is often used for various treatments including treatment for gastrointestinal diseases and also used as an alcoholic extract for the narcotic purposes [8]. There is also data which suggests that it possibly supports the treatment of breast cancer in humans [9] and the possibility of the control of parasites in farm animals [10, 11].

Methods

In order to estimate the possibility of using these types of plants for energy purposes numerous calculations have been done including: the yield per hectare, the density of growth, dry matter content during the harvest, bulk density, ash content and calorific value. The dry matter content was determined by weighing, after drying at 105°C to stabilize the mass. The ash content was determined after dry mineralization at 550°C for 5 hours. The calorific value was determined using calorimeter machine KL-10 by PN-81/G-04513. Elementar analysis of the biomass was determined by a CHNS analyser varioMACRO cube made by the Elementar Company. The demand for the fuel needed was also determined during the heating season based on sample farms using a biomass stove. Additionally, an economic analysis was also performed based on the energy 1 MJ compared to other commonly used fuels.

Results and discussion

Characteristics of the obtained biomass

The results indicate a relatively high yields of plants studied (Table 1), in spite of the lack of agrotechnology, including no fertilization, weed control or irrigation.

In typical energy plant farming, in order to improve yields, some typical agricultural practices are used: weeding (especially important in the first period after planting crops), irrigation and fertilization. This type of growing is sometimes seen as the area used for neutralizing the municipal wastewater. This practice leads to the degradation of soil and groundwater contamination by biogens and easily soluble organic matter [12]. The calculated dry matter yield of goldenrod (Table 1) occurring in its wild state are comparable to the yields derived from willow crops, which stands at 6.8-14.7 Mg d.m./ha [13]. Only a few authors discussing the usage of goldenrod for energy purposes highly appreciate both the yield per hectare as well as their potential energy [14, 15]. The monoculture that we are dealing with, particularly in the case of goldenrod or mugwort, allows us after a few seasons to work out the optimum harvest date depending on the course of the aura in the growing season. The tractor rotary mower is sufficient for harvesting, a trimmer in small or hard to reach areas or, in special cases, even a scythe. Mowed plants can be moved manually or a loader wagon can be used. Basically, it should be done in the month of December, when the plants are already dried (later in decreased humidity) and the seeds sprinkled (Table 1). Mowed biomass shall be transported under the canopy where set in the form of loose sheaves or heaps should be dried for a period of 7-10 days, depending on the weather. The exception is the spring harvest, for which additional drying is not necessary. Subsequently, air dry biomass should be pre-shred into pieces 15-20 cm in length. This can be done manually or using simple machines formerly used to prepare the chaff. Grinding can be done also at the time of the harvest by a green fodder mower so-called “Orkane”, but in this case, another drying method of fragmented biomass should be considered. The best in that case would be a ventilated and dry place.

Table 1

Main characteristic of analyzed plants (n = 3)

Plant	**Density [plants/m^2]**	**Yield [Mg d.m./ha]**	**Moisture of harvested plants [%]**			
			15.10	**29.10**	**16.11**	**2.12**
Solidago	121.7(29.0)	15.9(3.8)	58.5(0.3)	54.0(1.3)	57.9(1.3)	32.8(2.4)
Artemisia	13.2(4.7)	6.0(2.1)	58.7(0.8)	47.6(6.1)	39.3(3.9)	33.5(4.4)

Energy use

The calorific value of the uncondensed biomass of the analysed plants was similar at 16.24-16.49 MJ/kg (Table 2). This data is about 1 MJ/kg higher than their calorific value compared to the straw [16] and sand reed (*Calamagrostis epigejos* L.) [17]. The lower calorific value in comparison to the test plants was also recorded for various assortments of biomass of Scots pine (*Pinus sylvestris* L.), where for the needles it was 14.9 MJ/kg, for trunks 14.7 MJ/kg and for brunches 14.6 MJ/kg [18]. Particularly important is the low level of humidity of the prepared biomass, which prevents the self-heating of a pile and increases the calorific value. The content of organic biomass of the test was high (Table 2), and thus there is little amount of the ash formed, which makes the plants perfect for thermal recycling. A disadvantage in the use of this fuel is its low bulk density which is an important factor causing a need to collect it in a relatively large room, ensuring free flow of air through the plant prism. A decline in the efficiency of the boiler, usually adapted to coal, must also be assumed. A high total carbon content (TC) (44.6-44.8%) is lower than the value of TC parameter for brown coal (Table 2), but in the biomass, the proportion of organic carbon (TOC) is higher, constituting 94.4-96.0 and 91.4% (respectively for biomass and coal). Particularly important is the low sulphur content and a relatively high hydrogen content. Burning the analysed biomass can therefore produce from 1.6 to

5.04 kg SO_2/Mg d.m., which is less than the combustion of brown coal (14.24 kg SO_2/Mg d.m.) or hard coal even after taking into account the lower calorific value.

Table 2

Characteristic of biomass as a solid fuel source (n = 3)

	Carbon (TC) [%]	**Hydrogen (TH) [%]**	**Nitrogen (TN) [%]**	**Sulphur (TS) [%]**	**Burning heat [MJ/kg d.m.]**	**Calorific value [MJ/kg]**	**Organic substances [% d.m.]**
Solidago	44.8 (0.17)	6.46 (0.01)	0.37 (0.05)	0.198 (0.056)	18.12 (0.50)	16.49a	97.9(0.2)
Artemisia	44.6 (0.21)	6.30 (0.03)	1.54 (0.61)	0.252 (0.025)	17.87 (0.02)	16.24a	97.9(0.6)
Brown coal (Belchatow, Poland)	52.6 (0.04)	5.08 (0.05)	0.60 (0.01)	0.712 (0.077)	22.26 (0.10)	20.90	96.(0.2)
Hard coal (fine grain)	76.4(1.1)	4.71 (0.07)	nd	nd	30.55 (0.21)	29.27	94.1(0.3)

a - after correction for 10% moisture and hydrogen content; nd - no data

Taking into account the calorific value of plants (Table 3), the amount of energy that can be obtained from 1 ha fief monoculture was calculated and the potential amount of energy that can be obtained from the biomass of the test plants, which comes from fallow land in the territories of individual farms (409.6 thousand hectares). For ecological reasons, the preferred species is tansy, which is a native species considered as a monoculture or can be mixed with mugwort. Seeds of *Artemisia* constitute a valuable supplementary of winter feeding base diet for national avifauna. Self-sown seed is also a complement of vegetative propagation, which occurs through the growth of the rhizomes.

Table 3

Calorific value of plant biomass and energetically comparable amount of hard coal with mean energy 26MJ/kg

	Calorific value [GJ/ha]	**Energy P[*] [PJ]**	**Hard coal [thow. Mg]**	**Ecological effect [thow. Mg CO_2]**
Solidago	288.4	118.13	4,543.4	7,752.2
Artemisia	107.2	43.91	1,688.8	2,881.5

[*] the potential amount of energy obtained from burning plant monoculture of private fallow lands

Conclusions

Analysed non-food biomass is characterized by high calorific value. Small soil requirements, lack of natural predators, resistance to disease, and the lack of need for a typical energy crop cultivation technology, greatly reduces the cost of biomass. No competition with crops, lack of the need to transport over long distances and lack of recycling the resulting biomass (*eg* pelletising, briquetting) are additional factors that appeal in favour of the analysed plants. Analyzed plants could be a good source of energy, especially on non highly-urbanized areas.

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POTENCJALNE MOŻLIWOŚCI WYKORZYSTANIA STAŁYCH BIOPALIW Z ROŚLIN NIESPOŻYWCZYCH

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Abstrakt: Dyrektywa 2009/28/WE w sprawie promowania stosowania energii ze źródeł odnawialnych (OZE) ustanawia obowiązkowe krajowe cele, tak aby możliwe było osiągnięcie 20% udziału energii ze źródeł odnawialnych w końcowym zużyciu energii brutto we Wspólnocie w 2020 roku. Celem dla Polski jest osiągnięcie w 2020 roku 15% udziału energii z OZE w końcowym zużyciu energii brutto. Zatem wykorzystanie paliw kopalnych do wytwarzania energii powinno być stopniowo ograniczane na rzecz odnawialnych źródeł energii. Zwykle jednak przy zmianie sposobu ogrzewania lub projektowaniu instalacji wykorzystujących źródła odnawialne należy ponieść znaczne koszty inwestycyjne. Ponadto rachunek ekonomiczny w czasie eksploatacji także bywa niekorzystny dla nowoczesnych, korzystnych dla ludzi i środowiska technologii. W związku z tym należy szukać tanich paliw odnawialnych, które będą mogły być wykorzystywane w szczególności w tych gospodarstwach domowych, gdzie nie ma możliwości wykorzystania paliw gazowych lub energii cieplnej dostarczanej ze źródeł energetyki zawodowej. W niniejszej pracy przeanalizowano możliwości wykorzystania biopaliw roślinnych nieprzetworzonych. Pod uwagę wzięto rośliny z rodzaju: nawłoć (*Solidago sp.*) oraz bylica (*Artemisia sp.*). Rośliny te, uważane za chwasty, posiadają wiele zalet umożliwiających szersze ich wykorzystanie do celów energetycznych.

Słowa kluczowe: rośliny niespożywcze, paliwo, nawłoć, bylica

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Franciszek CZYŻYK[1] and Agnieszka RAJMUND[1]

LEACHING OF BIOGENIC ELEMENTS (NPK) FROM FERTILIZED LIGHT SOIL

WYMYWANIE PIERWIASTKÓW BIOGENNYCH (NPK) Z NAWOŻONEJ GLEBY LEKKIEJ

Abstract: This review presents results of investigation from 6 years studies of an annual light soil fertilization with mineral fertilizer influence on quantity amount of nitrogen (N), phosphorus (P) and potassium (K) penetration to water environment. The studies were done in lysimeters filled with light-clayey sand. In this studies two variants of compost fertilization (K1 - 10 and K2 - 15 g N $\cdot$ m^{-2}) were used. Additionally two variants of NPK with equivalent doses of nitrogen as an ammonium nitrate supplemented with PK as a superphosphate and potassium salt were applied. The results showed the increase of concentrations of nitrogen in the soil leachates, with increasing doses of fertilizer. During the time of fertilization there was observed intense increase of quantity of nitrogen eluted from the soil, which indicated on the presence of this element in the soil. The total quantity of nitrogen drained in leachates from soil were 14.9-17.3 $g \cdot m^{-2}$ of the total quantity of nitrogen supplied to the soil in the variants with compost fertilization, and 19.6-27.3 $g \cdot m^{-2}$ in the variants with mineral fertilization. Content of the phosphorus in the leachate from the soil, in contrast to the nitrogen, were relatively small and did not show depending on the type and dose of fertilizer, or the passage of time from the application of fertilizer. Leaching P were negligible 0.11-0.14 $g \cdot m^{-2}$ and independent of the type of fertilizer used. Loss of K as well as N were higher in variants with mineral fertilizers (28.4-31.7 $g \cdot m^{-2}$) than in the case of the use of compost (21.4-23.9 $g \cdot m^{-2}$).

Keywords: fertilization, biogenic components, leachate from the soil, lysimeters

Introduction

In recent years, growth in crop production caused a significant increase in the consumption of fertilizers [1, 2]. Crop production is seen as one of the main sources of pollutants leaching into groundwater [3]. In the current agricultural practice annual fertilization is used, and doses of fertilizers are usually determined according to the nutritional requirements of plants. Soil richness in digestible nutrients is not taken into account [4]. Fertilization currently used in agriculture - intensive crop production often deviates from accepted agricultural practices. Doses of fertilizers, especially nitrogen, often exceed the recommended level for sustainable agriculture [5]. This creates an excess of soluble components of fertilizers in the soil as well as their leaching and pollution of the water environment [6]. It concerns light soils, characterized by good permeability and generally low sorption properties [7]. Fertilizers used in agriculture are not fully utilized by the plants and are not discharged from the crop. Significant parts of component of fertilizers, both organic and mineral, are rinsed from the soil. The nutrients supplied to the soil in the mineral fertilizers are easily soluble and easily available to plants [8]. Nitrogen (N) supplied to the soil in mineral fertilizers, especially nitrate, is easily leached from the soil into the groundwater. Under anaerobic conditions it undergoes a process of denitrification which is associated with the formation of gaseous forms of NO, N_2O and N_2,

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which afterwards are oxidized to the atmosphere [9, 10]. Annual fertilization with phosphorus (P) may cause accumulation of P in the soil [11], and then its release into the water in an uncontrolled manner. Szara et al [12] write that P in mineral fertilizers used by the plant in about 20%. The remaining part of P in the soil undergoes changes into forms with different availability to plants [13]. Depending on soil conditions (pH, humidity), some of them may transform into inedible compounds and is locked. The use of potassium fertilizers (K) is necessary in sustainable fertilization of plants. The interaction between K and N is important. Unequal fertilization N/K leads to an increase in the concentration of nitrate in plant tissue. Potassium in mineral fertilizers is an element quite easily eluted from the soil, especially light soil [14]. The compost from sewage sludge used as a fertilizer is rich in organic matter and nutrients, which are released gradually over a longer period of time [8]. Organic nitrogen supplied to the soil undergoes complex and dynamic changes in which the nutrients are released in the forms of available for plants. Biogenic components, which will not be taken up by the plant are usually leached into the groundwater [15]. The scale of the threat depends on the size of the doses of compost, but is much smaller than at fertilization equivalent doses of mineral fertilizers.

In this paper presents the results of six-year study showing the degree of fertilizers components leaching from sandy soil annually fertilized by moderate doses of compost and mineral fertilizers.

Conditions and methods

The studies were conducted in 2002-2007 in lysimeters filled with clayey sand containing *ca* 14% of the earthy particles (fraction < 0.02), 0.7% organic carbon and 0.1% of the total nitrogen. Conditions in the lysimeters are similar to the natural field conditions. Lysimeters with a diameter of 100 cm and a depth of 130 cm are completely submerged in the ground. Every year the soil was fertilized by compost made from sewage sludge rural and vegetable waste, containing *ca* 2.5% total nitrogen, 20% organic carbon and 63% organic matter. Compost mixed with the top layer of soil. Each year, before the spring application of compost to fertilize, it was determined the content of the major components of fertilizers. Two variants of compost fertilization (K1 - 10 and K2 - 15 g $N \cdot m^{-2}$) were used. Additionally two variants of NPK with equivalent doses of nitrogen as an ammonium nitrate supplemented with PK as a superphosphate and potassium salt were applied (NPK1 - 10 g $N \cdot m^{-2}$ + 2.5 g $P \cdot m^{-2}$ + 6 g $K \cdot m^{-2}$ + 4 g $P \cdot m^{-2}$ + 9 g $K \cdot m^{-2}$). All variants were used in three replications. In the following years of research lysimeters were sown by: a mixture of grasses, corn, sugar beets, white mustard (to seeds), triticale and canola. Systematically there were investigated the volume of precipitation and leachates as well as the samples of water for laboratory analysis were collected. Chemical analyzes were performed according to the method currently in force and widely used [16, 17].

Results and discussion

Individual years of research considerably differ in terms of the amount of precipitation (Table 1). Leachate volume in the lysimeters was also varied and mainly depending on the amount of precipitation and to a lesser extent on the variants of fertilization and the type of fertilizer (Table 2). Leachate from the lysimeters occurred mainly during the late autumn and winter. In growing season the volume of leachate was much smaller and they only occurred after heavy precipitation, *eg* in May and July 2005 and August 2006. Depending on the moisture of the content of the soil, the leachate typically occurs after a period from several hours to two days after the onset of intense precipitation.

Precipitation in the growing seasons in different years were significantly higher than in the autumn-winter period and accounted for 60-70% of annual precipitation. The volume of leachate from the soil during vegetation season was significantly lower than in the autumn and winter periods. Of the total five-year amount of leachate, in particular variants of fertilization, only 24-29% occurred on vegetation seasons, and 71-76% for the autumn-winter period.

Table 1

Atmospheric precipitation at research station of the Lower Silesian Research Centre in Kamieniec Wroclawski in the years 2002-2007

Year	**Precipitation in months**											
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
2002	23.8	59.2	15.7	32.9	37.1	68.2	49.5	78.7	52.2	61.2	52.3	16.2
2003	40.0	2.8	18.7	11.9	80.5	24.3	58.8	55.3	42.4	51.5	27.8	47.3
2004	31.2	60.6	56.6	24.5	35.2	40.5	88.5	50.8	21.8	45.6	81.4	15.4
2005	46.2	51.2	11.5	27.0	150.8	46.8	122.6	54.4	24.9	6.7	31.0	106.4
2006	28.9	43.7	26.1	54.2	21.9	56.6	12.0	179.3	20.3	68.4	65.6	36.7
2007	64.6	53.3	55.5	3.6	57.6	79.5	124.1	42.0	51.6	26.5	56.0	27.9

Source: own studies

Table 2

Annual amount of precipitation and volume of leachates [dm^3] from particular fertilization variants in the period of IV 2002 - VII 2007

Year	**Annual amount of precipitation [mm]**	**Volume of leachates from particular variants**			
		K1	**K2**	**NPK1**	**NPK2**
2002	547.0	37.1$^{1)}$	28.9$^{1)}$	41.7$^{1)}$	45.2$^{1)}$
2003	461.3	66.0	64.3	64.5	54.6
2004	552.1	97.1	94.3	101.1	108.0
2005	679.5	112.1	123.2	119.7	122.6
2006	613.7	95.1	88.0	109.4	105.5
2007	642.2	64.8$^{2)}$	62.0$^{2)}$	69.7$^{2)}$	64.4$^{2)}$

$^{1)}$ 1 IV - 31 XII 2002 $^{2)}$ 1 I - 30 VI 2007

Explanations: K1, K2 - variants of soils fertilized with compost 10 and 15 g N$\cdot$m^{-2}, respectively;
NPK1 - 10 g N$\cdot$m^{-2} + 2.5 g P$\cdot$m^{-2} + 6 g K$\cdot$m^{-2}; NPK2 - 15 g N$\cdot$m^{-2} + 4 g P$\cdot$m^{-2} + 9 g K$\cdot$m^{-2}.

Source: own studies

The tested leachates from the soil are characterized by varying concentration of the NPK components (Table 3). In the leachates from all variants of fertilization, the largest there were concentrations of potassium and smallest of phosphorus. The variability of the concentrations of individual elements was at the average level (Table 4). Variation of concentrations of potassium, was lower than in the case of nitrogen and phosphorus.

Average nitrogen concentrations in the leachate from the lysimeters varied and depending on the variants of fertilization. These concentrations were calculated as the weighted average of the volume of leachate and content of analyzed components (NPK).

The concentrations of nitrogen in the leachate from the soil fertilized by compost were significantly lower than in the leachate from the soil fertilized with an equivalent dose of N as an ammonium nitrate [18].The concentration of N in the leachate from the soil increased with increasing doses of fertilizers, and also significantly increased over the years of use of fertilizer. This indicates the formation of excess N in the soil as a result of the use of annual fertilization, and thus increasing the water pollution with that component.

Table 3

Average annual concentrations of N, P and K in leachates from different variants of fertilization in IV 2002-VI 2007 [$mg \cdot dm^{-3}$]

Year	K1			K2			NPK1			NPK2		
	N	P	K	N	P	K	N	P	K	N	P	K
2002¹⁾	5.7	0.19	38.4	6.3	0.19	40.9	8.2	0.22	45.5	17.9	0.20	49.8
2003	13.8	0.19	38.7	16.8	0.23	41.5	18.4	0.20	47.0	24.4	0.27	49.5
2004	25.5	0.19	37.5	27.2	0.22	41.6	28.3	0.18	46.7	38.1	0.21	50.4
2005	26.3	0.24	37.1	29.0	0.26	43.2	33.4	0.26	44.7	42.1	0.27	48.6
2006	31.3	0.17	37.9	38.3	0.17	37.4	37.3	0.19	41.3	45.9	0.17	49.8
2007²⁾	33.9	0.10	36.5	45.3	0.10	38.0	41.9	0.10	41.4	55.1	0.14	51.2

¹⁾ 1 IV - 31 XII 2002 ²⁾ 1 I - 30 VI 2007

Explanations: K1, K2 - variants of soils fertilized with compost 10 and 15 $g\ N \cdot m^{-2}$, respectively;
NPK1 - 10 $g\ N \cdot m^{-2}$ + 2.5 $g\ P \cdot m^{-2}$ + 6 $g\ K \cdot m^{-2}$; NPK2 - 15 $g\ N \cdot m^{-2}$ + 4 $g\ P \cdot m^{-2}$ + 9 $g\ K \cdot m^{-2}$.
Source: own studies

Table 4

Average multiannual concentration and coefficient of variation of N, P and K in the leachate samples collected in 2002-2007 [$mg \cdot dm^{-3}$]

Components	Concentration of nutrients in different versions							
	K1		K2		NPK1		NPK2	
	n = 45		*n* = 46		*n* = 45		*n* = 51	
	x	*V%*	*x*	*V%*	*x*	*V%*	*x*	*V%*
N	22.9	52.9	27.7	39.1	27.3	51.0	36.5	42.5
P	0.22	52.4	0.20	57.3	0.21	42.6	0.22	46.0
K	42.9	44.4	43.3	29.6	49.5	33.2	51.6	39.2

Explanations: *n* - number of samples, *x* - mean concentration, *V%* - variability coefficient.
Source: own studies

Effluent of the nitrogen into groundwater can be reduced by precise determination of doses of fertilizers. It is important to consider not only the nutritional needs of plants, but also the content of mineral nitrogen in the soil [4]. It is also necessary to maintain optimum pH of the soil for plants and control conditions of the water in soil. These factors provide a greater use of fertilizers by plants. One of the most effective ways to reduce nitrogen leaching from soil is the use of aftercrops. They reduce the leaching of soil nitrogen 36-62% [19].

Content of the P in the leachate from the soil (Table 3), in contrast to the N, were relatively small and did not show depending on the type and dose of fertilizer, or the passage of time from the application of fertilizer. Movement of phosphorus in the soil is very small and usually remains in the layer of soil, which has been introduced [20, 21].

Phosphorus is mainly kept in the subsurface, the biologically active layer of the soil profile. Penetration of P into the deeper layers of the soil and groundwater is generally low. Significant leaching of P by precipitation can occur in the case of very light and highly acidic soils [22]. The potassium concentration in the leachate from the soil, as well as the concentration of N was dependent mainly on variants of fertilization and increased with increasing doses of fertilizers. However it was not increased over the years the use of fertilizer. It indicates that the part of the delivered doses of K that is not used by plants is annually eluted by rain water.

Tables 5 and 6 shows the total amounts of N, P and K supplied in fertilizers and precipitation to the soil and discharged from the leachate during the entire period of study. The amount of N in the soil brought from the rain was an average 3.4 $g \cdot m^{-2}$ per year [23]. The amount of K was significantly lower and was annually 0.82 $g \cdot m^{-2}$. [24] The amount of P in precipitation was trace and did not take into account in the calculation. The amount of N discharged from the leachate was significantly higher in variants with mineral fertilization than fertilizer with equivalent doses of N in compost. The loss of N, in the 6-year study, caused by the leaching to groundwater, were on average 14.9-17.3 $g \cdot m^{-2}$ in compost and 19.6-27.3 $g \cdot m^{-2}$ for the fertilizer with equivalent doses of ammonium nitrate. P losses were however negligible 0.11-0.14 $g \cdot m^{-2}$, and independent of the type of fertilizer used K was most significantly leached. Its amount leached from the soil increased depending on the size of rainfall, volume of leachate and increasing doses of fertilizers. K and N losses, were higher in the variants with mineral fertilizers (28.4-31.7 $g \cdot m^{-2}$) than in the case of the use of compost (21.4-23.9 $g \cdot m^{-2}$).

Table 5

Amounts of nitrogen and phosphorus drained from leachate during the period of IV 2002 - VII 2007

Year	**The amounts of individual variants of fertilization [mg · m^{-2}]**											
	K1			**K2**			**NPK1**			**NPK2**		
	N	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
2002[1)]	269	9	1815	232	8	1505	436	12	2417	1031	12	2867
2003	1160	16	3254	1376	19	3400	1512	16	3862	2080	19	3442
2004	3154	24	3639	3268	26	4998	3645	24	6015	5944	29	6934
2005	3756	34	5098	4551	41	6780	5093	40	6816	6857	42	7593
2006	3792	21	4592	4294	19	4193	5199	27	5756	6868	23	6691
2007[2)]	2799	8	3013	3578	8	3001	3721	9	3540	4521	12	4200
Sum	14930	112	21411	17299	121	23877	19606	128	28406	27301	137	31727

[1)] 1 IV - 31 XII 2002 [2)] 1 I - 30 VI 2007

Explanations: K1, K2 - variants of soils fertilized with compost 10 and 15 $g N \cdot m^{-2}$, respectively;
NPK1 - 10 $g N \cdot m^{-2}$ + 2.5 $g P \cdot m^{-2}$ + 6 $g K \cdot m^{-2}$; NPK2 - 15 $g N \cdot m^{-2}$ + 4 $g P \cdot m^{-2}$ + 9 $g K \cdot m^{-2}$.
Source: own studies

Table 6

Amounts of nitrogen and phosphorus supplied to the soil and drained from leachate during the period of IV 2002 - VII 2007 [$g \cdot m^{-2}$]

The amounts of N P K	**K1**			**K2**			**NPK1**			**NPK2**		
	N	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
in fertilizers	60.0	15.0	32.1	90.0	21.6	48.2	60.0	15.0	36.0	90.0	24.0	54.0
of precipitation	20.5	-	4.9	20.5	-	4.9	20.5	-	4.9	20.5	-	4.9
total	80.5	15.0	37.0	110.5	21.6	53.1	80.5	15.0	40.9	110.5	24.0	58.9
leachate discharged from	14.9	0.11	21.4	17.3	0.12	23.9	19.6	0.13	28.4	27.3	0.14	31.7

Conclusions

1. Annual fertilization of the soil, both by the compost and mineral fertilizers, in doses determined only by the nutritional needs of the plants cause a systematic increase of contamination of the water environment by nitrogen. Concentrations of total nitrogen in the leachate from the soil fertilized by compost were significantly lower than in the leachate from the soil fertilized by equivalent doses of nitrogen in the form of ammonium nitrate.
2. Loss of nitrogen and its penetration into the water environment are smaller in the case of using organic fertilizer in the form of compost, than in the case of a mineral fertilizer. This is due to the mineralization process of organic matter in the soil and gradual supply of available forms of nitrogen to the plants.
3. The concentrations of nitrogen in the leachate from the soil are increased with increasing doses of fertilizers, as well as their use over the years. This indicates the formation of an excess of nitrogen in the soil due to application of fertilizer annually, and thereby increasing the pollution of environment by the soluble forms of this component.
4. The concentration of phosphorus in the leachate from the soil were relatively small and do not show depending on the type of fertilizer, the dose and over the years the application of fertilizer. Losses of phosphorus and the amount of leaching into groundwater does not exceed 0.11-0.14 $g \cdot m^{-2}$ of the applied doses.
5. Potassium concentrations in the leachate from the soil are much higher than concentrations of nitrogen and phosphorus. The total amount of potassium leached from the fertilized soil depends mostly on the size of precipitation and volume of doses of fertilizer.

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WYMYWANIE PIERWIASTKÓW BIOGENNYCH (NPK) Z NAWOŻONEJ GLEBY LEKKIEJ

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Abstrakt: Przedstawiono wyniki 6-letnich badań wpływu corocznego nawożenia gleby lekkiej nawozami mineralnymi i kompostem na ilości azotu (N), fosforu (P) i potasu (K) przenikających do środowiska wodnego. Badania prowadzono w lizymetrach wypełnionych piaskiem słabo gliniastym. W badaniach zastosowano dwa warianty nawożenia kompostem (K1 - 10 g N·m^{-2} i K2 - 15 g N·m^{-2}). Dodatkowo zastosowano dwa warianty nawożenia mineralnego (NPK) z równorzędnymi dawkami azotu w postaci saletry amonowej z uzupełnieniem PK w postaci superfosfatu i soli potasowej. Badania wykazały zwiększanie się stężenia azotu w odciekach z gleby wraz ze zwiększeniem dawek nawozu. Stężenia N w odciekach z gleby nawożonej kompostem były zdecydowanie mniejsze niż w odciekach z gleby nawożonej równorzędnymi dawkami N w postaci saletry. Wystąpiło też zdecydowane zwiększenie się ilości wymywanego z gleby N wraz z upływem lat stosowania nawozów, co wskazuje na tworzenie się coraz większych jego nadmiarów w glebie. Łączne ilości azotu odprowadzone w odciekach z gleby wynosiły 14,9-17,3 g·m^{-2} ogólnej ilości azotu dostarczonej do gleby w wariantach z nawożeniem kompostem i około 19,6-27,3 g·m^{-2} w wariantach z nawożeniem mineralnym. Zawartości P w odciekach z gleby w przeciwieństwie do N były stosunkowo małe i nie wykazywały zależności od rodzaju nawozów i ich dawek ani też od upływu lat stosowania nawożenia. Wymywanie P było znikome, wynosiło 0,11-0,14 g·m^{-2} niezależne od rodzaju stosowanych nawozów. Straty zarówno K, jak i N były większe w wariantach z zastosowaniem nawozów mineralnych (28,4-31,7 g·m^{-2}) niż w przypadku stosowania kompostu (21,4-23,9 g·m^{-2}).

Słowa kluczowe: nawożenie, składniki biogenne, wymywanie z gleby, lizymetry

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ESTIMATION OF MASS RELOCATION INTENSITY IN VICINITY OF THE FORMER CEMENT WORKS

OSZACOWANIE SKALI PRZEMIESZCZANIA SIĘ MATERII NA OBSZARZE BYŁEJ CEMENTOWNI

Abstract: Estimation of possible mass relocation in environment is crucial in assessment of threats resulting from industrial activity. In many regions the abandoned industrial estates can be found, which can be a source of harmful substances in environment. In such areas composition of soils can be changed as a result of industrial activity. Among others, the radioactive isotopes in significant concentrations could appear in processing of mineral materials as well as in branches of production where fossil fuels are combusted. Surface soil samples were collected in the area of the former cement plant "Piast", situated within Opole city borders. Concentrations of gamma radioactive isotopes were determined in the samples. A number of naturally occurring radioisotopes were found as well as artificial Cs-137. In results studies methods designed for compositional data analysis were utilized. It was found that the radioisotopes from the radium and thorium decay series were close to equilibrium state. Concentrations of U-235 and its daughter Th-231 are rather poorly related with each other. Concentration of K-40 is somewhat bound with concentrations of radionuclides from thorium decay chain. The Cs-137 concentrations were not related to concentrations of other radioisotopes. The results obtained suggest lack of an intense mass relocation in the area close to former cement works. Neither significant deposition or elution/leaching of matter in the investigated area can be supposed.

Keywords: industrial pollution, radioisotopes, relocation

Introduction

Knowledge about mechanisms and ways of matter transport may provide information about current and future state of environment. Among others, estimation of possible mass relocation in environment is crucial in assessment of threats resulting from industrial activity.

Radioactive isotopes of elements are common in environment. They can be found in living organisms and in mineral components. In low concentrations they are harmless for organisms. Because of their specific physical and chemical properties, the radioisotopes can be helpful in assessment of matter relocation in environment. Many radioisotopes can be arranged in decay series in which irreversible sequences from parent to daughter radioisotope is defined. Chemical properties of radioisotopes in decay chains are changed in each decay step. It is a consequence of change in the atomic number of the radionuclide, which determines chemical properties of an element.

Change in chemical properties of a radionuclide leads to change in chemical properties of parent compound. This change affects fate of the corresponding element in environment. It could be expected that investigation of common relationships between concentrations of radionuclides in the environment components will provide information about matter

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transport mechanisms, matter sources, matter distribution in space and/or in time, and assessment of potential future threats related to matter dispersion in an investigated region.

In this work the results of soil investigations which surface layers were collected in the area of former cement works are described.

Materials and methods

Soil samples were collected in the area of the former cement plant “Piast”, situated in southeastern part of Opole (Poland). This plant was founded in 1906. Due to mineral deposits depletion and high harmfulness for the city, after over 70 years of cement production it was closed. From this area 11 samples of surface soil layers were collected.

Activity concentrations of radioisotopes were carried out by means of a gamma-spectrometer with a germanium detector HPGe (Canberra) of high resolution. Geometry of samples container was Marinelli, and measuring process and analysis of spectra were computer controlled with use of the software GENIE 2000. The radiation spectrum was recorded day and night.

Computations

Activity concentrations a of radionuclides were recalculated to their mass concentrations from the formula:

$$c = \frac{t_{1/2} M}{10^3 \ln 2 N_A} a \tag{1}$$

where: $t_{1/2}$ is the half life time of the radionuclide, M is its molar mass of radionuclide, N_A is Avogadro constant and c is mass fraction (concentration) of the radionuclide in sample.

The sample space of the calculated concentrations data can be regarded as a set of compositions with lower data limit equal to 0 and upper data limit equal to 1. This data type shows some specific features. The compositional data are not independent on each other. If content of one of components increases the others have to decrease. It is the main reason for which results of standard statistical analysis of the relationships between raw components or parts are spoiled by spurious effects [1, 2]. For this reason application of standard statistical techniques on such data in raw form lead to faulty statistical inferences. For example, a variance of concentration provides only poor (or not at all) information about variability in a component abundance. In the variance of a single component, variabilities of all other components are involved.

The main principles of compositional data analysis are:

1. Scale invariance - composition provides information only about relative values and not about the absolute ones.
2. Subcompositional coherence - the same results of statistical inference of common parts regardless of whether full composition or subcomposition was used in computations.

Utilization of variables ratios (or logratios) in computations supports both principles.

Compositional data occupy a restricted space where variables can vary only from 0 to a given constant. For the geometrical representation of sample space, the unit simplex was proposed. In such space a distance between points and basic operations are defined [3].

Linear relationship between variables is usually estimated on base of the Pearson correlation coefficient. This parameter cannot be used in comparison of compositional variables. As a result of constant sum of all concentrations in a system, phenomenon of "spurious correlation" appears in compositional data analysis [4]. Co-variability of two compositional variables A and B can be tested using variance of ratio VR:

$$VR = \mathrm{var}\left(\ln \frac{\boldsymbol{c}_A}{\boldsymbol{c}_B}\right) \tag{2}$$

where: $\boldsymbol{c}_A$ and $\boldsymbol{c}_B$ are vectors of, respectively, A and B concentration values. If A increase linearly with increase in B then $\boldsymbol{c}_A/\boldsymbol{c}_B$ ratio is constant and its variance is 0. If there is a weak or negative linear relationship between A and B then the VR value increases.

To study common relationships between concentrations a Principal Component Analysis (PCA) was utilized [5]. In this method an orthogonal basis is selected for data description so that the first coordinate (principal component, PC) represents the direction of maximum variability and then the consecutive PCs are sorted in order of decreasing variabilities.

Though PCA method cannot be directly applied to study compositional data, within some limitations it can be used in transformed data interpretation [6, 7]. Among possible compositional data transformations the centered logratio, clr, is one of the commonly used:

$$\mathrm{clr}(\boldsymbol{c}) = \ln\left(\frac{\boldsymbol{c}}{g(\boldsymbol{c})}\right) \tag{3}$$

where $g(\boldsymbol{c})$ is geometrical mean of $\boldsymbol{c}$.

Results and discussion

In soil samples the following radionuclides were determined: K-40, Cs-137, Pb-210, Bi-212, Pb-212, Bi-214, Pb-214, Ac-228, Th-231 and U-235. The isotopes Pb-210, Bi-214 and Pb-214 are members of radium decay series. The Bi-212, Pb-212 and Ac-228 isotopes belong to thorium series, and actinium decay chain contains Th-231 and U-235. The K-40 radionuclide is constituent in natural potassium, with constant abundance of 0.017%. This isotope is the main radioactivity source for organisms. The Cs-137 is an artificial radioisotope. It was introduced into environment with the global fallout, resulting from nuclear tests, particularly intensified in 1963-1964, as well as the nuclear power plants fallouts, for example Chernobyl 1986 [8] and Fukushima Daiichi 2011 [9]. Half-life times of these radioisotopes are shown in Table 1 [10].

Table 1

Half-life times of the radionuclides determined in soil samples. Membership in decay series is marked as follows: * - radium series, ** - thorium series, *** - actinium series

isotope	**K-40**	**Cs-137**	**Pb-210** *	**Bi-212** **	**Pb-212** **	**Bi-214** *	**Pb-214** *	**Ac-228** **	**Th-231** ***	**U-235** ***
$t_{1/2}$	$1.248\cdot10^9$ a	30.08 a	22.2 a	25 min	10.64 h	19.9 min	26.8 min	6.15 h	25.52 h	$7.04\cdot10^8$ a

In Table 2 the statistical parameters of the calculated radionuclides concentrations are shown. In table *min* and *max* are minimum and maximum values of concentrations, q_2 is median, q_1 and q_3 are, respectively, lower and upper quartile. Arithmetic mean of concentrations is *mean* and standard deviation is *SD*. Coefficient of variation (*CV*) is the ratio of *SD* (nominator) and *mean* (denominator).

Table 2

The statistical parameters of the calculated radionuclides concentrations

	K-40 $\cdot10^{7}$	**Cs-137** $\cdot10^{14}$	**Pb-210** $\cdot10^{14}$	**Bi-212** $\cdot10^{21}$	**Pb-212** $\cdot10^{19}$	**Bi-214** $\cdot10^{21}$	**Pb-214** $\cdot10^{21}$	**Ac-228** $\cdot10^{19}$	**Th-231** $\cdot10^{19}$	**U-235** $\cdot10^{8}$
min	6.90	0.69	0.55	4.80	1.93	4.10	6.43	1.37	1.27	1.25
q_1	8.34	1.03	0.68	6.86	2.76	6.12	8.57	1.93	1.58	2.12
q_2	8.89	1.34	0.94	7.46	2.96	9.49	13.5	2.07	1.98	3.37
q_3	9.35	3.25	1.34	9.73	3.18	12.4	18.4	2.38	2.57	3.87
max	15.7	5.32	2.14	13.1	5.10	16.10	21.6	3.54	5.85	6.75
mean	9.28	2.09	1.08	8.31	3.03	9.53	13.8	2.16	2.34	3.29
SD	2.28	1.51	0.51	2.70	0.82	3.79	5.35	0.59	1.28	1.59
$CV\cdot10^{2}$	2.46	7.23	4.73	3.24	2.71	3.98	3.87	2.72	5.50	4.82

The lowest determined concentrations were of 10^{-21} and the biggest were of 10^{-7} order of magnitude. Significant differences between mean and median q_2 indicate skewed distributions of Cs-137 concentrations. Also variability in Cs-137 concentrations was the highest among the radionuclides determined.

Relationship between pairs of radionuclides concentrations are illustrated by values of *VR* parameters shown in Table 3.

Table 3

The *VR* parameter values calculated for pairs of radionuclide concentrations

	K-40	**Cs-137**	**Pb-210**	**Bi-212**	**Pb-212**	**Bi-214**	**Pb-214**	**Ac-228**	**Th-231**
Cs-137	0.58								
Pb-210	0.27	0.27							
Bi-212	0.10	0.50	0.09						
Pb-212	0.04	0.45	0.14	0.03					
Bi-214	0.22	0.33	0.05	0.07	0.10				
Pb-214	0.17	0.36	0.06	0.05	0.07	0.01			
Ac-228	0.05	0.49	0.14	0.02	0.00	0.09	0.07		
Th-231	0.20	0.70	0.45	0.32	0.28	0.36	0.33	0.28	
U-235	0.27	0.40	0.18	0.20	0.22	0.10	0.11	0.20	0.21

The results presented in Table 3 suppose that the radioisotopes from the radium and thorium decay series were close to equilibrium state. Despite of significantly longer half-life time of Pb-210 in comparison with $t_{1/2}$ of Pb-214 and Bi-214, concentrations of these isotopes were proportional in soil samples. It supposes lack of Pb-210 deposition or leaching in the investigated area.

The thorium series is represented by short living radioisotopes. But in decay chain unstable Ac-228 is separated from short living Bi-212 and Pb-212 by moderately stable Th-228 ($t_{1/2}$ = 1.913 a). Concentration proportionality of the determined radioisotopes from thorium series supposes absence of processes significantly influencing thorium content in soil.

In opposite to radium and thorium decay series, concentrations of U-235 and its daughter Th-231 from actinium series are rather poorly related with each other. The U-235 long-living radioisotope is the straight ancestor of rather unstable Th-231. Though concentrations proportionality of these radioisotopes could be expected, the data analysis reveals considerable values of measurement uncertainty, particularly for Th-231. For this reason proportionality of radium and thorium concentration could be masked.

Concentration of K-40 is somewhat bound with concentrations of radionuclides from thorium decay chain. It could be a result of K abundance in minerals containing Th-232, the prime, long-living ancestor in thorium series.

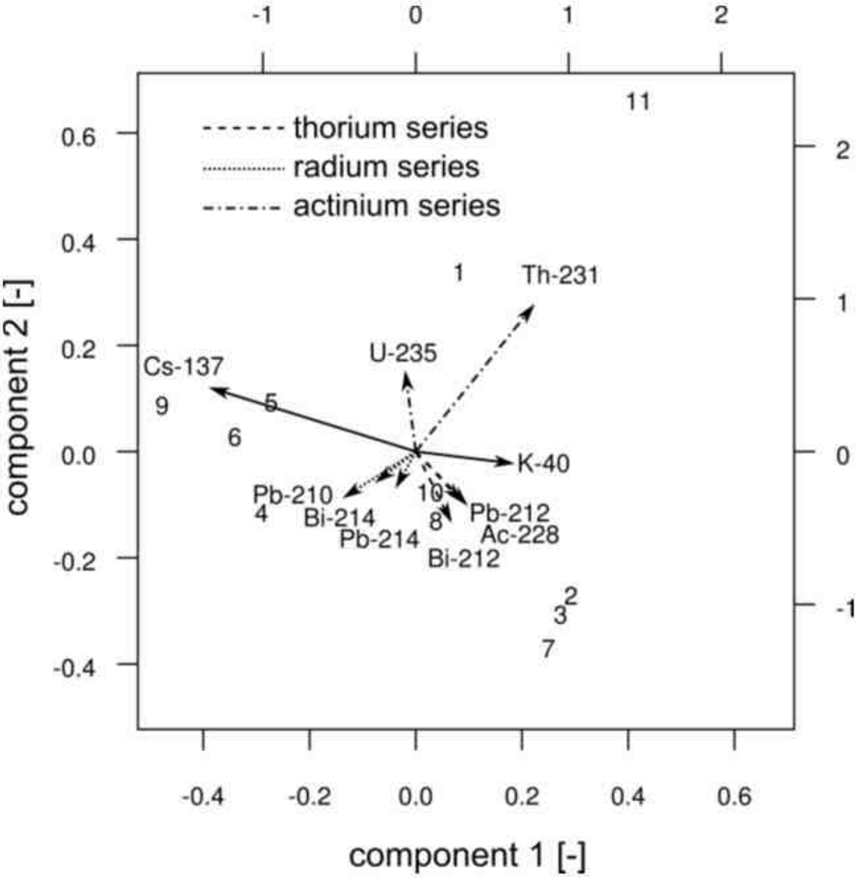

Fig. 1. Variables factor map of principal components calculated from clr transformed isotope concentrations

The Cs-137 concentrations were not related to concentrations of other radioisotopes. This radionuclide is still deposited from air on the soil surface. The dust containing Cs-137, deposited on the soil surface, can be locally relocated by floating water from rains or by breaths of wind. This phenomenon occurs mainly on the ground surface, leaving practically unaffected somewhat deeper soil layers.

To investigate common relationship between radionuclide concentrations, the principal component analysis of clr transformed concentrations was carried out. The results obtained are illustrated in biplot (Fig. 1).

The results of PCA confirm conclusions drawn from the *VR* values. In the biplot small distances between arrowheads indicate positive co-variability between concentrations. Such relationship is observed for radionuclides of thorium series and for the radionuclides belonging to radium decay chain.

Conclusions

In the investigated area the radioisotope composition of soil surface layer remains nearly unchanged. It supposed absence of significant matter translocation in ground. It could be supposed that a possible soil contaminant, introduced in period of industrial activity, remains in soil. However due to contaminants specific chemical or physical properties (good solubility in water, high volatility) it could be moved outside borders of the polluted area. But such phenomenon would be finished soon after stopping contaminant delivery. Pollution bound with inorganic components of soil is well immobilized.

Acknowledgments

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OSZACOWANIE SKALI PRZEMIESZCZANIA SIĘ MATERII NA OBSZARZE BYŁEJ CEMENTOWNI

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Abstrakt: Oszacowanie skali wielkości przemieszczania się masy ma kluczowe znaczenie w ocenie zagrożeń wynikających z działalności przemysłowej. W wielu regionach znajdują się pozostałości po zakładach przemysłowych, które mogą być źródłem szkodliwych substancji w środowisku. Na tych obszarach można oczekiwać podwyższonego poziomu skażeń, których źródłem jest wcześniejsza działalność przemysłu. Na takich terenach mogą występować m.in. zwiększone ilości izotopów promieniotwórczych, powstających w wyniku przetwarzania surowców mineralnych lub spalania paliw kopalnych. Do badań pobrano próbki gleby zebrane na obszarze byłej cementowni „Piast", znajdującej się w granicach miasta Opole. W badanych próbkach oznaczono aktywności naturalnych izotopów gamma-promieniotwórczych. W próbkach gleby znajdował się także sztuczny izotop Cs-137. Podczas analizy danych wykorzystano metody analizy zmiennych złożonych. Stwierdzono, że izotopy z szeregu radowego i torowego znajdowały się w stanie zbliżonym do równowagowego. Aktywności U-235 i Th-231 były słabo ze sobą związane. Aktywność K-40 była w pewnym stopniu związana z aktywnościami radionuklidów pochodzących z szeregu torowego. Aktywność Cs-137 nie ma związku z aktywnościami pozostałych izotopów promieniotwórczych. Uzyskane wyniki sugerują ograniczone przemieszczanie się materii na obszarze byłej cementowni. Zarówno depozycja, jak i wymywanie/ługowanie materii na badanym obszarze jest ograniczone.

Słowa kluczowe: zanieczyszczenia przemysłowe, radioizotopy, translokacja

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Janina GOSPODAREK[1]

EFFECT OF SOIL POLLUTION WITH OIL DERIVATIVES ON THE OCCURRENCE OF *Harpalus rufipes* DEG.

ODDZIAŁYWANIE ZANIECZYSZCZENIA GLEBY ROPOPOCHODNYMI NA WYSTĘPOWANIE *Harpalus rufipes* DEG.

Abstract: The research aimed at investigating the effect of oil derivatives during the process of their bioremediation on dynamics of *Harpalus rufipes* Deg. (Coleoptera, Carabidae) occurrence. The following objects were established: control - unpolluted soil; soil polluted with petrol; soil polluted with diesel fuel and soil polluted with used engine oil (dose: 6,000 mg of fuel · kg^{-1}d.m. of soil). Experiment was set up in two series: with natural and supported bioremediation. *H. rufipes* was trapped using Barber's traps, during the periods from June to October 2010, from May to October 2011 and 2012. Activity of *Harpalus rufipes* Deg. species representatives under conditions of soil polluted with oil derivatives depended on the kind of pollutant substance and on the time which passed from the moment of the soil pollution. Petrol had the least negative effect - it was visible only during the first four months after the pollution. Negative effect of diesel and engine oil was observed even 14 months from the moment of pollution. Application of supported bioremediation on the soil polluted with diesel oil contributed to increasing the number of trapped *H. rufipes* beetles after 14 months from the moment of its application and from the moment of the soil contamination, whereas after two years the same measure neutralized the effect of intensified activity of the above mentioned beetles under conditions of the soil polluted with diesel and engine oil.

Keywords: oil derivatives, soil, bioremediation, Carabidae, *Harpalus rufipes* Deg.

Harpalus rufipes Deg. beetle from the Carabidae family is often counted among the species dominating the epigeal fauna of arable fields [1-4]. It has been stated that it is both herbivourous species (particularly in spring) and may feed on animal food [5]. Both feeding systems may have positive aspects, because consumed plants and their seeds often include weeds, whereas aphids are among the eaten animals [6]. Great number of beetles from this species and their distribution on crops on a majority of soils may prove very important from the perspective of pest and weed control. Quality of the soil environment is of crucial importance for the epigeal fauna presence. Among many kinds of soil pollution, contamination with oil derivatives has been the least identified as to its effect on invertebrates connected with the soil environment. At the same time the occurrence of many Carabidae species is known to be connected both with the type of soil and soil management [7, 8].

The research aimed at investigating the effect of oil derivatives during the process of their bioremediation on dynamics of *Harpalus rufipes* Deg. (Coleoptera, Carabidae) occurrence.

Materials and methods

The research was carried out in 2009-2012 at the Experimental Station of the University of Agriculture in Mydlniki near Krakow. In the experiment containers with

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a capacity of 1 m^3 were used. The containers were dug into the soil, so that their upper area was on the same level with the surrounding soil. In June 2010 the soil in containers was polluted with the following oil derivatives: petrol, diesel oil and used engine oil (dose 6,000 mg of fuel · kg^{-1}d.m. of soil). A week later half of the containers was subjected to bioremediation with the use of ZB-01 biopreparation, specially prepared for this purpose. The experiment was performed in four replications. Four objects were created in two series (natural and supported bioremediation): 1. Control - unpolluted soil, 2. Soil contaminated with petrol, 3. Soil contaminated with diesel oil and 4. Soil contaminated with used engine oil. Epigeal fauna including *Harpalus rufipes* Deg. was trapped using Barber's traps (0.9 dm^3 jars dug even with the soil level and protected against atmospheric precipitation with a plastic roof) placed in the central point of each container. The traps were emptied once a week during the periods from June to October 2010, from May to October 2011 and 2012. Trapped carabid beetles were classified using appropriate keys [9]. Statistical computations were made using Statistica 10.0 PL computer programme. Means were diversified using NIR Fisher test at significance level $\alpha = 0.05$.

Results and discussion

The dynamics of *H. rufipes* trappings points to its most numerous presence in the summer months (July, August, September) and generally at the same time a significant diversification in the trapped number was observed depending on the applied pollutant substance (Figs. 1 and 2, Table 1).

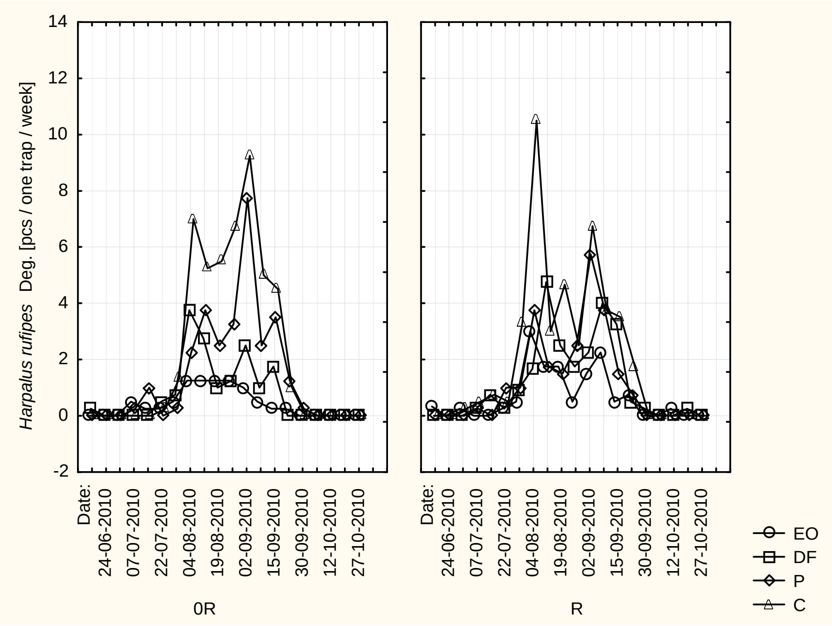

Fig. 1. Course of dynamics of *Harpalus rufipes* Deg. occurrence trapped using Barber's traps in 2010. EO - soil contaminated with used engine oil, DF - soil contaminated with diesel fuel, P - soil contaminated with petrol, C - unpolluted soil, 0R - series without bioremediation, R - series with bioremediation

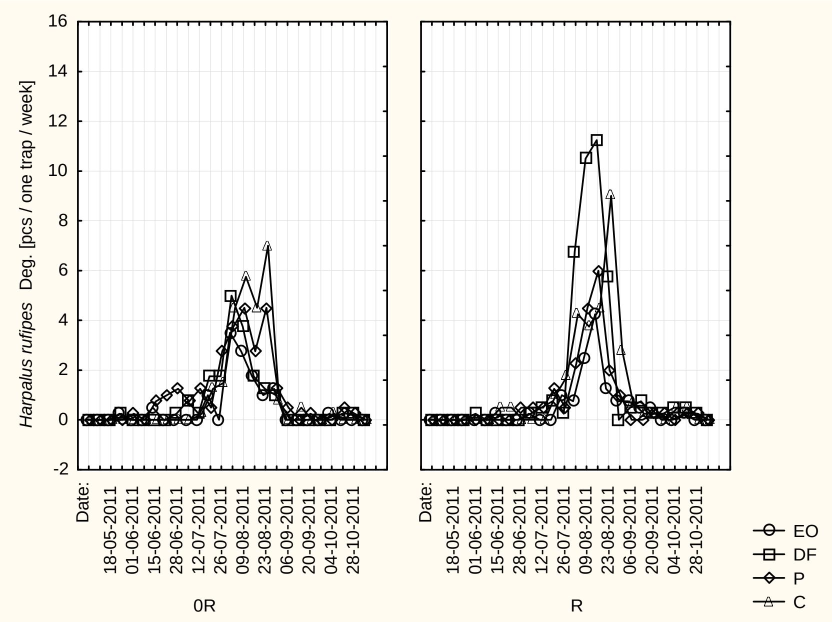

Fig. 2. Course of dynamics of *Harpalus rufipes* Deg. occurrence trapped using Barber's traps in 2011. The symbols as in Figure 1

Table 1

Occurrence of *Harpalus rufipes* Deg. trapped using Barber's traps in individual months after soil contamination. The symbols as in Figure 1

Number of months from the moment of soil contamination	***Harpalus rufipes* Deg. [pcs/trap/month]**							
	Control		**Petrol**		**Diesel fuel**		**Engine oil**	
	0R	**R**	**0R**	**R**	**0R**	**R**	**0R**	**R**
1	0.00 a*	0.75 a	0.25 a	0.25 a	0.26 a	0.25 a	0.50 a	0.58 a
2	7.17 b	15.08 c	3.50 ab	5.75 ab	5.00 ab	3.42 ab	2.25 a	3.75 ab
3	26.75 b	16.92 ab	17.25 ab	11.50 a	7.50 a	11.25 a	4.75 a	5.50 a
4	10.50 d	9.00 cd	7.50 bcd	6.00 a-d	2.75 ab	8.00 bcd	1.00 a	3.50 abc
5	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.25 a	0.00 a	0.25 a
11	0.00 a	0.00 a	0.25 a	0.00 a	0.25 a	0.25 a	0.25 a	0.00 a
12	0.00 a	1.00 a	3.00 b	0.50 a	0.25 a	0.00 a	0.50 a	0.25 a
13	3.00 a	2.75 a	5.25 a	2.75 a	4.50 a	1.75 a	1.00 a	1.25 a
14	22.50 ab	24.25 ab	16.75 ab	15.75 a	12.75 a	34.25 b	10.25 a	9.50 a
15	0.50 ab	1.17 ab	0.75 ab	0.50 ab	0.00 a	1.75 b	0.00 a	1.75 b
16	0.25 a	1.00 a	0.75 a	0.50 a	0.50 a	1.25 a	0.25 a	0.25 a
23	1.50 b	0.50 ab	0.75 ab	0.25 a	0.50 ab	0.00 a	0.75 ab	0.50 ab
24	2.50 a	1.25 a	2.50 a	1.25 a	1.50 a	2.25 a	1.25 a	2.00 a
25	5.00 ab	2.83 a	7.25 ab	3.50 a	18.83 d	10.50 bc	15.08 cd	5.83 ab
26	1.00 a	1.00 a	0.83 a	3.50 bc	1.83 ab	5.00 c	1.50 ab	3.42 bc
27	1.25 c	0.92 bc	0.00 a	0.25 ab	0.33 ab	0.08 ab	0.50 abc	0.00 a
28	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a

*Means in lines marked with the same letters do not differ significantly according to NIR test at $\alpha = 0.05$; factors contamination x remediation

A similar seasonal course of the dynamics was noted by Huruk [1]. The author states that characteristic for *H. rufipes* is the model of dynamics in which the species activity intensifies with passing vegetation season and reaches the peak in August or September, then decreases. Only in few cases the peak activity were observed in other months. It results from the fact that it is a species of autumnal type of development, wintering as a larvae or pupa. Further development ensues in the subsequent year, so that the peak appearance of particularly the beetles which winter as larvae, fall in the summer months. During the first 5 months from the pollution (in the 2010 season) considerably limited number of trapped beetles from this species was registered under the influence of all applied substances (Fig. 1). Petrol reduced *H. rufipes* presence the least, whereas engine oil the most. In laboratory experiments on the effect of soil derivative polluted soil collected from the presented experiment 2 months after pouring the oil derivatives, no negative effect on the beetle viability or changes of their body weight was noted, except for the engine oil which decreased beetle survivability by about 30% after 4 weeks of culturing. Also inhibited activity of the enzymes connected with functioning of the defence system in these insects was noted, specific for the pollutant substance [10]. Statistical analysis of the results obtained in respective months passing from the moment of pollution revealed significantly less trapped specimens under conditions of soil polluted with diesel oil in months 3 and 4, while in polluted with engine oil in months from 2 to 4, in comparison with the unpolluted soil (Table 1). Application of biopreparation contributed to an increase in the number of trapped specimens in the control soil in the 2nd month of the experiment. In the objects with polluted soil greater number of *H. rufipes* were caught in the series with bioremediation, however the differences were not statistically proven. No statistically significant differences were registered either in the 1st or in the 5th month of the experiment, but it is due mainly to low activity of beetles in this period. There were June and October months when according to many authors, dynamics of *H. rufipes* trappings is characterized by a sharply outlined peak number preceded by a very low number before the peak and equally low after the maximum has been reached [1, 11, 12]. Analysis of results for the whole period from the 1st to the 5th month after contamination revealed a marked decrease in the number of trapped *H. rufipies* for all analysed kinds of pollution, the highest for the engine oil, the lowest for petrol. On the other hand, no significant effect of the applied bioremediation was noticed (Fig. 4).

During the subsequent research period (2011), *ie* after 11-16 months from the contamination, the course of *H. rufipes* trapping dynamics still indicated a negative effect of the engine oil (Fig. 2). Even 14 months after the contamination, half as many of these insects were trapped in the soil polluted with it than in the control soil. Similar as in the 2010 season, the peak number of the trapped specimen was “sharp” and fell in August, with very low number in May, June, September and October. Statistical analysis of the results from individual months indicated a significant effect of the applied bioremediation in case of the soil polluted with diesel oil. During the period of maximum presence of the insects, over twice more specimens were trapped in the soil contaminated with diesel oil and subjected to bioremediation than on the same object when the measure was not applied (Table 1). It was also reflected in the statistical analysis of results for the entire 2011 season (Fig. 4).

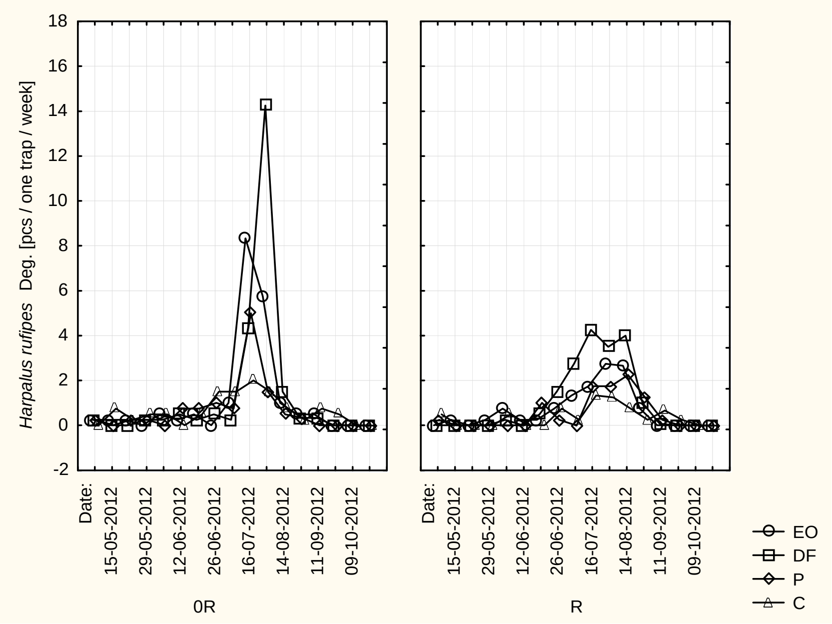

Fig. 3. Course of dynamics of *Harpalus rufipes* Deg. occurrence trapped using Barber's traps in 2012. The symbols as in Figure 1

In the 2012 season, *ie* after 23-28 months from the moment of the oil derivatives pouring, no negative effect of the pollutants on *H. rufipes* activity was registered. In the soil contaminated with diesel or engine oil at the peak number, even more of *H. rufipes* beetles were trapped than on the control (Fig. 3). In this case bioremediation was neutralizing, since the number of trapped insects in the objects polluted with diesel oil and engine oil after application of this measure was similar as in the unpolluted soil (Table 1). Similar relationships between the objects were obtained after subjecting the results obtained for the whole 2012 research period to statistical analysis.

H. rufipes Deg. is counted among the species which easily adapt to unfavourable conditions resulting from human activity. In the research on the effect of urbanization on the occurrence of Carabidae, the species was counted among the most numerous in the environment under the strongest human pressure [13]. *H. rufipes* was regarded also as one of the potential species which could be environmental indicator of Carabidae biodiversity. However, the research of Doring et al [2] revealed that the presence of none of the Carabidae species which they investigated were directly connected with this family species diversity. Huruk [1], who analysed the effect of soil chemical properties on the number of *H. rufipes* trappings, found a negative correlation between the number of captured specimens and C, K, Mg and N concentrations in the soil, whereas the correlation was positive for Ca. As results from his investigations *H. rufipes* is a species preferring soils with low content of organic matter, loose but warm. It may partially explain higher number of trappings in 2012 in the object with soil polluted with engine oil. It might result from a simultaneous decreasing of substances repellent for *H. rufipes* [14] and faster warming of soil resulting from darker colouring of the polluted soil. On the other hand, Sadej et al [15], while analysing the influence of fertilization and soil tillage method on Carabidae occurrence, caught the greatest number of *H. rufipes* on the 12-year-old fallow. The other factors, such as N and C concentrations, soil pH or C:N ratio did not reveal any direct relation with this species occurrence.

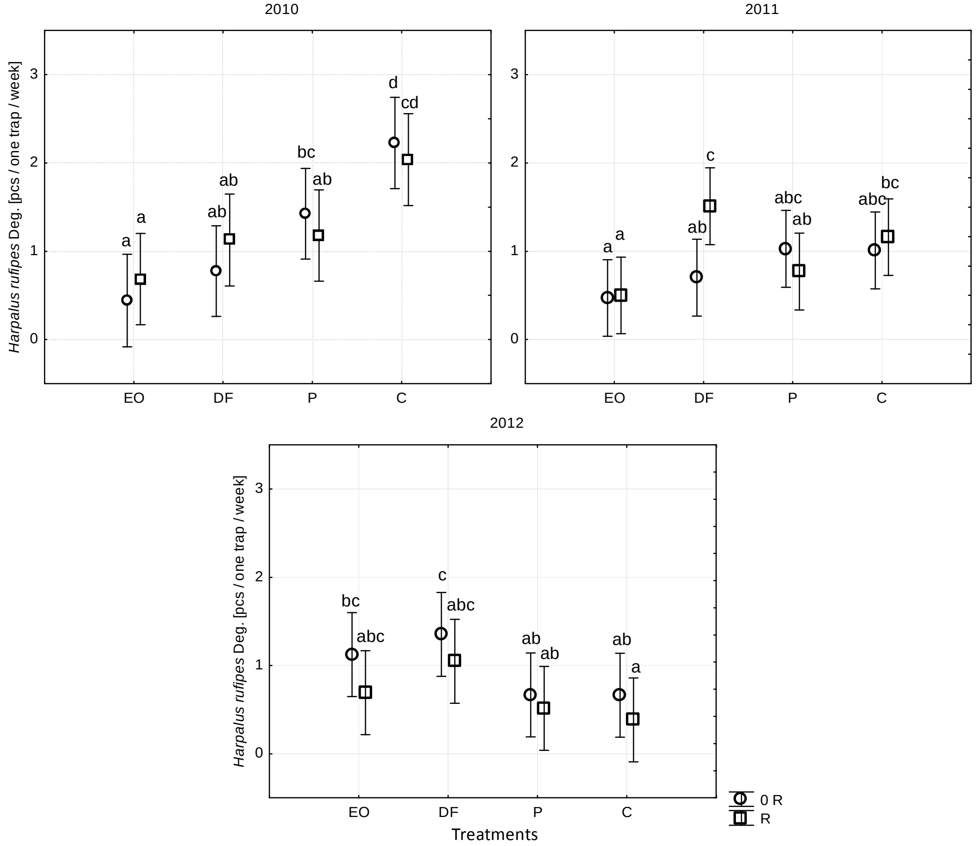

Fig. 4. Occurrence of *Harplaus rufipes* Deg. trapped using Barber's traps mean in the years 2010, 2011, 2012. The symbols as in Figure 1. Means marked with the same letters do not differ significantly according to NIR test at $\alpha = 0.05$; factors contamination x remediation. ⌶ Mean ±0.95 confidence interval

Conclusions

1. Activity of *Harpalus rufipes* Deg. species representatives under conditions of soil polluted with oil derivatives depended on the kind of pollutant substance and on the time which passed from the moment of the soil pollution. Petrol had the least negative effect - it was visible only during the first four months after the pollution. Negative effect of diesel and engine oil was observed even 14 months from the moment of pollution.

2. Application of supported bioremediation on the soil polluted with diesel oil contributed to increasing the number of trapped *H. rufipes* beetles after 14 months from the moment of its application and from the moment of the soil contamination, whereas after two years the same measure neutralized the effect of intensified activity of the above mentioned beetles under conditions of the soil polluted with diesel and engine oil.

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ODDZIAŁYWANIE ZANIECZYSZCZENIA GLEBY ROPOPOCHODNYMI NA WYSTĘPOWANIE *Harpalus rufipes* DEG.

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Abstrakt: Celem pracy było zbadanie oddziaływania substancji ropopochodnych w trakcie procesu bioremediacji gleby na przebieg dynamiki występowania *Harpalus rufipes* Deg. (Coleoptera, Carabidae). Utworzono następujące obiekty: kontrola - gleba niezanieczyszczona; gleba zanieczyszczona benzyną; gleba zanieczyszczona olejem napędowym oraz gleba zanieczyszczona zużytym olejem silnikowym (dawka: 6000 mg paliwa · kg^{-1} s.m. gleby). Eksperyment został przeprowadzony w dwóch seriach: z naturalną i wspomaganą bioremediacją. *H. rufipes* był odławiany z użyciem pułapek Barbera w okresie od czerwca do października 2010 oraz od maja do października 2011 i 2012 roku. Aktywność przedstawicieli gatunku *Harpalus rufipes* Deg. w warunkach gleby zanieczyszczonej ropopochodnymi zależała od rodzaju substancji zanieczyszczającej oraz od czasu, jaki upłynął od momentu skażenia gleby. Najmniej negatywnie oddziaływała benzyna - jej wpływ widoczny był tylko przez początkowe 4 miesiące po zanieczyszczeniu. Negatywne działanie oleju napędowego i silnikowego obserwowano jeszcze po upływie 14 miesięcy od momentu skażenia. Zastosowanie bioremediacji wspomaganej na glebę zanieczyszczoną olejem napędowym przyczyniło się do zwiększenia liczby odławianych chrząszczy *H. rufipes* po upływie 14 miesięcy od momentu jej przeprowadzenia oraz od momentu skażenia gleby, natomiast po upływie dwóch lat zabieg ten neutralizował efekt zwiększenia aktywności wspomnianych chrząszczy w warunkach gleby poddanej zanieczyszczeniu olejem napędowym i silnikowym.

Słowa kluczowe: ropopochodne, gleba, bioremediacja, Carabidae, *Harpalus rufipes* Deg.

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PRELIMINARY REMOVAL OF PHOSPHORUS FROM MUNICIPAL WASTEWATER

WSTĘPNE USUWANIE FOSFORU ZE ŚCIEKÓW MIEJSKICH

Abstract: The results of municipal wastewater treatment by chemical coagulation with PIX and electrocoagulation in a recirculation system with aluminum electrodes were discussed and interpreted. Those processes were analyzed as: a) preliminary processing before biological treatment, or b) the only treatment procedure. The results of chemical coagulation and electrocoagulation indicate that both methods guarantee the required level of phosphorus and suspended solids removal from wastewater. Despite the above, the COD of treated wastewater decreased to only 200 mg·dm^{-3} which exceeds the norm for effluents discharged from treatment plants (125 mg·dm^{-3}). Our results indicate that municipal wastewater treatment cannot rely solely on chemical coagulation and/or electrocoagulation. In successive parts of the study, wastewater was treated with significantly smaller doses of a ferric coagulant (PIX) and an aluminum electrocoagulant (Al electrodes). The above treatments lowered the phosphorus load of wastewater to a level ensuring its nearly complete removal in successive stages of biological treatment. The practical application of the resulting database was evaluated in view of the existing regulations as well as more stringent requirements which could be introduced in the future, in particular with regard to total phosphorus loads.

Keywords: municipal wastewater, chemical treatment, electrocoagulation

Introduction

Global phosphorite and apatite resources will be depleted by around 2050 [1]. This fact and the introduction of increasingly stringent wastewater treatment standards will necessitate the recovery of potential fertilizer nitrogen from treated wastewater. Phosphorus recirculation in the <wastewater sludge - fertilizer - food - phosphorus in wastewater> system can become an important, if not the only source of phosphorus fertilizers.

In Poland, selected municipal wastewater treatment plants are not under obligation to control phosphorus concentrations in processed effluents. The above applies to effluents discharged to a body of flowing water, such as a river, which does not intersect stagnant water bodies, including lakes and ponds [2]. Polish regulations continue to raise many objections in the Baltic states, in particular in Scandinavian countries. Wastewater treatment standards determine research trends, including on the industrial and semi-technical scale. The majority of laboratory research focuses on the development of new methods for preliminary chemical coagulation [3-5] and electrocoagulation [6-8] of wastewater are becoming more and more interesting even for these WWTPs. In view of searching for more efficient and more economical methods for phosphate removals, a comparison of the effectiveness of chemical coagulation and electrocoagulation is interesting. This study discusses the preliminary research on such a comparison with

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a wastewater which is not required to remove phosphorous today, but which might get such a requirement in the future.

Materials and methods

Reszel WWTP in Poland serves an estimated population of 5000 and is not required to control phosphates in its effluents today. The WWTP has mechanical and biological (SBR) unit process. Wastewater samples were collected after the sand trap one day before the experiments and refrigerated until the following morning. The effluent discharges meets the COD and suspended solids (SS) discharge norms, while the effluent phosphorous concentrations, which is not subject to a control, remains around 3.5 $\text{mg}\cdot\text{dm}^{-3}$.

Wastewater samples were brought to the temperature of 21°C. Several cm^3 of saturated NaCl solution was added to 1 dm^3 of wastewater to increase its specific conductance to 0.004 $\Omega^{-1}\cdot\text{cm}^{-1}$. In the jar test procedure, the sample and the coagulant were mixed rapidly for 2 minutes (at approximately 400 rpm) and slowly for 15 minutes (at approximately 20 rpm). The mixture was left to stand for 30 minutes. Analytical samples were collected after sedimentation from the liquid above the sludge layer. The applied coagulant was PIX-113 [www.kemipol.com.pl] was used as the coagulant and the experiments were conducted at this time without any pH adjustments.

A diagram of the electrocoagulation reactor with a recirculation system is presented in Figure 1.

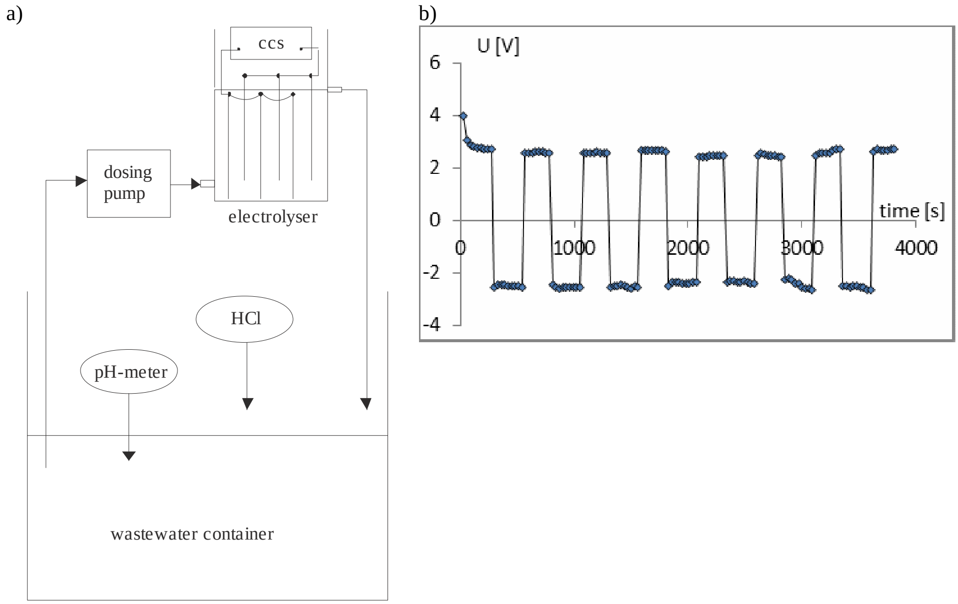

Fig. 1. Diagram of the electrocoagulation reactor (a) and change in applied voltage (b)

Electrocoagulated wastewater was recirculated between the electrolyzer and the reservoir. The process was performed chronopotentiometrically at constant current intensity of 0.3 or 0.15 A, and changes in voltage were registered (Fig. 1b). The direction of the current was reversed automatically every 256 s [9] to clean the electrodes. Wastewater samples of 1 or 2 dm^3 were recirculated within 22 minutes to 1 hour. The pH of wastewater samples was maintained at 6.0 through the addition of 2M HCl. Analytical specimens were collected every 5-15 minutes. Their colour, suspended solid concentrations and turbidity were determined, and the specimens were fed back into the recirculated wastewater reservoir to minimize changes in system volume.

The pH, specific conductance, colour, turbidity, suspended solid concentrations, total phosphorus and total nitrogen concentrations, and COD were determined in raw and processed wastewater samples. Specific conductance was measured in the Delta OHM HD 2156.2 conductometer, pH was determined with the Hanna Instruments HI 8242 pH meter, and the remaining measurements were performed with the use of the Hach Lange DR 2800 spectrophotometer [10].

Results and discussion

The results of chemical coagulation of wastewater, applied as the only treatment method, are presented in Figure 2.

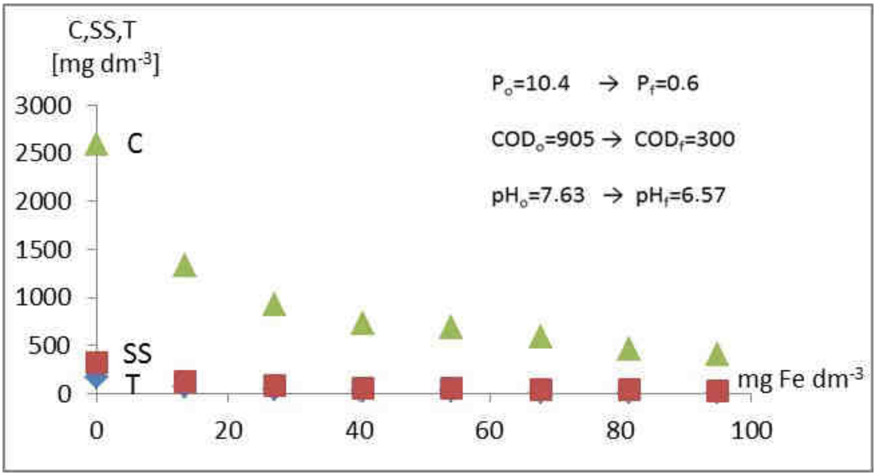

Fig. 2. PIX coagulation of municipal wastewater as the only treatment method; (C - Colour, SS - Suspended Solids, T - Turbidity)

The chemical coagulation process with PIX is presented in Figure 2. Changes in the colour, suspended solid concentrations and turbidity of wastewater samples at increasing coagulant doses are shown here. The first PIX dose of 13.55 mg $Fe\cdot dm^{-3}$ (0.24 $mmol\cdot dm^{-3}$) removed 49% colour, 60% suspended solids and 61% turbidity from coagulated wastewater. The highest PIX dose of 95 mg $Fe\cdot dm^{-3}$ (1.7 $mmol\cdot dm^{-3}$) reduced colour in 85% (from approximately 2600 $mg\cdot dm^{-3}$ in raw wastewater to 403 $mg\cdot dm^{-3}$ in treated wastewater), suspended solids - in 95% (from approximately 320 to 31 mg·dm^{-3}) and turbidity in 93% (from 173 to 12 mg·dm^{-3}). The highest PIX dose lowered the pH of wastewater from pH_o = 7.63 to pH_f = 6.57. Total phosphorus concentrations decreased by 94% from P_o = 10.4 to P_f = 0.6 mg·dm^{-3}, and COD decreased by 67% from COD_o = 905 to COD_f = 300 mg·dm^{-3}. The effluent wastewater satisfied Polish discharge regulations [2] with regards to total phosphorus and suspended solid concentrations, but the COD of the analyzed effluent insignificantly exceeded the statutory threshold of 125 mg·dm^{-3}.

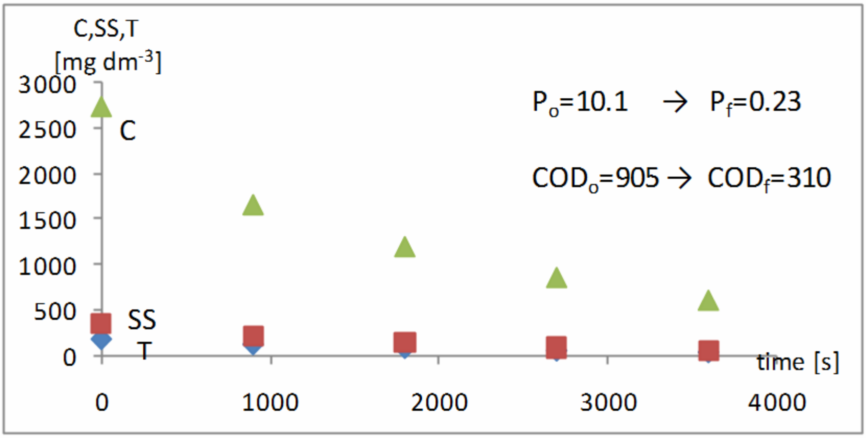

Fig. 3. Electrocoagulation of municipal wastewater in a recirculation system as the only treatment method (C - Colour, SS - Suspended Solids, T - Turbidity)

The results of electrocoagulation of wastewater in a recirculation system, applied as the second treatment method, are presented in Figure 3. Changes in the colour, suspended solids concentrations and turbidity of wastewater samples at increasing does of the aluminum are shown here. A dosage of electrocoagulant was proportionall to the time of electrolysis according to Faraday's law, $m = k \cdot i \cdot t$, where the chemical equivalent of aluminum is:

$$k = 27/(96500 \cdot 3) = 9.3 \cdot 10^{-5}\ \mathrm{g \cdot A^{-1} \cdot s^{-1}}$$

The intensity of the electrical current was kept constant at 0.3 A. The degree of wastewater purification was checked every 15 minutes (900 s). The first (minimal) dose of the aluminum electrocoagulant (approximately 25 mg Al·dm^{-3} = 0.92 mmol of Al·dm^{-3}) removed colour and suspended solids in 40% and turbidity in 33%. The highest Al dose of approximately 100 mg Al·dm^{-3} (3.7 mmol·dm^{-3}) removed 78% colour, 83% suspended solids and 88% turbidity. Total phosphorus concentrations decreased by 98% from P_o = 10.1 to P_f = 0.23 mg·dm^{-3}, and COD decreased by 65% from COD_o = 905 to COD_f = 310 mg·dm^{-3}. The system's pH increases during electrolysis [11], and approximately 5 cm^3 of 2M HCl was added per 1 dm^3 of the sample to maintain a constant pH of 6.0 during one hour of electrocoagulation. Treated wastewater met the prescribed values for total phosphorus concentrations, whereas suspended solid concentrations and COD of the analyzed effluents insignificantly exceeded the statutory thresholds.

The biological treatment normally removes 50-60% of the total phosphorus in the WWTP studied. We wanted to study the requirement of coagulation processes to supplement this removal to achieve the discharge standards. For the comparison purposes we have thus studied the coagulation requirements to remove about 50% of the total phosphates.

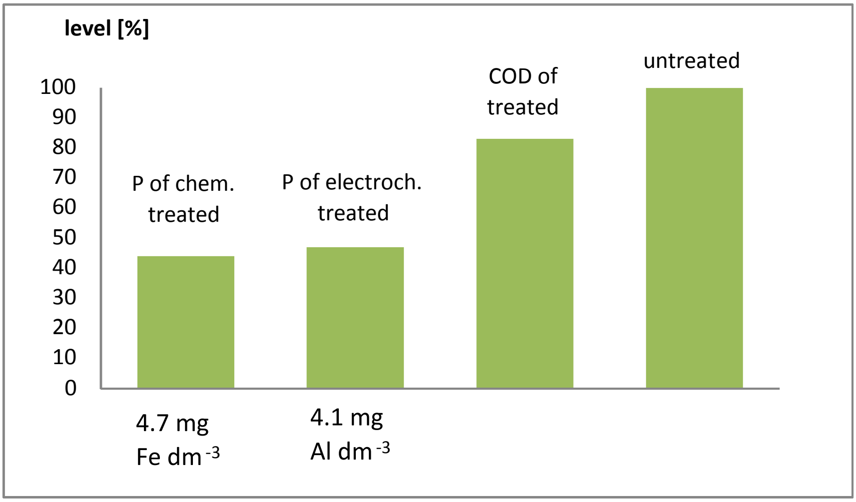

Fig. 4. Preliminary PIX chemical coagulation and preliminary electrocoagulation of municipal wastewater in a recirculation system

The results of preliminary PIX chemical coagulation and electrocoagulation are presented in Figure 4. Approximately 4.7 mg Fe·dm^{-3} (0.084 mmol·dm^{-3}) was required to reduce the total phosphorus load by 50%. The above implies that a treatment plant with daily processing capacity of 1000 m^3 wastewater would consume minimum 12 tons of PIX per year.

A 50% reduction in the total phosphorus load of electrocoagulated waste was achieved by applying an electrocoagulant dose of 4.1 mg Al·dm^{-3} (0.15 mmol·dm^{-3}) and consuming 10^{-5} kWh·dm^{-3} of electricity at $I = 0.15$ A and mean $U = 1.5$ V. This implies that a treatment plant with daily processing capacity of 1000 m^3 wastewater would consume minimum 1.2 tons of scrap aluminum (electrodes) and 300 kWh energy per year.

A 50% reduction in total phosphorus load (by coagulation and electrocoagulation) decreases COD by approximately 17% on average.

Conclusions

Coagulation experiments documented by using 0.084 mmol-Fe·dm^{-3} of PIX-113 (conventional coagulation) or 0.15 mmol-Al·dm^{-3} (electrocoagulation) it is possible to achieve comparable removals of total phosphorus, which resulted in about 50% reduction. Such a supplementary removal, in addition to the removals by the biological treatment processes, will then satisfy the Polish discharge standards. In practical terms, 12 tons of

PIX or 1.2 tons of scrap aluminum (electrodes) and 3000 kWh of energy will be required to achieve the above targets annually. It will produce about 3 tons of phosphorus annually, which is equivalent to 13 tons of fertilizer with chemical formula $CaHPO_4$.

Acknowledgements

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WSTĘPNE USUWANIE FOSFORU ZE ŚCIEKÓW MIEJSKICH

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Abstrakt: W pracy opisano i zinterpretowano rezultaty procesów oczyszczania ścieków miejskich koagulowanych chemicznie PIX-em oraz elektrokoagulowanych recyrkulacyjnie na elektrodach glinowych. Procesy te potraktowano jako: a) etap wstępny, przed biologicznym oczyszczaniem badanych ścieków oraz b) jedyne i ostateczne oczyszczanie ścieków. Wyniki badań procesu koagulacji chemicznej oraz elektrokoagulacji badanych ścieków pokazały, że metody te zapewniają wymagany poziom usunięcia zawiesin i fosforu z oczyszczanych ścieków. Jednakże ChZT tak oczyszczonych ścieków obniżało się tylko do ok. 200 mg·dm^{-3} przy wymaganym poziomie 125 mg·dm^{-3} w odpływie z oczyszczalni. Fakt ten nie pozwala więc traktować koagulacji chemicznej lub/i elektrokoagulacji jako jedynego i ostatecznego etapu oczyszczania badanych ścieków miejskich. Stąd w dalszej części badań ścieki traktowano znacznie mniejszymi dawkami koagulantu żelazowego (PIX) i elektrokoagulantu glinowego (elektrody Al). Prowadziło to do częściowego usunięcia fosforu, zapewniając takie obniżenie poziomu P, aby kolejny etap biologicznego oczyszczania mógł zapewnić stężenie tego biogenu w odpływie bliskie zeru. Dokonano oceny wartości aplikacyjnej uzyskanej bazy danych w świetle aktualnych norm i uwarunkowań, jak również w szerszej perspektywie zaostrzenia wymagań w zakresie stopnia oczyszczenia ścieków, szczególnie w zakresie usuwania fosforu.

Słowa kluczowe*:* ścieki miejskie, chemiczne oczyszczanie, elektrokoagulacja

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and Teresa KRZYŚKO-ŁUPICKA[1]

BACTERIAL BIOAEROSOL IN THE WAREHOUSING AREA OF FOOD INDUSTRY FACILITY

BIOAEROZOL BAKTERYJNY W STREFIE MAGAZYNOWEJ ZAKŁADU PRZEMYSŁU SPOŻYWCZEGO

Abstract: In food industry is observed increase of attention to step related with warehousing of food product and raw materials in the food processing chain. Microbial contamination of raw materials and food products due to warehouse errors could generate economical losses for the facility. The aim of study was estimation of the bacterial contamination in the bioaerosol from warehousing area of the selected food processing plant. Research materials were microorganisms isolated from the air in the food warehousing area of the selected food processing plant. Air analysis was performed in four closed spaces: warehouse of food products (MPI and MPII), technical warehouse (MT) and the warehouse of unit packages (MPJ). Analysis was carried out three times by Koch's sedimentation method. Petri dishes with PCA agar medium (BTL, Poland) were placed at a height of 1.3 m for 15 minutes. Samples were incubated at 30ºC for 48 hours (PN-89/Z-04111/03). Results were given in $\mathrm{cfu \cdot m^{-3}}$. Each room was analyzed three times in May, July and October. Presence of catalase-positive and catalase-negative strains was determined by biochemical tests with use of hydrogen peroxide in concentration of 3%. Gram-negative rods were divided into two groups: oxidase-positive and oxidase negative by using the reaction of tetramethyl-1,4-phenylenediamine dihydrochloride oxidation. Analysis showed that the most polluted bioaerosol was observed in first warehouse of products (MPI), where in May and October assessed number of bacteria was $1 \cdot 10^5$ $\mathrm{cfu \cdot m^{-3}}$. Similarly high numbers of bacteria were determined initially in a technical magazine (MT), which then has 4-fold decrease. In the second warehouse of product (MPII) and the warehouse of unit packages (MPJ) the air contamination of bacteria ranged from $3.7 \cdot 10^4$ $\mathrm{cfu \cdot m^{-3}}$ in MPII space to $2 \cdot 10^4$ $\mathrm{cfu \cdot m^{-3}}$ in MPJ space.

Keywords: food industry, bioaerosol, bacteria, storage area

Introduction

The food industry, in relation to the secondary product contamination caused by microflora associated with the production, suffers high economic losses resulting from a decrease in the quality of the final product. Both bacteria and filamentous fungi may pose a threat not only to the final product during storage but also to the health of workers [1]. The degree of exposure to the adverse impacts in a significant extent is dependent on the composition of bioaerosol in warehouses. Persistence of pathogens in food products depends on processing conditions, habitats of microorganisms and transmission of microbial cells and spores [2]. Olborska and Lewicki [3] suggest, that 70% of microbial contamination of finished product is caused by spreading of microorganisms in the air. High risk of the microbial transmission to food commodities leads to increased interest in monitoring of the food processing environment [4].

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[2] Scholarship holder of programme: Stypendia doktoranckie - inwestycja w kadrę naukową województwa opolskiego co-financed by European Union under European Social Fund

[*] Contribution was presented during ECOpole'14 Conference, Jarnoltowek, 15-17.10.2014

Bioaerosol is heterogeneous mixture consisting of the biological materials including: viruses, bacteria, fragments of filamentous fungi and spores [1, 5, 6]. In addition to it, bioaerosol contain metabolic products of microorganisms such as endotoxins, enterotoxins, enzymes and mycotoxins. [6, 7]. Bioaerosol is composed of particles with diameters from 0.02 to 100 μm [6]. Individual bioaerosol fractions is characterized by various particles size. In the cause of bacteria, cells diameter range from 0.5 to 2.0 μm *eg Bacillus, Pseudomonas, Xanthomonas* and *Arthrobacter*. Size of fungal spores is related to isolated species e.g. *Aspergillus fumigatus* (3.5-5.0 μm), *Aspergillus niger* (3.0-0.5 μm), *Pencillium brevicompactum* (7-17 μm), *Cladosporium macrocarpum* (5-8 μm) *Epiccocum nigrum* (15.0-25 μm) and *Trichoderma harzianum* (2.8-3.2 μm) [8]. Small particles of diameter ranged from 0.1 to 5.0 are capable to resist in the air for hours and may be transfer at a large distance from their source [9]. Bacterial cells and spores transferred with airborne particles may pose source of contamination of processing surfaces and indirectly raw materials or final products [10].

The aim of the study was to evaluate the state of bacteriological bioaerosols in warehouses in selected food plant.

Materials and methods

The research material was the bacterial microflora isolated from the air of the storage area in the selected food plant. The following rooms were analyzed: products storage rooms (MPI, MPII), technical storage room (MT) and primary packages storage room (MPJ). Samples were taken by settle plate method in triplicate. Petri dishes with PCA medium (BTL, Poland) were exposed on height of 1.3 meters by time of 15 minutes. Each room was analyzed three times in the months of May, July and October. Then, the sample was incubated at 30°C for 48 hours [11]. The number of bacteria in the air was calculated according to Polish Standard Omelianski's formula as modified by Gogoberidze (PN-89/Z-04111/03) and is shown in $\text{cfu}\cdot\text{m}^{-3}$ [12]:

$$X = \frac{z \cdot 100 \cdot 100}{\pi \cdot r^2 \cdot 0.2 \cdot t} \quad \left[\text{cfu} \cdot \text{m}^{-3}\right]$$

where: X - the number of microorganisms in the 1 m^3 of air, z - the number of colonies in the Petri plate, r - diameter of Petri plate, t - time of Petri plate exposure.

Bacteria were divided into morphological groups using Gram staining method. Pure strains of bacteria after 48 hours incubation at 30°C were stained to isolate a group of Gram-positive and Gram-negative bacteria. Presence of catalase-positive and catalase-negative strains was determined by biochemical tests with use of hydrogen peroxide in concentration of 3%. Gram-negative rods were divided into two groups: oxidase-positive and oxidase-negative by using the reaction of tetramethyl-1,4-phenylenediamine dihydrochloride oxidation [13, 14].

Results and discussion

The analysis of composition of bioaerosols during the five-month study period showed that the number of bacteria in different storage areas remained almost unchanged. The highest difference was observed in the case of MT, which in May the number amounted to

$1 \cdot 10^5$ cfu·m^{-3}, and during the subsequent measurements it was at the level of $3 \cdot 10^4$ cfu·m^{-3}.The number of bacteria also in the case of MPI was slightly different from the others, namely in July it amounted to $4.4 \cdot 10^4$ cfu·m^{-3}, and in May and October $1 \cdot 10^5$ cfu·m^{-3}. In the remaining storage rooms, that are MPII and MPJ, the bacterial bioaerosol remained constant (Fig. 1). The level of microbial contamination of air was set by Interdepartmental Commission for Maximum Admissible Concentration and Intensities for Agents Harmful to Health in the Working Environment and should not exceed $5 \cdot 10^4$ cfu·m^{-3} [15, 16]. According to guidelines, the number of bacteria in the bioaerosol was higher than the recommended level in cases of MT and MPI.

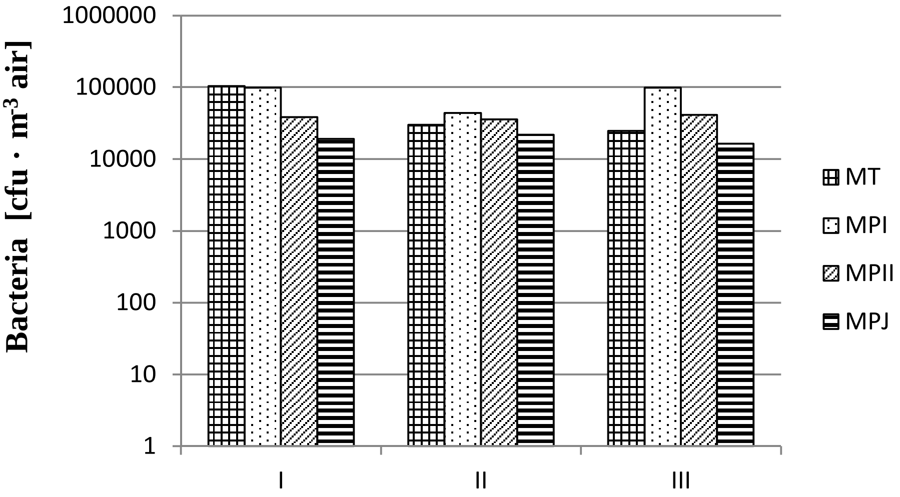

Fig. 1. Total number of bacteria [cfu·m^{-3}] in bioaerosols from storage facilities of the food plant (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected I - in May; II - in July; III - in October

Table 1

The occurrence of each morphological groups of bacteria, depending on the storage room (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected in May, July and October [%]

			MT	MPI	MPII	MPJ
Gram-negative	Rods	May	12	55	50	25
		July	0	25	77	30
		October	22	30	9	50
Gram-positive	Bacilli	May	61	34	25	50
		July	91	19	0	30
		October	33	42	53	50
	Coccus	May	27	11	25	25
		July	9	56	23	40
		October	44	28	38	0

Morphological division showed that the Gram-negative bacteria dominated only in MPII. In MPI similar levels of Gram-positive and Gram-negative bacteria were recorded. In other storage rooms, most bacterial microflora were Gram-positive bacteria. Bacilli accounted for the largest population of bacterial bioaerosols in MT and MPJ. In MPI most frequently cocci and bacilli, on the MPII dominated bacilli (Table 1). Karbowska-Berent et al, in their study also noted a predominance of Gram-positive bacteria over the Gram-negative [15]. Predominance of sporulated bacteria may pose a threat for health of workers in the storage area. The group of bacilli contains species which may be cytotoxic. Species belonging to *Bacillus* genera may product high temperature resistant cytotoxic metabolites [17].

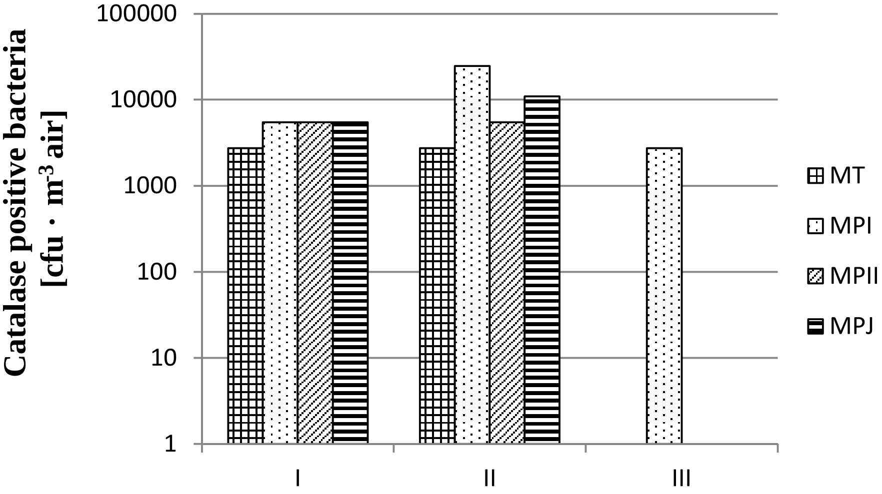

Fig. 2. Total number of catalase positive bacteria in bioaerosols from storage facilities of the food plant (MPI and MPII - products storage rooms, MT - technical storage room and the MPJ - primary packages storage room) in samples collected I - in May; II - in July; III - in October

Analysis of bacterial strains collected in May indicated that number of catalase-positive bacteria was at similar level in all storage areas. This group of bacteria accounted for 30% of the MPJ bacterial microflora of the air. In other areas the number of catalase-positive bacteria compared to the total number of bacteria was less than 15%. In July was an increase in the number of catalase-positive strains from $5.5 \cdot 10^3$ to $2.5 \cdot 10^4$ $\text{cfu} \cdot \text{m}^{-3}$ in the case of first product storage area (MPI) and from $5.5 \cdot 10^3$ to $1.1 \cdot 10^4$ $\text{cfu} \cdot \text{m}^{-3}$ in the warehouse of unit packages (MPJ). These group of bacteria accounted for 50% of the total number of bacteria in both warehouses. In October, catalase-positive bacteria occurred only in MPI and accounted for 3% of the total number of bacteria isolated from the first product warehouse (MPI) (Fig. 2, Table 2). Results showed that the highest rate of contamination of catalase-positive bacteria was observed in first warehouse of products (MPI) and storage room of units package. The contamination of bioaerosol at a level higher than $1 \cdot 10^3$ cfu·m^{-3} may pose source of final product cross-contamination and may cause adverse human health effects. The group of catalase-positive cocci includes genus of *Staphylococcus*, which not only pose threat to human health but also may form biofilm on the food related surfaces [18]. Among the bacteria of this group the most dangerous pathogen of food is *Staphylococcus aureus*. Antibiotic-resistant strains of *S. aureus* are frequently isolated from food. In addition, some of these strains produce the thermostable, protease resistant and dangerous to human health enterotoxin. *Staphylococcus* species are often isolated from the air, dust, waste water, milk, food and equipment in the food plant [19].

Table 2

The ratio of catalase-positive to total number of bacteria, depending on the storage room (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected in May, July and October [%]

Month	**MT**	**MPI**	**MPII**	**MPJ**
May	3	6	14	29
July	9	56	15	50
October	0	3	0	0

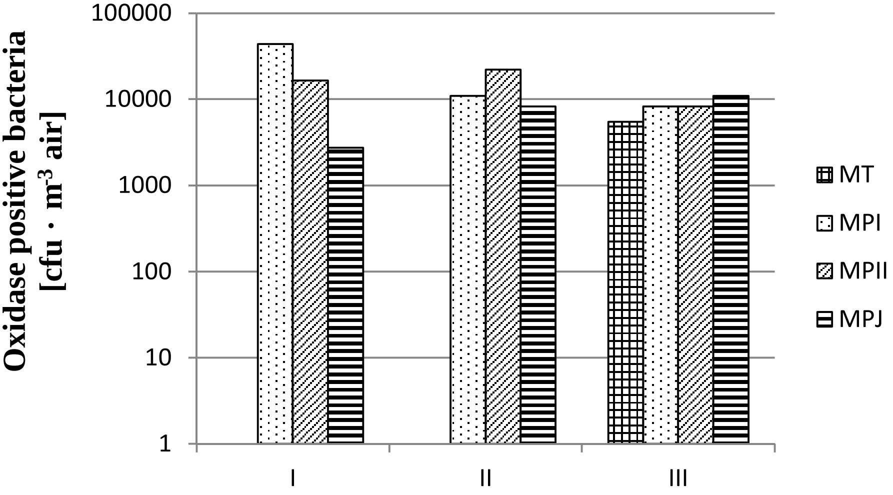

Fig. 3. Total number of oxidase positive bacteria in bioaerosols from storage facilities of the food plant (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected I - in May; II - in July; III - in October

In the samples collected in May the highest number of oxidase-positive bacteria in bioaerosol was $2.5 \cdot 10^4$ cfu·m^{-3} (MPI), and it accounted for 44% of the bacterial microflora. In the following months, number of oxidase-positive bacteria and its percentages compared to the total number of bacteria in the air in the MPI, were lower. In the case of MPII highest content of oxidase-positive bacteria was observed in July and it amounted to $2.2 \cdot 10^4$ cfu·m^{-3}. In this period also was noted the highest percent of oxidase-positive strains compared to total bacteria number and it amounted to 62%. Storage room MT was characterized by an increase in the number of oxidase-positive bacteria during the 5-month study period ranged from $2.8 \cdot 10^3$ cfu·m^{-3} in May to $1.1 \cdot 10^4$ cfu·m^{-3} in October. Number of oxidase-positive bacteria in relation to the total number of bacteria in bioaerosol of MT storage space increased from 14 to 67%. Oxidase-positive bacteria in MPT occurred only during the third collection trial, and their number was at a level similar to remaining rooms. Higher content of oxidase-positive bacteria in the air was observed in the summer months. This may be due to the higher intensity of production in these months. Group of oxidase-positive bacteria contain strains of the genus *Pseudomonas*, *Legionella* or *Campylobacter*. However, the oxidase-negative rods include a large group of pathogens contaminating food as *Salmonella*, *Shigella*, *Escherichia*, *Listeria* and *Yersinia* [19-22]. The biggest problem of air pollution from gram-negative is related to meat processing plants [23].

Table 3

The ratio of oxidase-positive to total number of bacteria, depending on the storage room (MT - technical storage room, MPI and MPII - products storage room and MPJ - primary packages storage room) in samples collected in May, July and October [%]

Month	MT	MPI	MPII	MPJ
May	0	44	43	14
July	0	25	62	37
October	22	8	20	67

Conclusions

- The composition of the bacterial bioaerosol remained at a similar level in each storage facility areas of the food plant.
- Gram-positive bacteria were the dominant group in most storage rooms.
- The results suggest that the bacterial bioaerosols can pose a potential threat to the final product.
- In case of the technical storage room in May and (MT) primary packages storage room (MPJ) in May and October the numbers of bacteria in the bioaerosol where higher than exceeds the guidelines set by the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment.
- Height number of sporulated bacteria in the air may generate a threat for the health of workers.

Acknowledgments

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BIOAEROZOL BAKTERYJNY W STREFIE MAGAZYNOWEJ ZAKŁADU PRZEMYSŁU SPOŻYWCZEGO

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Abstrakt: Branża spożywcza zwraca coraz większą uwagę na etap magazynowania surowców i produktów gotowych. Mikrobiologiczne zanieczyszczenie surowca czy produktu gotowego w wyniku błędów przy magazynowaniu może generować straty ekonomiczne dla przedsiębiorstwa. Celem pracy była ocena stanu bioaerozolu bakteriologicznego magazynów w wybranym zakładzie przemysłu spożywczego. Materiał badawczy stanowiła mikroflora bakteryjna wyizolowana z powietrza w strefie magazynowej wybranego zakładu przemysłu spożywczego. Analizie zostały poddane cztery pomieszczenia: magazyny produktów (MPI, MPII), magazyn techniczny (MT) oraz magazyn opakowań jednostkowych (MPJ). Badania wykonano metodą sedymentacyjną Kocha w trzech powtórzeniach. Płytki Petriego z podłożem agarowym PCA (BTL, Polska) eksponowano na wysokości 1,3 m przez okres 15 minut. Następnie próby inkubowano w temperaturze 30°C przez 48 godziny (PN-89/Z-04111/03). Liczebność bakterii podano w jtk·m^{-3}. W każdym pomieszczeniu dokonano analizy trzykrotnie w miesiącach maj, lipiec i październik. Równolegle przeprowadzono wstępne testy biochemiczne. Przy użyciu 3% nadtlenku wodoru bakterie podzielono na dwie grupy: posiadające i nieposiadające katalazy. Gram-ujemne pałeczki podzielono na oksydazo-dodatnie i oksydazo-ujemne przy zastosowaniu reakcji utleniania dichlorowodorku tetrametylo-1,4-fenylenodiaminy. Największym stopniem zanieczyszczenia charakteryzował się pierwszy magazyn produktu gotowego (MPI), gdzie w maju i październiku oznaczono liczebność bakterii na poziomie $1\cdot10^5$ jtk·m^{-3}. Podobnie wysoką liczebność bakterii oznaczono początkowo w magazynie technicznym (MT), która następnie uległa 4-krotnemu obniżeniu. W drugim magazynie produktu gotowego (MPII) i magazynie opakowań jednostkowych (MPJ) zanieczyszczenie powietrza bakteriami utrzymywało się na stałym poziomie $3{,}7\cdot10^4$ jtk·m^{-3} w MPII i $2\cdot10^4$ jtk·m^{-3} w MPJ.

Słowa kluczowe: przemysł spożywczy, bioaerozol, bakterie, przestrzeń magazynowa

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and Zbigniew ZIEMBIK[1]

ISSUES OF RADON (Rn-222) EXHALATION MEASUREMENTS

PROBLEMATYKA POMIARÓW EKSHALACJI RADONU (Rn-222)

Abstract: The Rn-222 isotope, a naturally occurring radioactive gas, is created in a radioactive decay of Ra-226. Both radionuclides belong to the radium decay chain in which U-238 is the prime parent. Radon is usually in plenty found in the earth crust, rocks, soil, water and air, therefore it is regarded as an indicator of ionizing radiation dose received by a population. Due to radon negative influence on people's health, measurements of its concentration are commonly performed. The presented research concerns assessment of measurements uncertainty of Rn-222 concentration exhaled from soil. The measurements were repeated at the same site. The experiment was carried out in natural conditions, in the city center and lasted about half a year. In Rn-222 concentration measurements the AlphaGUARD radon monitor was used. The device was operating in the 10-minutes flow mode, with the air pump and exhalation-box arranged in the closed cycle. The studies have shown a significant differences between results of radon exhalation measurements, which were carried out sequentially in the same place. Generally, increase in measurements number in a series lowers the result uncertainty. Consequently, the number of measurements in a series can be adjusted to the desired uncertainty of result. What is more, the distribution of the results in series is well described by the Poisson distribution.

Keywords: radon, exhalation, concentration, measurements uncertainty, AlphaGUARD

Introduction

Radon is an only gaseous radioactive element common in the whole world. It constitutes about 40% of an average annual radiation dose to the world population, what means it is a main source of ionizing radiation affecting the human [1-4]. Consequently, the presence of radon in the environment is not neutral for people's health. It can lead to some illnesses like lung cancer in specific conditions [5-8]. Therefore, an effective method of radon measurements interpretation is needed.

From a chemical point of view, radon is situated in the 18th column of the periodic table. Its density is 7.5 times bigger than the one of air, so this gas accumulates near the ground. It has no smell, taste or color, that is why people cannot detect radon with their senses. What is more, it is chemically inert and well soluble in water. This element has above 30 isotopes, but the most important is Rn-222, because of the longest half-life, which comes to 3.8 days. It is produced in U-238 decay series, directly from Ra-226. Both elements are common in soil, rocks and crust, therefore these are natural sources of radon [6, 9, 10].

Radon is able to migrate among the atmosphere, lithosphere and hydrosphere. There are three main steps of its migration. Emanation is a process in which radon moves from mineral granules to the intergranular space during radium disintegration in rocks and crust. Transportation consists relocation to the soil, which takes place with other gases or in

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liquids. Exhalation happens when radon is exuded from the soil to the air on the surface of the ground [6, 11-14].

Radon accumulates in closed spaces such as mines, caves, tunnels and even houses. Its migration is caused by diffusion and convection supported by the stack effect. This effect occurs when there is higher pressure outside than inside houses. Lower pressure inside is a result of heating in winter and air condition all over the year [6, 15-17].

Due to radon low concentration in the environment and its chemical inactivity, its activity concentration can be determined using some specialized equipment. This devices measures number of alpha-particles emitted from some kind of samples [5, 6, 18-25].

In measurements the radon monitor AlphaGUARD (Saphymo, DE) was used. It determines radon specific activity concentration and, simultaneously, air temperature, pressure and humidity. It has optimal sensitivity coming to 5 cpm at 100 Bq/m^3 and linear response from 2-2 000 000 Bq/m^3. This set consists of the radon monitor and an air pump in the basic version [21, 26-28].

The aim of this research was to study distribution of repeated measurement results of radon concentration in the air exhaled from soil and to assess the measurements uncertainty.

Materials and methods

The study of Rn-222 activity concentration *a* was conducted during 7 months, from December 2013 to June 2014, in one testing area located in the Opole University site, near the Kominka Street. It is an ordinary piece of ground situated in the city center. The territories around this area are built-up with residential buildings, so there is no close industry influence. It spanned wide range of temperatures and weather conditions. The place in which the measurements were carried out remain unchanged, so an influence of local factors, like soil type, on results can be omitted.

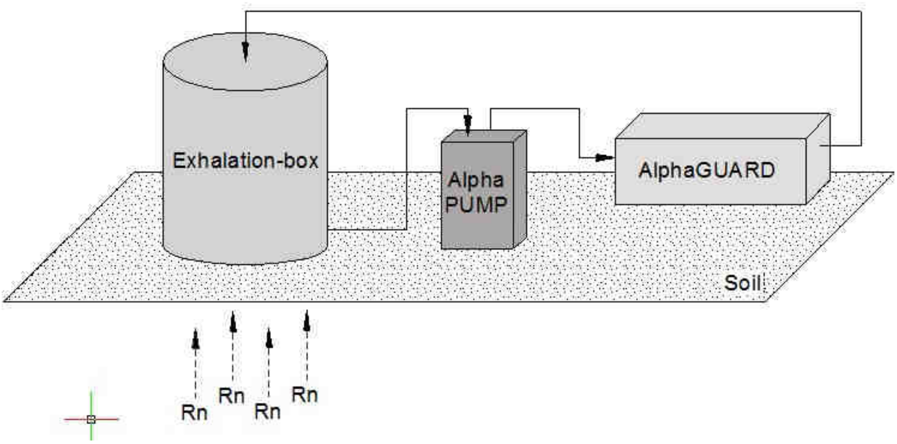

Fig. 1. Scheme of the radon exhalation measurements stand

The main device used in the research was the radon monitor AlphaGUARD. It was operating in 10-minute flow mode with the air pump AlphaPUMP, which enabled air transport from a radon-box to AlphaGUARD. Air flow rate was 1 dm^3 per minute. The exhalation-box was a specially prepared container partially hammered into the ground surface. It limited the volume of analyzed air. After the air sample analysis, it was transported back to the exhalation-box, so all the components remain in the closed volume. The AlphaGUARD was calibrated during its production and it has guaranteed 5 years stability, so recalibration was not needed. In Figure 1 scheme of the stand used in measurement is shown.

The measurements were carried out in different dates, but always in the same place. Each series of measurements lasted 2 hours and 10 minutes and included 13 measurements. Every measurement result was an arithmetic mean from 10-minute continuous examination.

Results and discussion

A chart in Figure 2 shows four exemplary measurement series from different days. The analysis indicates large variation of radon concentration during each series. There is not also any common trend between radon concentration and measuring time among other series. It suggests the influence of many factors as weather conditions and soil parameters on radon exhalation process. They can lead either to the increase or to decrease in number of radon particles, exhaled in the tested area.

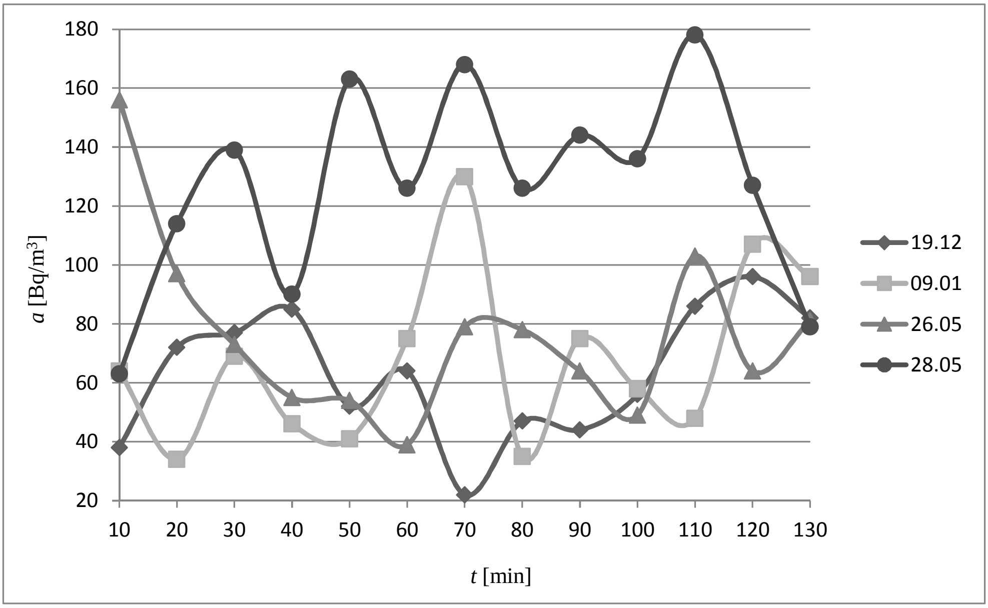

Fig. 2. Four examples of radon concentration variability over measurement series

In Figure 3 basic statistic parameters of all measurement series are presented. These are minimum and maximum values depicted as narrow vertical lines, first and third quartiles forming an upper and lower edge of the box and also the median forming horizontal cut of the box. As it can be seen from the comparison of 20 measurement series, interquartile ranges (middle fifties) are not the same. It might be very small as in the 5th one or big as in the 8th one. Interquartile range containing large range of radon concentration values, as for example on day 10.03.2014, is probably caused by a change of the temperature and low air humidity during measurements time. Then low range of radon concentration values containing in the middle fifty might be induced by stable and optimal weather conditions. Furthermore, it is worth noting, that radon concentration values were relatively stable during winter months (until the beginning of March), when the temperature did not exceed 10°C. Both in spring and early summer it was variable.

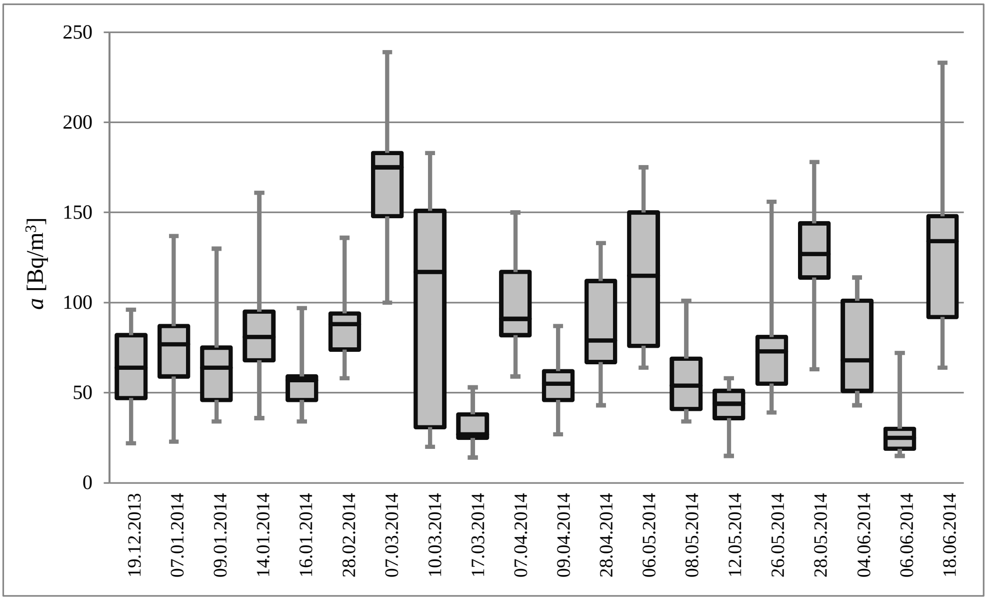

Fig. 3. Box-plot of radon concentration for all measurement series

Statistical analysis revealed a significant correlation between amount of measurements in one place and the size of measurement uncertainty. Figure 4 shows how the probability of obtaining a result similar to the average and dispersion of these results changes when there is less measurements in one place done, for example only: 3, 4, 5, 6, 7, 8, 9 or 10. The results showed that the more measurements are made, the lower measurement uncertainty is. This dependence is generally known, but the analysis confirms legitimacy of multiple measurements performing.

The charts (Figs. 4, 5) below show an analysis of one randomly selected measurements series. Rectangles present a histogram of radon concentration density distribution. It was created basing on the results obtained during experiment (centers of horizontal lines). The full lines are the centers extended on both sides about half the value of measurement uncertainty, so they demonstrate values representing real values with the biggest probability. Narrow vertical lines shows Poisson distribution for this measurements series. It is a discrete probability distribution, which can be used to describe nucleuses decay, because it expresses the probability of series of events occurring in determined time, when the average of events occurring frequency is known and this events occur independently of the time since the last event [29, 30]. It should be noticed that radon concentration density distribution is described well by Poisson distribution.

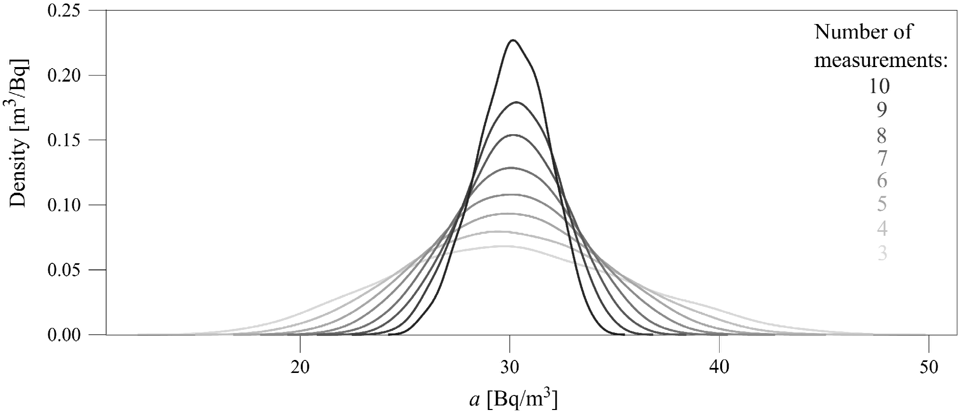

Fig. 4. Distribution of the means from several measurements

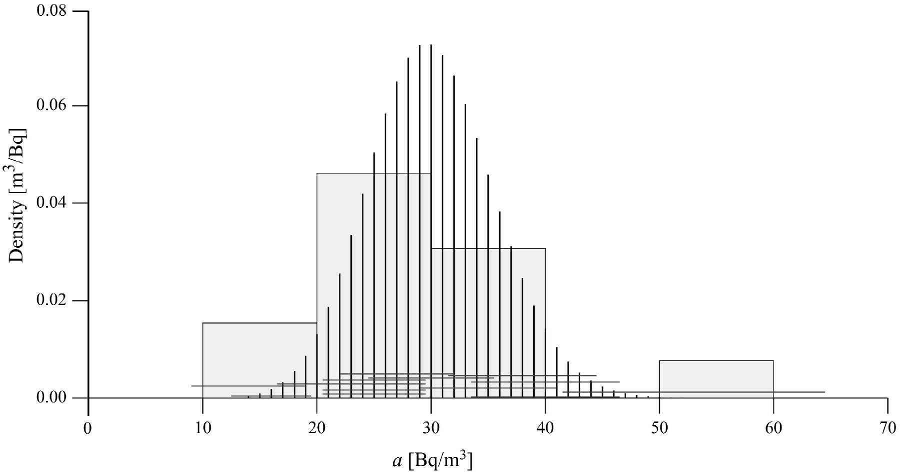

Fig. 5. Histogram of the measurements results distribution with their uncertainties and Poisson distribution

Conclusions

The study of radon (Rn-222) exhalation measurements allowed to enunciate the following conclusions:

1. There is a large variation between radon concentration results in one series.
2. There is not any common trend between different radon concentration measurements series.
3. Decrease in the result uncertainty might be gained by an increase in measurements number in a series.
4. The number of measurements in a series can be adjusted to the desired uncertainty of result.
5. Poisson distribution well describes distribution of radon activity concentration in series.
6. It is possible to optimize the Rn-222 concentrations measurement procedures to reduce consumed time (and costs) of the works.

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PROBLEMATYKA POMIARÓW EKSHALACJI RADONU (Rn-222)

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

Abstrakt: Izotop Rn-222, naturalnie występujący radioaktywny gaz, powstaje na skutek rozpadu promieniotwórczego Ra-226. Oba radionuklidy należą do szeregu uranowo-radowego, który rozpoczyna U-238. Radon występuje w dużych ilościach w skorupie ziemskiej, skałach, glebie, wodzie i powietrzu, dlatego jest on traktowany jako wskaźnik dawki promieniowania jonizującego otrzymywanego przez ludzi. Ze względu na negatywny wpływ radonu na zdrowie ludzi pomiary stężenia tego gazu są często wykonywane. Zaprezentowano wyniki badań niepewności pomiarowej stężenia Rn-222 w powietrzu ekshalowanym z gleby. Pomiary powtarzano wielokrotnie w tym samym miejscu. Eksperyment przeprowadzony został w warunkach naturalnych, w centrum miasta i obejmował okres około pół roku. Do pomiarów stężenia radonu wykorzystano monitor radonowy AlphaGUARD. Pracował on w 10-minutowym trybie przepływowym, z pompą oraz komorą ekshalacyjną w obiegu zamkniętym. Badania wykazały znaczne różnice pomiędzy wynikami pomiarów, wykonywanych w całym okresie badawczym. W pojedynczej serii większa liczba pomiarów zmniejsza niepewność pomiarową. Dzięki temu ilość pomiarów w serii może być dostosowana do oczekiwanej niepewności pomiarowej wyniku. Stwierdzono, że rozkład wyników aktywności radonu w serii jest dobrze opisywany przez rozkład Poissona.

Słowa kluczowe: radon, ekshalacja, stężenie, niepewność pomiarowa, AlphaGUARD

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THE ROLE OF EXTRACELLULAR METABOLITES PRODUCED BY BACTERIA IN THE PROCESS OF FUNGI GROWTH INHIBITION

UDZIAŁ ZEWNĄTRZKOMÓRKOWYCH METABOLITÓW BAKTERYJNYCH W ZAHAMOWANIU WZROSTU GRZYBÓW

Abstract: In the process of biological control of plants pathogens, special biological preparations containing cells of microorganisms or their metabolites are applied. The aim of conducted research was to assess the effect of *Bacillus subtilis* culture or its supernatant on the mycelial growth of *Fusarium culmorum*. The antagonistic properties of *B. subtilis* were assessed with the culture-plate method. The research was conducted on two different media, containing glucose or sucrose, in order to consider the effect of the source of carbon on the inhibitory process. The culturing process was conducted at 25±2ºC for 7 days and every 2-3 days the diameter of the mycelium was measured while the fungistatic activity of *B. subtilis* was determined against the mycelial growth rate index. The obtained results showed that the measured values of the growth rate index in the control trials were similar regardless of the carbon source in the medium. In tested trials no significant inhibition of the mycelial growth have been noted in the presence of glucose. Measured values of the growth rate index in tested trials were lower of 0.2-1.7 units compared with the control trials. The growth inhibition in the cultures on the medium with sucrose obtained 20% with an addition of the supernatant and 40% with an addition of *B. subtilis* culture.

Keywords: *Bacillus subtilis*, *Fusarium culmorum*, antifungal activity

Introduction

In recent years, there has been a growing interest in a potential application of the nonpathogenic microorganisms isolated from natural habitats in the process of plant protection. The microorganisms may be an alternative to chemical substances which according to the EU Directives [1] should be systematically eliminated from use in order to reduce their level in the environment. One of the reasons for such policy is the fact that pesticides are not selective when applied and what is more they may reduce biological variety in the agricultural production systems and their particles remaining in the field crops may pose hazard to consumers' health. Another issue is the fact that pathogens have the ability to develop resistance against pesticides. Therefore, the research has been conducted in order to find microorganisms which could protect plants against diseases. Such microorganisms belong to the group of plant growth promoting microorganisms (PGPR) which synthesize enzymes hydrolyzing cell wall of fungi. In consequence, the process leads to protoplasts fusion and degradation of phytopathogens. The microorganisms in rhizosphere may induce plant's resistance against diseases. The factors activating such reaction are chemical compounds called elicitors, which are recognized by specific plants receptors and induce biochemical defense reactions. A highly important aspect of such reaction is the fact that it is potentially non-specific in relation to the pathogen, contrary to classic (direct) methods of biological control, which apply the substance usually active

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against one or at most several pathogens. Among microorganisms showing such properties there is a group of bacteria *Bacillus*, which produce metabolites very actively. Many species of *Bacillus* spp. are widely known for their antagonistic properties against the following fungi: *Alternaria, Fusarium, Rhizoctonia, Phytophthora* and *Pythium*, but also as factors stimulating plants' growth [2-5]. The application of biological methods may reduce the amount of applied synthetic pesticides and in some cases may lead to their elimination as chemical substances are dangerous to biological balance in the soil as well as the quality of plants grown.

The aim of this paper was an assessment of fungistatic properties of *B. subtilis* against strain *F. culmorum*, one of the most frequently isolated plant pathogen, causing many diseases called fusariosis.

Materials and methods

In the research, a fungistatic activity of *B. subtilis* for the growth control of *F. culmorum* has been assessed. The bacteria were grown in the nutrient broth at 30ºC for 48 hours. The suspension of bacterial cells at an optical density of 1.0 or 2.0 was used to inoculate the tubes with nutrient broth and incubated for 24 hours. For the further studies the supernatants obtained from the bacterial cultures were used as well as bacterial cultures.

The antagonistic activity of *B. subtilis* against *F. culmorum* was assessed *in vitro* tests with a dual culturing-plate method using two different sources of carbon - glucose or sucrose. In the experiment the following culturing media were used: PDA consisting of [g/dm^3]: glucose 20.0, potato extract 4.0, agar 15.0 and Czapek consisting of [g/dm^3]: sucrose 30.0, $MgSO_4 \times 7H_2O$ 0.5, KH_2PO_4 1.0, KCl 0.5, $NaNO_3$ 3.0, $Fe_2(SO_4)_3 \times 7H_2O$ 0.01, agar 15.0. In the test trials the media were inoculated with either 0.5 cm^3 of the bacterial culture or 0.5 dm^3 of the supernatants. Next, the media were inoculated with 10 mm discs overgrown with 7-days old mycelium of tested *F. culmorum* strains. The control trials contained only tested strains of *F. culmorum*. All plates were incubated at 25ºC for 7 days. The diameters of the colony on the plates were measured every 2-3 days for the period of 7 days until the mycelium of *F. culmorum* in the control trial, reached the edge of the plate. The experiment was run in triplicate, where one replicate was represented by one culturing plate with the culturing medium and one mycelial disc.

The influence of metabolites produced by *B. subtilis* on the growth of *F. culmorum* was determined against the growth rate index, calculated according to the formula below [6]:

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

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

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

Results and discussion

B. subtilis is acting as an antagonist by competing for nutrients and space as well as by producing hydrolases (amylase, protease, pullulanase, chitinase, xylanase, lipase) and natural substances such as: subtilisin, fengycin, bacilysocin, surfactin or iturine [7-10]. Therefore, in the pilot research it has been assessed how the bacterial culture or the cell free supernatant containing metabolites produced by *B. subtilis* affects the growth of *F. culmorum.* Additionally, the density of bacterial inoculum and the type of the medium (source of carbon) were taken into account.

In the conducted tests no significant differences were noted in obtained values of the growth rate index in the control trials. Regardless of the source of carbon in the culturing medium the growth rate index amounted over 43 units (Figs. 1, 2). However, the control trials have proved that the activity of *B. subtilis* was different depending on the parameters analysed in the experiment. Therefore, the value of the growth rate index for the supernatant was similar to the value noted in the control trial when the optical density of the inoculum was 1.0 and glucose was present in the medium. Whereas, the value of the growth rate index was 2-fold lower in the bacterial culture and 48% of the growth reduction was obtained (Fig. 1). Different values have been noted for the cultures with sucrose providing carbon. The value of the growth rate index decreased significantly in case of both the supernatant and the bacterial culture and amounted 33 and 38% respectively (Fig. 1).

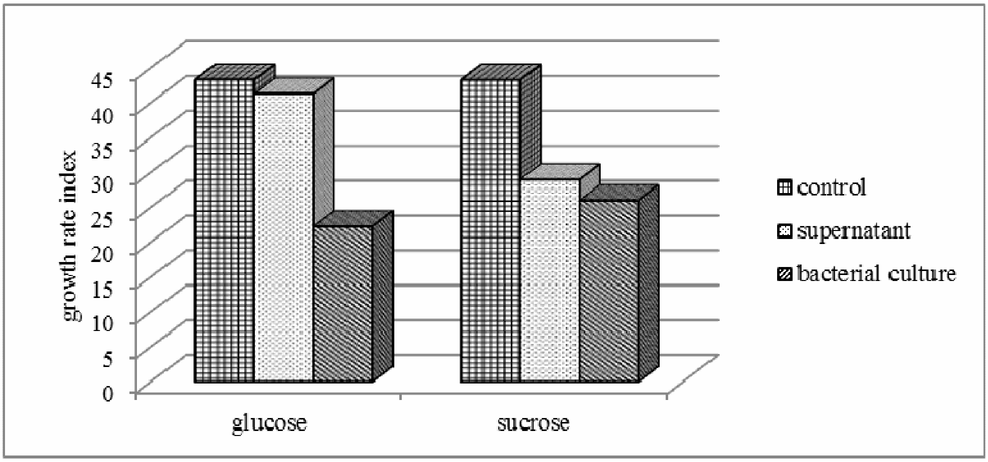

Fig. 1. The growth rate index for the trials with the inoculum at optical density 1

Similar relationship can be observed in trials with the inoculum at initial optical density equal 2.0. However, the inhibitory properties of *B. subtilis* are lower despite the 2-fold increase in the amount of the inoculum. On the medium with glucose no significant differences have been noted between the control trial and test trials. The highest measured value of the growth inhibition index was noted on the medium with sucrose, where the inhibition amounted 21% for the supernatants and over 41% for the bacterial culture (Fig. 2).

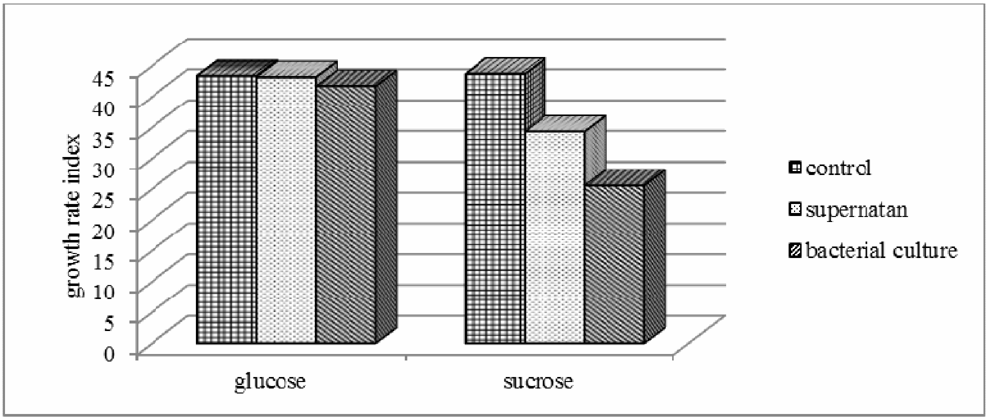

Fig. 2. The growth rate index for the trials with the inoculum at optical density 2

In the author's own research, beside the growth rate index enabling the assessment of mycelial growth in time, also the degree of the linear growth inhibition of *F. culmorum* has been take into account (Fig. 3). Higher values of the inhibition has been noted in the medium with sucrose. Regardless of the initial density of inoculum, the mycelial growth inhibition for both the supernatant and the bacterial culture has reached 20-38%, although the application of the latter proved to be more efficient. In the presence of glucose, 46% reduction has been obtained also in case of bacterial culture, with the initial density of inoculum equal 1 (Fig. 3).

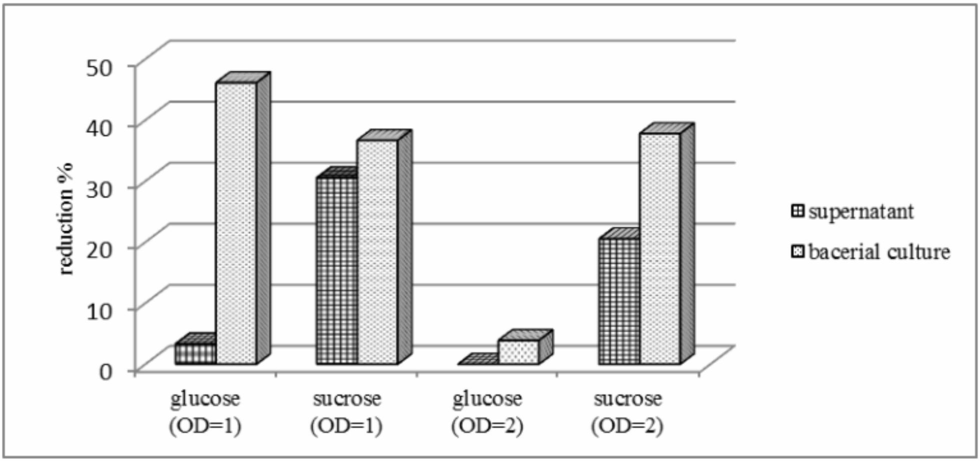

Fig. 3. *B. subtilis* affecting the linear growth of *F. culmorum.* (OD - *optical density*)

According to many authors, strains of *B. subtilis* are applied in the process of biological protection of plants due to their ability to produce antimicrobial chemical compounds, including predominantly peptides as well as a couple of non-peptidic compounds such as polyketides, an aminosugar, and a phospholipid [11-13]. Antifungal activity of *Bacillus* spp. results from their ability to produce also other cyclic lipopeptides and the cell-wall degrading enzymes, of which the most valuable and important compounds belong to the group of peptide antibiotics. They are: surfactin, iturin and fengycin, which have a great potential of biotechnological and biopharmaceutical applications. Iturins and fengycins display a strong antifungal activity, however, surfactins are not fungitoxic by themselves but could synergistically impact the antifungal activity of other lipopeptides. Possibly lipopeptides affect the cell membrane of filamentous fungi to alter its permeability, resulting in release of cell contents [13-15]. According to other authors, *B. subtilis* might also act on pathogenic fungi by either producing antifungal substances or colonizing microsites faster than the surface fungi [16].

Conclusions

In conducted pilot research, strain *B. subtilis* showed the ability to affect the growth of *F. culmorum* in a varied way depending on the parameters under study. One, which played an important role in the metabolic activity of *B. subtilis*, was the source of carbon in the culturing medium. In most analysed trials, a significant growth reduction of *F. culmorum* has been noted in the presence of sucrose. In the trials, a higher degree of the growth reduction has been obtained with the inoculum at optical density 1.0. The results were also more favourable in case of bacterial cultures containing bacterial cells and their metabolites when compared with the results noted after the application of the supernatant.

A fungistatic activity of *B. subtilis* against *F. culmorum* has been proved in *in vitro* research and allows to conduct further studies at a wider range.

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UDZIAŁ ZEWNĄTRZKOMÓRKOWYCH METABOLITÓW BAKTERYJNYCH W ZAHAMOWANIU WZROSTU GRZYBÓW

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

Abstrakt: W metodach biologicznej walki z fitopatogenami roślin stosuje się między innymi biopreparaty, które jako substancję czynną zawierają żywe komórki mikroorganizmów lub ich metabolity. Celem przeprowadzonych badań było określenie wpływ hodowli bakteryjnej *Bacillus subtilis* oraz jego supernatantu na wzrost grzybni *Fusarium culmorum*. Ocenę właściwości antagonistycznych *B. subtilis* przeprowadzono metodą hodowlano-płytkową. Badania przeprowadzono na dwóch różnych podłożach, zawierających glukozę lub sacharozę, uwzględniając w ten sposób wpływ źródła węgla na proces inhibicji. Hodowle prowadzono w temperaturze 25±2ºC przez 7 dni, dokonując co 2-3 dni pomiaru średnicy grzybni, a aktywność fungistatyczną *B. subtilis* określono w oparciu o indeks tempa wzrostu grzybni. Na podstawie uzyskanych wyników stwierdzono, iż w próbach kontrolnych indeks tempa wzrostu był na zbliżonym poziomie niezależnie od źródła węgla zawartego w podłożu. W próbach badanych nie odnotowano znaczącego zahamowania wzrostu grzybni w obecności glukozy. Wartości indeksu tempa wzrostu dla prób badanych były niższe jedynie o 0,2-1,7 jednostki w porównaniu do prób kontrolnych. Natomiast w hodowlach prowadzonych na podłożu zawierającym sacharozę uzyskano około 20% zahamowanie po zastosowaniu supernatantu i ponad 40% w obecności hodowli bakteryjnej *B. subtilis*.

Słowa kluczowe: *Bacillus subtilis*, *Fusarium culmorum*, aktywność przeciwgrzybowa

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ACTIVITY CONCENTRATION OF RADON-222 IN THE BUILDINGS OF THE OPOLE UNIVERSITY

AKTYWNOŚĆ RADONU-222 W BUDYNKACH UNIWERSYTETU OPOLSKIEGO

Abstract: Rn-222 activity concentration is on average several times higher in confined spaces than outside. Given that approx. 75% of the time we spend indoors, there is a need to draw attention to the activity concentration of Rn-222 in rooms in which we live. The paper presents the results of measurements of Rn-222 activity concentration in the period September-October 2014, in the buildings of the Opole University. The measurements were performed using a portable spectrometer AlphaGUARD. In the tested buildings Rn-222 activity concentrations differs in the ranged from 4 to 38 Bq/m^3. These differences may be related to the local properties of the soil, the properties of the materials used in buildings construction and the method of building ventilation. The measurements of Rn-222 activity concentrations in the buildings of the Opole University showed no potential health risks resulting from radon.

Keywords: Rn-222 activity, AlphaGUARD spectrometer

Radioactivity is an integral part of the natural environment [1]. Statistical Polish resident receives annually from all sources of radiation effective dose average of 3.35 mSv, of which 74.2% comes from natural sources. Much of this dose (about 1.36 mSv/year) is caused by the radioactive gas radon [2].

Radon is a chemical element with atomic number 86 and is in 6th period and 18 group (helium group) of the periodic table of chemical elements. It is the heaviest gas from helium group, about 8 times heavier than atmospheric air with an average composition under normal conditions [1, 3, 4]. Entirely filled electron shell, making a noble gas radon, very poorly chemically reactive [5, 6]. Radon gas is non-flammable, tasteless and odorless [3, 4]. It has 33 isotopes, all of them are radioactive, and four of them occur naturally in nature and belong to the three natural radioactive series - uranium - radium (Rn-222 and Rn-218), thoron (Rn-220 - thoron) and uranium - actin (Rn-219 - actinon). The most important in environment is the isotope Rn-222, primarily because of its longest half-life (3.8224 days) [3].

Radon atoms formed in rocks and soil by spontaneous nuclear transformation emit during the decay an alpha particle, which is accompanied by a low-energy gamma rays [3].

In soil, the mineral grains release up to 70% of radon. The rate of radon transfer to the surface depends on porosity and soil moisture, atmospheric pressure and other meteorological factors. Radon migration processes is also affected by geological terrain, sediments structure, tectonic and erosion of the substrate, which manifests all sorts of gaps and cracks [5, 6].

The radon concentration is much greater inside the building than outside. In Poland, the mean activity concentration of radon in homes is about 40 Bq/m^3 and is, unlike in the

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fresh air, larger in winter than in summer, which probably stems from the frequent airing of rooms in summer than in winter [5, 6].

Radon infiltrates from the soil substratum and construction materials into the interior of buildings, what causes increase in his concentration inside the closed rooms. It penetrates into the cellars throughout the cracks of foundations, during the leaks in basing of water-supply tubes and sewerage as well as the system of drainage. The little differences of pressure between the ground under foundation and interior of accommodation, evoked by winds and differences of temperatures, cause that the house "sucks" the radon coming from the ground [7, 8].

The International Commission on Radiological Protection gives two reference values (for dwellings), above which action must be taken to reduce radon concentrations: 400 Bq/m^3 - for houses already built and 200 Bq/m^3 for newly built homes [9]. Recent recommendation of International Commission on Radiological Protection for the reference level for radon gas in dwellings is 300 Bq/m^3 [10].

Table 1 shows the mean values in many countries with recommended in these countries limit values of radon both in existing buildings and new build.

Table 1

Mean values calculated in many countries with recommended in these countries, radon concentration limit values for both existing buildings and new-build [11]

Country	The average concentration of radon [Bq/m^3]	Recommended radon concentration limits [Bq/m^3]	
		existing buildings	new build
Poland	38	400	200*
Finland	120	800	200
Germany	40	-	-
USA	65	150	10
Slovakia	-	500	500
Sweden	800	800	140
Switzerland	60	800	800
United Kingdom	28	400	80

* European Commission recommendation

The aims of this study were the analysis of the risk arising from the activity of Rn-222 in the buildings of the Opole University.

Material and methods

In this study the results of Rn-222 activity concentration measurements, in the period September-October 2014, in different buildings of the Opole University (UO): Collegium Biotechnologicum (CB), building on Dmowskiego str. (D), building on Kominka str. (K), building of Department of Physics (F), were presented. In each building the measurements were carried out on floors 0-III (Table 2).

Determination of radon activity concentrations in air were performed using the portable spectrometer AlphaGUARD, located at about 1.5 m above the ground level.

Table 2

Location of Rn-222 activity concentration measurements

Location			
The old building at Kominka street	K0	Collegium Biotechnologicum	CB0
	KI		CBI
	KII		CBII
	KIII		CBIII
Building at Dmowskiego street	D0	Department of Physics	F0
	DI		FI
	DII		FII
	DIII		FIII

Results and discussion

The results of measurements in each building are shown in Figure 1. The highest values were recorded in the building at Kominka street, which is a small building equipped with limited air exchange system. The relatively high activity concentrations of Rn-222 in the air were recorded on the lower floors, as a result of radon specific density bigger than that of air.

The results obtained Rn-222 activity in the buildings of the Opole University were used to estimate the effective doses from radon, received by UO students, within 1 academic year.

The calculations assumed the following:

1. 1-hour exposure to radon 1 Bq/m^3, with a balance factor $F = 0.4$ corresponds to an effective dose of 3.2 nSv ($3.2 \cdot 10^{-6}$ mSv) (equilibrium factor F is defined as the ratio of the radon concentration to potential radiation energy of shortliving α decay products of radon) [12].
2. Students spend 800 hours (full time), 500 (part-time studies) at the university in one year.

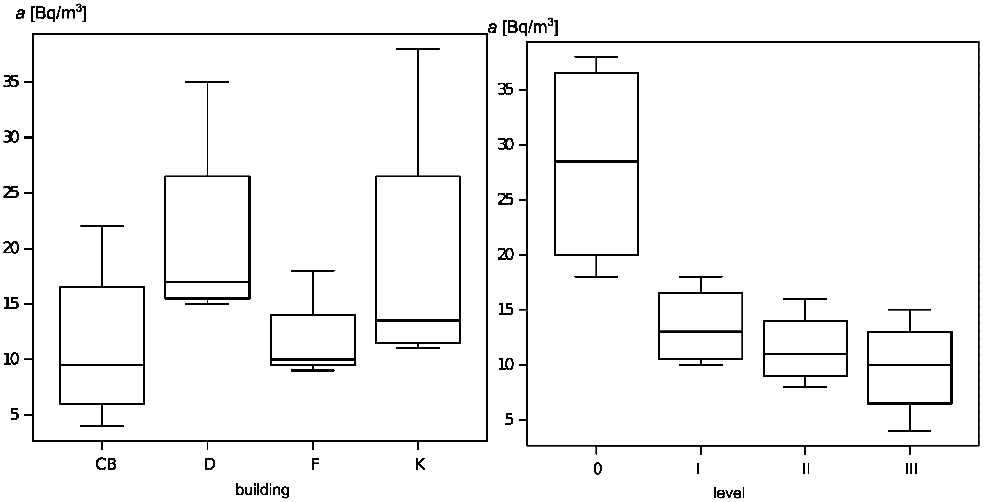

Fig. 1. The concentration of Rn-222 in the particular measurement places and on different floors

Annual dose of radon exposure in relation to full time and part time students presents Figure 2.

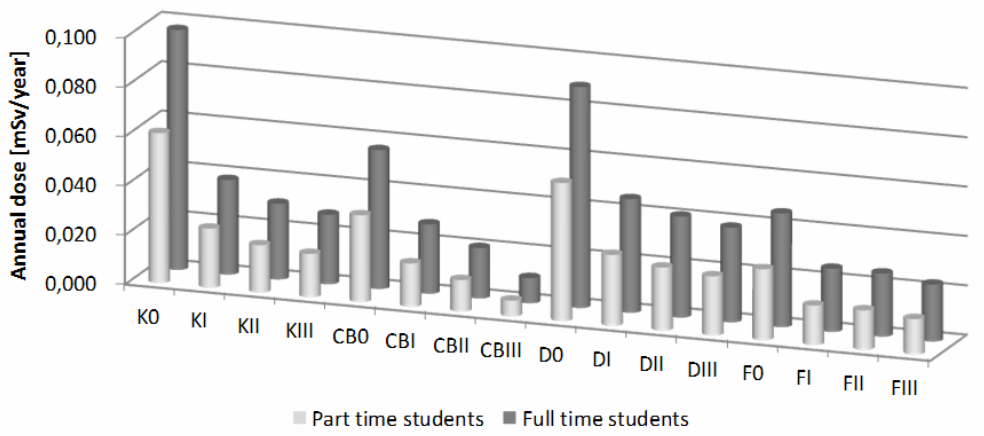

Fig. 2. The radiation dose from Rn-222 inhaled with air

Conclusions

Can be seen from the graph that the annual dose exposure to radon is the largest in the old buildings of the Opole University, on the lowest floors. Full-time students, due to the greater number of hours spent at the university are more exposed to radon than part-time students. However, the calculated doses do not exceed the standards adopted by an International Atomic Energy Agency.

The Rn-222 activity measurements performed using a portable spectrometer AlphaGUARD in buildings of Opole University showed no potential health risk from radon contamination. Based on the research it was observed that humans staying on the lower floors are more vulnerable to radiation associated with Rn-222 decay, due to the physical properties of the element.

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AKTYWNOŚĆ RADONU-222 W BUDYNKACH UNIWERSYTETU OPOLSKIEGO

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Abstrakt: Aktywność Rn-222 w pomieszczeniach zamkniętych jest średnio kilkakrotnie większa niż na zewnątrz. Ponieważ ok. 75% czasu spędzamy w pomieszczeniach zamkniętych, istnieje potrzeba zwrócenia uwagi na aktywność Rn-222 w pomieszczeniach, w których przebywamy. W pracy przedstawiono wyniki pomiarów aktywności Rn-222, w okresie wrzesień-październik 2014 r., w budynkach Uniwersytetu Opolskiego. Pomiary wykonano za pomocą przenośnego spektrometru AlphaGUARD. Zaobserwowano zróżnicowane aktywności Rn-222 w badanych budynkach, które mieściły się w przedziale od 4 do 38 Bq/m^3. Różnice te mogą być związane z lokalnymi właściwościami gruntu, właściwościami wykorzystanych materiałów budowlanych, a także sposobem wentylacji. Pomiary aktywności Rn-222 w budynkach Uniwersytetu Opolskiego wykazały brak potencjalnego zagrożenia zdrowia, wynikającego z zanieczyszczenia radonem.

Słowa kluczowe: Rn-222, aktywność, spektrometr AlphaGUARD

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ANALYSIS OF EUTROPHICATION PROCESS IN UPLAND AND LOWLAND STREAMS IN POLAND

ANALIZA PRZEBIEGU PROCESU EUTROFIZACJI W CIEKACH KRAJOBRAZÓW WYŻYNNYCH I NIZINNYCH NA OBSZARZE POLSKI

Abstract: Transformations occurring in the aquatic environment as a result of anthropogenic discharge of nutrients lead to intensification of eutrophication processes and secondary pollution of surface waters. An effect of this phenomenon is the deterioration of physical, chemical and biological characteristics of water. The increasing intensity of eutrophication processes and the specificity of their course in various types of water bodies require the development of specific control methods and ways to protect aquatic ecosystems. In view of the fact that eutrophication is a process that is characterized by high dynamism and depends on many interacting factors such as: morphological, hydrological, hydrobiological, climatic and other conditions, the assessment of trophic status of water is a very complicated task. In particular, it concerns the rivers and streams, because the symptoms of this process and its course vary according to the type of the river. Traditionally eutrophication is assessed on the basis of the boundary values of indicators or indices developed by various authors mostly for stagnant waters or estuaries. Among the various ways of trophic status assessment it is definitely a strong lack of a reliable and simple assessment methods for running waters. The paper presents the results of research concerning the course of eutrophication and evaluation of trophic state in selected rivers and streams in the upland and lowland areas of Poland on the base of the author's methodology. The method used is fundamentally different from traditional approach and based on the definition of trophic status as the existing state of the biotic balance in waters. It is characterized by easy interpretation of results and low costs. Comparative analysis of different methods for assessing the trophic status of running waters is also presented. Long-term dynamics of trophic state changes in the investigated upland and lowland rivers is also described.

Keywords: biogenic substances, anthropogenic eutrophication, upland and lowland rivers, biotic balance, eutrophication indices, trophic state assessment

Introduction

The problem of eutrophication since the seventies of the last century takes on a global scale because of its negative consequences, which could result in total loss of economic and biospheric functions of aquatic ecosystems.

In European Union water policy the problem of eutrophication is regarded as a priority issue. Many EU directives contain a requirement of assessment and control of this process and the necessity of water protection measures implementation in order to prevent its development. These requirements are contained, for example, in Directive 91/271/EEC (Directive on Urban Waste Water Treatment), Directive 91/676/EEC (Directive concerning the nitrates from agricultural land) and Directive 2000/60/EEC (Water Framework Directive). These directives contain neither uniform definition of eutrophication, no universal approach for assessing the trophic state [1-3].

In Poland the requirements of EU directives are reflected in the Water Act and three Regulations of the Ministry of Environment, according to which the evaluation of trophic

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status is carried out on the base of traditional indicators in the frames of general water state assessment, and only in terms: "subjected to eutrophication" or "not subjected to eutrophication". Moreover, different Regulations contain divergent normative values of eutrophication indicators [4-6].

Specific characteristics of eutrophication in running waters

Length of the river network in Poland is quite large and amounts 74,714 km, 52% of which are regulated rivers. Positive feature of river regulation is responding the economy needs, however such a drastic interference into environment leads to the loss of natural values of the river, changes of structure of riverine biota, and consequently - to decrease of self-purification ability, progressing eutrophication and secondary contamination.

Eutrophication process pattern is best studied in stagnant waters. Much less is known the eutrophication processes in running water ecosystems which are functioning in different way due to the following specific features:

- flow of water, which plays a significant role as a limiting factor of eutrophication;
- more intensive exchange between water and land, that makes the rivers to be an “open ecosystem” with heterotrophic type of metabolism;
- more uniform distribution of dissolved oxygen in rivers and therefore the lack of thermal or chemical stratification [7].

Specific features of river ecosystems condition the different course of eutrophication process as compared to limnetic ecosystems, and therefore it cannot be described accurately on the basis of methods established for lakes and coastal areas.

Materials and methods

The approach proposed by the authors of this paper is different from generally used methods, which focus mainly on nitrogen and phosphorus enrichment and primary production. The proposed method starts from the assumption, that trophic state can be reflected by the state of biotic balance, *ie* the balance between the processes of production and destruction of organic matter synthesized by water plants. Hence, the indicator of trophic status of aquatic ecosystem should reflect the integral result of processes of organic matter production and destruction. Similar view is presented also by other authors [8-11].

Dystrophic waters are characterized by exceeding of organic matter decomposition rate (V_d) in relation to the rate of its production (photosynthesis) (V_p), and then $V_p/V_d < 1$; while oligotrophic are characterized by the balanced rates of these processes: $V_p/V_d \approx 1$; and in waters subjected to eutrophication the rate of organic matter production is higher than the rate of decomposition, so $V_p/V_d > 1$ [12].

Index of trophic status of waters ITS (Index of Trophic State) proposed by the authors is based on theoretical assumptions, according to which the imbalance of the rates of water vegetation production and its decomposition in any water ecosystems leads primarily to changes in the quantitative ratios of the concentrations of O_2 and CO_2. The details of the theoretical foundations of ITS index are presented in [12, 13].

In the light of this assumption the problem seems to be the choice of indicator, which reflects the balance between the production and destruction of organic matter in surface waters during the annual cycle. Index of Trophic State can be calculated according to equation:

$$\mathrm{ITS} = \sum \mathrm{pH_i}/n + a\left(100 - \sum [\mathrm{O_2}\%]/n\right) \tag{1}$$

where: $\mathrm{pH_i}$ - pH value; $[\mathrm{O_2}\%]$ - oxygen saturation measured synchronously with pH; a - an empirical coefficient; n - number of measurements [13].

The state of biotic balance and the values of ITS in waters of different trophic conditions are shown in Table 1.

Table 1

Values of ITS in fresh waters of different trophic state [13]

State of biotic balance (V_p/V_d)	Trophic state	ITS
Negative ($V_p/V_d < 1$)	Dystrophic Ultraoligotrophic	< 5.7 ± 0.3 6.3 ± 0.3
Balanced ($V_p/V_d = 1$)	Ologotrophic	7.0 ± 0.3
Positive ($V_p/V_d > 1$)	Mezotrophic Eutrophic	7.7 ± 0.3 > 8.3 ± 0.3

The researches were carried out in order to use Index of Trophic State for the purposes of trophic state assessment of running waters. The assessment was realized for different types of running waters determined in Poland according to Frame Water Directive [1]. There were appointed 26 types of courses in Poland, among them are: 3 types of mountain streams, 12 types of upland rivers, 7 types of lowland rivers, 4 types of rivers independent on ecoregion and group of indefinite types.

Table 2

Measurement points analyzed in the research

Type of the river	Measurement point	Period of monitoring	Description of river type
5	Katowice Bolina before Przemsza 0.3 km	2000-2009	Upland landscape: small silicate stream with fine-grained substrate
6	Kielce Silnica Bialogon 0.9 km	2000-2009	Upland landscape: small carbonate stream with fine-grained substrate
9	Krakow Rudawa Podkamycze 9.3 km	2000-2009	Upland landscape: small carbonate river
10	Lodz Pilica Sulejow 159.8 km	2000-2009	Upland landscape: middle river
14	Krakow Czarna Orawa Jablonka 25 km	2000-2009	Upland landscape: small river on flysc structures
17	Bialystok Krynka bondary profile 4.5 km	2000-2009	Lowland landscape: small sand stream
20	Olsztyn Guber Sepopol 76 km	2000-2009	Lowland landscape: medium gravel river
21	Warszawa Narew Pultusk 64 km	2000-2008	Lowland landscape: great river in the plains
22	Gdansk Lupawa Rowy 0.7 km	2001-2009	Estuary part of the river under the influence of saline water
23	Lublin Czyzowka Janow Podlaski 4.2 km	2000-2009	Small streams in the valleys or large lowland rivers under the influence of peat-forming processes
24	Zielona Gora Pliszka before Odra 0.3 km	2000-2008	Small rivers in the valleys or large lowland rivers under the influence of peat-forming processes

The researches were carried out for selected measurement points located on the rivers of different types on the base of long-term monitoring database obtained in the frames of Polish State Monitoring System (Table 2).

The results of comparative trophic state assessment carried out on the base of ITS and boundary values of traditional indices, elaborated by different authors, presented in Table 3. The assessment was made on the base of average annual values of parameters measured during 10-year monitoring period [14].

Table 3

Results of comparative trophic state assessment in measurement points
(o - oligotrophic, m - mesotrophic, e - eutrophic)

	Type of the river	**5**	**6**	**9**	**10**	**14**	**17**	**20**	**21**	**22**	**23**	**24**
Indicators of trophic state	ITS value	m	e	m	e	e	m	e	e	e	m	m
	N - Burns	e	e	e	e	e	e	e	e	e	e	e
	P- Burns	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - Burns	e	e	m	e	m	m	e	e	e	e	e
	P - Carlson	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - Carlson	m	e	o	m	o	m	m	e	e	m	m
	P - Chapra Dobson	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - Chapra Dobson	m	e	o	m	m	m	e	e	e	e	e
	P - DillonRigler	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - DillonRigler	e	e	m	e	m	m	e	e	e	e	e
	N - Dodds	e	e	e	e	m	e	e	e	e	e	m
	P - Dodds	e	e	e	e	m	e	e	e	e	e	e
	Chl-a - Dodds	o	m	o	o	o	o	m	m	e	o	m
	N - Forsberg Ryding	e	e	e	e	e	e	e	e	e	e	e
	P - Forsberg Ryding	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - Ryding	m	e	o	m	o	o	e	e	e	m	e
	P - OECD	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - OECD	m	e	o	m	m	m	e	e	e	e	e
	P - New Hampshire	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - New Hampshire	m	e	o	m	o	o	m	e	e	m	m
	N - Nurnberg	e	e	e	e	e	e	e	e	e	e	e
	P - Nurnberg	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - Nurnberg	m	e	o	m	o	m	e	e	e	e	e
	N - Vant	e	e	e	e	e	e	e	e	e	e	e
	P - Vant	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - Vant	e	e	o	e	o	o	e	e	e	e	e
	P - Vollenweider	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - Vollenweider	m	e	m	m	m	m	e	e	e	e	e
	P - Welch Lindell	e	e	e	e	e	e	e	e	e	e	e
	Chl-a - Welch Lindell	o	m	o	o	o	o	o	m	e	o	m

Results of the research

Analysis of Table 3 shows high compliance of assessments made on the basis of ITS and boundary values of traditional indicators of eutrophication, developed by different authors. On the base of the assessment conducted by ITS for selected points located on rivers of 5, 9, 17, 23 and 24 types, it can be concluded, that these measurement points belong to mesotrophic waters. However, water in the points located on the rivers of 6, 10, 14, 20, 21 and 22 types are characterized as eutrophic. Wherein, the measurement points located on small silicate streams and carbonate rivers of upland landscape, small sandy rivers of lowland landscape and small streams and rivers in the peaty areas were classified as mesotrophic waters. Waters in the points on small and medium rivers of upland landscape, medium and large rivers of plains and estuarine rivers under the influence of saline waters were classified as eutrophic waters. Trophic state assessment realized on the base of ITS index allowed to follow the long-term dynamics of trophic level in selected measurement points (Figs. 1, 2).

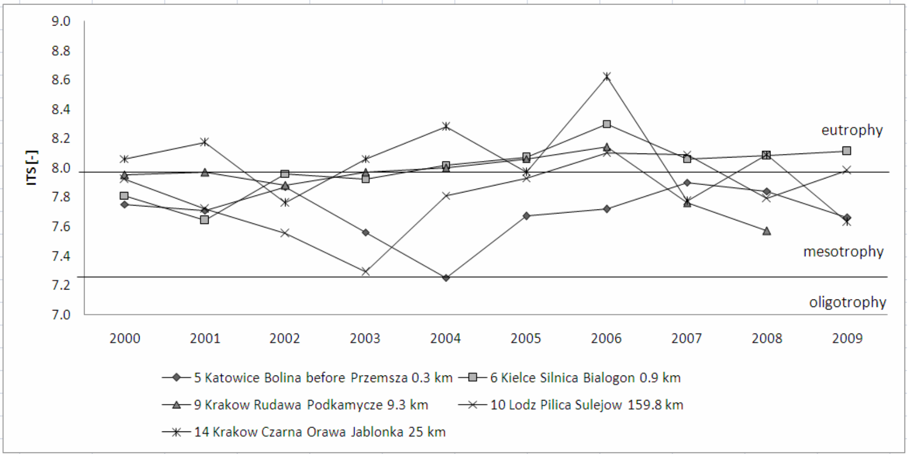

Fig. 1. Long-term dynamics of trophic level in measurement points on upland rivers

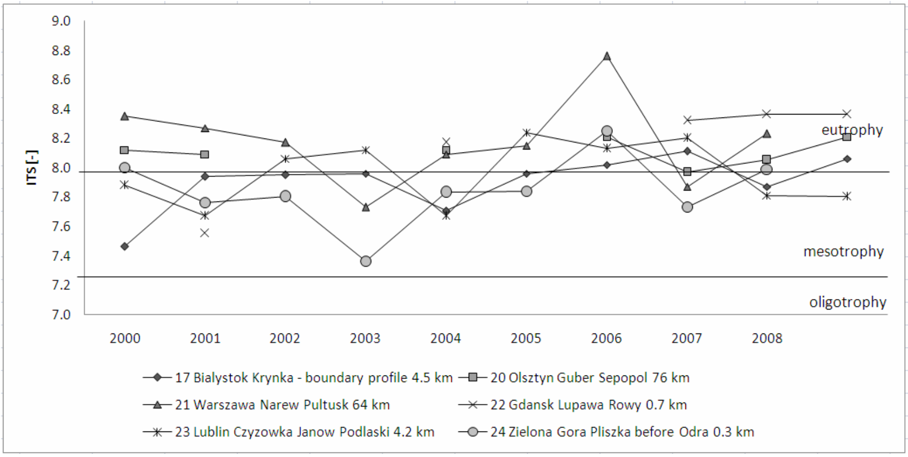

Fig. 2. Long-term dynamics of trophic level in measurement points on lowland rivers

Discussion

The conducted studies showed that the examined water monitoring points are characterized by a high content of total nitrogen and phosphorus, qualifying these waters as eutrophic. But it was also stated that the enrichment of river waters in nutrients does not always lead to a corresponding increase of primary production. Chlorophyll content indicated the mesotrophic conditions, and in some cases - oligotrophic conditions. These findings correlate with the results obtained by other authors concerning the limiting role of nitrogen and phosphorus in eutrophication process in the rivers [8, 15]. Assessment of trophic status made on the basis of ITS in the majority of measurement points agrees with the assessments based on chlorophyll content. It can be explained by the fact that both of these indicators reflects the integrated ecosystems response to the effects of pressure factors, whereas the content of nitrogen and phosphorus - only the eventuality of plant biomass growth.

The above mentioned considerations mean that nutrient content may not always be a reliable indicator of eutrophication, as trophic status is determined not only by the content of these substances, especially in riverine ecosystems. Other authors also confirm the diversity of eutrophication processes in running waters [8, 16]. The concentration of chlorophyll-a also may lead to low reliability of trophic level assessment in running water due to its high seasonal volatility, mobility in rivers and large variety of factors determining the development of aquatic vegetation [7, 10, 17]. This indicator can be used rather for eutrophication assessment in so called “chlorophyll-type rivers”. In Poland there are only 5 types of such rivers: 21 (great lowland rivers), some rivers of 19 type (lowland sandy-clay rivers), 20 (medium lowland gravel rivers), 24 (small valleys rivers under the influence of peat-forming processes) and 25 (lakes connecting rivers).

Due to the complex nature of eutrophication in running waters further studies are needed in order to verify the preliminary results of the researches.

Conclusion

The studies presented in this paper were devoted to very complicated problem of trophic state assessment of running waters. The results of statistical analysis based on long-term monitoring data concerning the rivers of different types allowed to verify the possibility of ITS usage and to state its conformance with modern requirements to environmental indicators. It allowed in simple and low-cost way to assess the trophic state and to evaluate the changes and tendency of eutrophication development in different types of rivers during 10-year period.

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ANALIZA PRZEBIEGU PROCESU EUTROFIZACJI W CIEKACH KRAJOBRAZÓW WYŻYNNYCH I NIZINNYCH NA OBSZARZE POLSKI

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Abstrakt: Transformacje zachodzące w środowisku wodnym w wyniku antropogenicznego dostarczania związków biogennych prowadzą do intensyfikacji procesów eutrofizacji i wtórnego zanieczyszczenia wód powierzchniowych. Skutkami tego zjawiska jest pogorszenie ich właściwości fizycznych, chemicznych i biologicznych. Nasilającą się intensywność procesów eutrofizacji oraz specyfika ich przebiegu w wodach różnego typu wymagają opracowania specjalnych metod kontroli i sposobów ochrony ekosystemów wodnych. W związku z tym, że eutrofizacja jest procesem, który charakteryzuje się dużą dynamicznością i zależy od zespołu wielu współdziałających między sobą czynników morfologicznych, hydrologicznych, hydrobiologicznych, klimatycznych i innych, ocena stanu troficznego wód jest zadaniem bardzo skomplikowanym. Zwłaszcza dotyczy to rzek i strumieni, ponieważ objawy tego procesu i jego przebieg różnią się w zależności od typu rzeki. Tradycyjnie eutrofizację wód przyjęto oceniać na podstawie granicznych wartości zespołu wskaźników lub indeksów opracowanych przez różnych autorów przeważnie dla wód stojących lub estuariów. Wśród rozmaitych sposobów oceny stanu troficznego zdecydowanie brakuje wiarygodnych i prostych sposobów jego oceny w wodach płynących. W pracy przedstawiono wyniki badań nad przebiegiem procesu eutrofizacji i oceny statusu troficznego w wybranych ciekach na obszarach wyżynnych i nizinnych Polski na podstawie autorskiej metody. Zastosowana metoda zasadniczo różni się od metod tradycyjnych i opiera się na definicji stanu troficznego jako stanu istniejącego bilansu biotycznego w wodach. Charakteryzuje się ona łatwą interpretacją wyników oraz niskimi kosztami. Przedstawiono analizę porównawczą różnych metod oceny stanu troficznego wód rzecznych. Opisano wieloletnią dynamikę zmian stanu troficznego badanych rzek wyżynnych i nizinnych.

Słowa kluczowe: substancje biogenne, eutrofizacja antropogeniczna, rzeki krajobrazów wyżynnych i nizinnych, bilans biotyczny, wskaźniki eutrofizacji, ocena stanu troficznego

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ESSENTIAL OILS IN THE NEEDLES OF SCOTS PINE (*Pinus sylvestris* L.)

WYDAJNOŚĆ OLEJKÓW ETERYCZNYCH Z IGLIWIA SOSNY ZWYCZAJNEJ (*Pinus sylvestris* L.)

Abstract: The aim of the study was to determine the content of essential oils in pine needles in 3 canopy zones (lower, middle and upper) of model trees, for 3 youngest needle cohorts (1, 2 and 3 years old). The research revealed a clear increase in the content of essential oils from the lower through the middle to the upper part of the crown. The lowest oil content was found in 3-year-old needles (0.40%), while 1-year and 2-year old needles contained 0.48 and 0.49% of oil, respectively.

Keywords: essential oil, pine needles, Scots pine

Natural essential oils, the possibility of their isolation and use in various industries, have been receiving increasing attention. Considering the highest share of pine stands in Poland, pine essential oils are of the greatest economic importance in our country. Results of earlier studies on the content of essential oils in needles indicate that their content depends on such factors as tree age, needle age, time of harvest, or atmospheric pollution [1-4].

In Scots pine, the highest concentration of essential oil is found in the needles, but it can also be isolated from shoots, cones, bark and wood. Annual production of the pine oil is about 40 tons, and the leaders are China and Russia. Poland used to be the greatest manufacturer of pine oil in the 1960s and 1970s, with annual production up to 30 tons [5].

The aim of the study was to determine the content of essential oils in the needles of Scots pine (*Pinus sylvestris* L.), divided into 3 cohorts (year 1, 2, and 3), collected from different parts of the crowns (lower, middle and upper zone).

Material and methods

The study was conducted in a pine stand located in the south-western Poland and characterized by a mountain climate of Sudety.

Study material were pine branches collected in 15 years old pine stands. At each site, 6 model trees were selected, growing in full sun in the fresh mixed mountain forest. The needles were collected from the lower, middle and upper crown of standing trees. The branches were gathered in the morning on rainless days. In the laboratory, the needles were separated from the shoots, divided into three cohorts, and pulverized in a cutting mill. The material was used to determine the content of essential oils by steam distillation. One sample contained 100 g of fresh needles. Distillation time since the boiling point was 70 min.

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Results and discussion

The study evaluated the changes in the content of essential oil in the needles of Scots pine, depending on the age of the needles and their location in the crown. Mean content of the essential oils, not accounting for the needle age and location within the crown, was 0.46%, with a minimum of 0.29% and a maximum of 0.72%. The mode for 27 analyzed samples was 0.36%, and the median 0.44%. Variability of mean analysis results reached 25% (Table 1).

Table 1

Content of the essential oils in the needles of 15 years old Scots pine (*Pinus sylvestris* L.)

Statistical characteristic	**Volatile oil content [%]**
$\bar{x}$	0.46
SD	0.11
Min	0.29
Max	0.72
Mode	0.36
Median	0.44
Coefficient of variation [%]	25.06

Comparable amounts of the essential oils obtained from pine needles have been reported by other authors [1, 5, 6], although their content is highly variable. According to Głowacki [1], it ranges from 0.3 to 0.7%. Essential oil content depends also on the sampling location. Numerous papers confirmed that pine needles obtained from the positions exposed to air pollution and from locations characterized by different soil type, differed not only in the essential oil content, but also in its chemical composition [2-4, 6-8]. Most authors [1, 9, 10], claim that the needles collected from young trees contain more essential oils. Głowacki [1] showed that mean content of the essential oil in the needles collected from 5 year old pines was 0.54%, in those from 15 year old young stand it was on average 0.52%, and in 100 years old stand it was 0.38%.

In this study, the analysis of the essential oil content was based on the age of the needles. A very similar content was detected in 1 and 2-year old needles, while the 3-year old needles were characterized by the lowest content of water and the oil. Mean oil content in 1-year old needles was 0.48%, in 2-year old needles 0.49%, and in 3-year old needles it amounted to 0.40% (Table 2, Fig. 1).

Table 2

Content of the essential oils in 1-, 2- and 3-year old needles of Scots pine (*Pinus sylvestris* L.)

Statistical characteristic	**Age of needles**		
	I 1-year-old	**II 2-year-old**	**III 3-year-old**
	Volatile oil content [%]		
$\bar{x}$	0.48	0.49	0.40
SD	0.15	0.09	0.05
Min	0.36	0.29	0.31
Max	0.72	0.63	0.46
Mode	0.36	0.49	0.44
Median	0.38	0.49	0.43
Coefficient of variation [%]	31.7	19.0	13.9

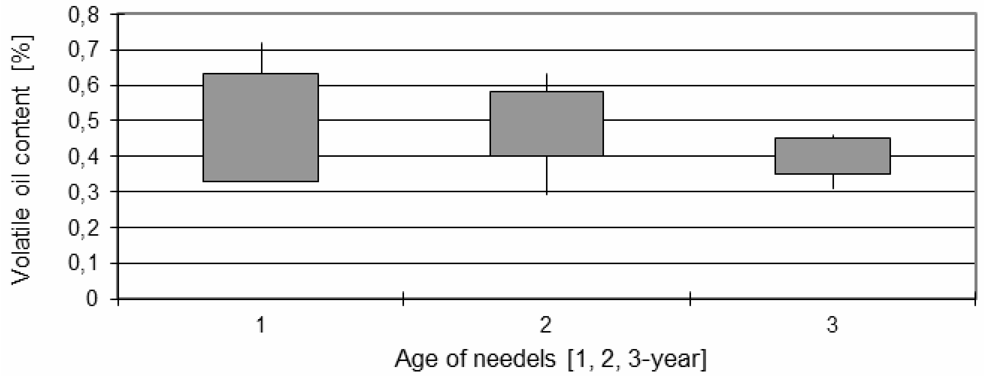

Fig. 1. Volatile oil content of Scots Pine needles in relation to the needles' age

A similar analysis concerning the content of essential oils was performed for the pine needles collected from the lower, middle and upper part of the crown. The age of the stands determines tree growth conditions. Crow density and closure may be very variable, thus providing different light conditions, especially at the crown base. When selecting the model trees, we tried to collect the samples from well-lit crowns, characterized by similar closure, understood as fulfilling the ecological space by the crown. Analysis of the needle samples revealed a linear distribution of the essential oil. The higher the needles were located in the crown, the more essential oils they contained. The oil content in the needles from the lower part of the crown was 0.36%, from the middle part 0.46%, and from the upper part 0.55% (Table 3, Fig. 2).

Table 3

Content of the essential oils in the needles of Scots pine (*Pinus sylvestris* L.) from different parts of the crown

Statistical characteristic	**Crown zones**		
	Bottom	**Middle**	**Top**
	Volatile oil content [%]		
$\overline{x}$	0.36	0.46	0.55
SD	0.05	0.09	0.11
Min	0.29	0.36	0.44
Max	0.49	0.63	0.51
Mode	0.36	0.43	0.51
Median	0.36	0.43	0.51
Coefficient of variation [%]	15.3	18.4	19.2

The subject literature provides different reports on the content of the essential oils depending on the needle position in the crown. For example, spruce needles coming from the upper and middle part of the crown contained respectively 0.21 and 0.19% of the oil, while those from the lower part were characterized by a 40% decrease in the oil content that amounted to 0.12% [11].

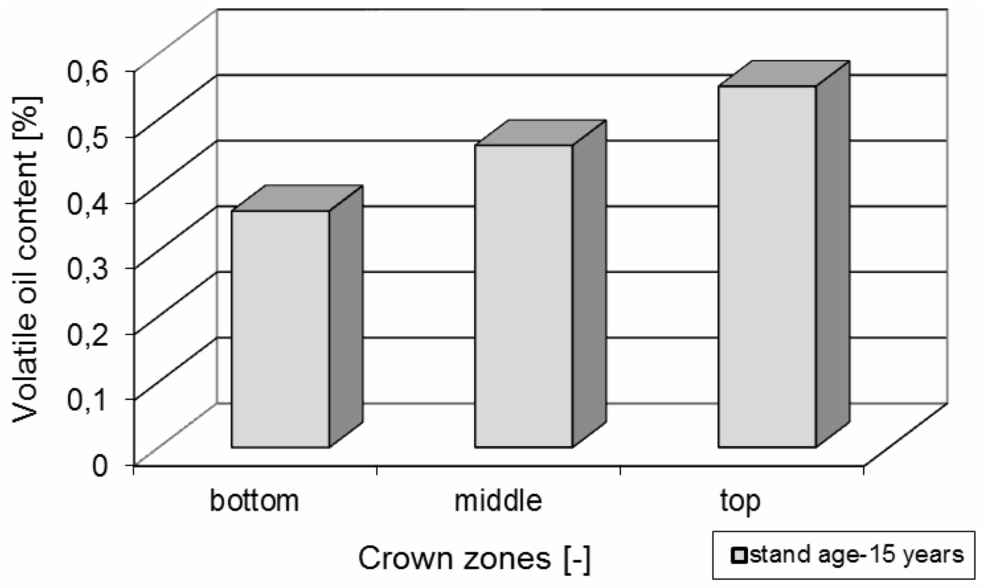

Fig. 2. Volatile oil content of Scots Pine (*Pinus sylvestris* L.) needles collected from three zones of the canopy

Conclusions

1. Mean oil content decreases with the age of the needles. The lowest content of the essential oil was found in 3-year old needles (0.40%), but similar amounts were detected in 1- and 2-year old needles.
2. In the stand of age class Ib (15 years old), the content of the essential oils increases at higher crown levels. The highest content of the oil was detected in the needles from the top part of the crows.

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WYDAJNOŚĆ OLEJKÓW ETERYCZNYCH Z IGLIWIA SOSNY ZWYCZAJNEJ (*Pinus sylvestris* L.)

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Abstrakt: Celem badań było określenie wydajności olejków eterycznych w igliwiu sosnowym w 3 strefach korony drzew modelowych (dolnej, środkowej i górnej) dla 3 najmłodszych roczników igliwia (1-, 2- i 3-letniego). W wyniku przeprowadzonych badań stwierdzono wyraźny wzrost wydajności olejków eterycznych w kierunku od dolnej poprzez środkową do górnej części korony. Najmniejszą zawartość olejku stwierdzono w igliwiu 3-letnim (0,40%), natomiast igliwie 1-roczne i 2-letnie zawierało odpowiednio 0,48 i 0,49%.

Słowa kluczowe: olejek eteryczny, igliwie, sosna zwyczajna

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HYDROLYSIS OF KERATIN MATERIALS DERIVED FROM POULTRY INDUSTRY

HYDROLIZA MATERIAŁÓW KERATYNOWYCH POCHODZĄCYCH Z PRZEMYSŁU DROBIARSKIEGO

Abstract: In 2011, the European Union produced about 45 million tons of meat and in this time Poland occupied the fourth place in Europe in the production of poultry and pork meat and seventh in production of beef. The consumption of poultry meat grows year by year, which results in an increase of its production. Feathers consist in 90% of keratin and they make up 5-7% of the total weight of adult chickens what causes that feathers waste is the main source of keratin. Feathers are discarded in the process of the converting of poultry as a waste product and contribute to environmental pollution. The development of the poultry industry in the world has led to the generation of more than 4 million tons per year of waste feathers. Average farm size in Poland produces about 7 tons of chicken feathers a day. Nationally, during each year 77,000 tons of waste is produced. Keratin has a high immunity to physical and chemical factors and it is the reason of searching for new methods of keratin waste conversion. It would help to avoid a problem with storage of feather wastes. Application of alkaline hydrolysis is one of the ways of feathers utilizations. During this hydrolysis the keratin swells and is subject to degradation. The increase in solubility of keratin in bases is caused by peptide and disulfide bond cleavage and it leads to increase of keratin plasticity and decrease of its strength. The aim of this study was to determine the effect of process parameters on the degree of degradation of keratin and optimize the process to achieve its maximum value.

Keywords: keratin, feathers, hydrolysis

Introduction

In 2011, the European Union produced about 45 million tons of meat and in this time Poland occupied the fourth place in Europe in the production of poultry and pork meat and seventh in production of beef.

The consumption of poultry meat grows year by year, which results in an increase of its production [1]. A considerable amount of waste are produced during slaughter of chickens. The main waste are: feathers, skin, blood, heads and legs and they belong to the III waste category and they may be used for feed and fertilizer after heat treatment. Poultry processing plants currently covert feather waste into low-nutritive animal feed [2]. Industrial methods of processing this waste are based on chemical (acid, base, catalyst) or enzymatic hydrolysis. Chemical hydrolysis requires more aggressive conditions of reaction (high temperature and pressure) and carries a greater risk to the environment. The product has a low nutritional value, because it contains small amounts of the essential amino acids. Enzymatic hydrolysis is more expensive and requires a longer time of operation.

The nutritional value of the product obtained by hydrolysis is determined by amino acids contained in it. These amino acids are methionine and histidine and their number decreases with age of chicken [3, 4]. In some countries legal regulations prohibit the use of waste feathers into animal feed and in most such cases, feathers are stored [5]. Odors

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formed from the degradation of substances contained in the biomass and greenhouse gases such as methane and carbon dioxide are the main problem of storing [6]. In addition, non-volatile products of decomposition can penetrate along with the water into the soil and contaminate groundwater [7].

Feathers consist in 90% of keratin and they make up 5-7% of the total weight of adult chickens what causes that feathers waste is the main source of keratin [8]. Feathers are discarded in the process of the converting of poultry as a waste product and contribute to environmental pollution. The development of the poultry industry in the world has led to the generation of more than 4 million tonnes per year of waste feathers. Average farm size in Poland produces about 7 tonnes of chicken feathers a day. Nationally, during each year 77,000 tonnes of waste is produced [7, 9].

Keratin is a biopolymer having a three-dimensional, fibrous structure with hierarchical character. It consists of small nano-amino acids which are polymerized in the known sequence to the molecular weight of protein order of 10-100 nm. The molecular weight of the feather keratin is approximately 10,500 Da. The content of cysteine/cystine in the amino acid sequence is about 7%. Keratin has in its structure about 40% hydrophilic and 60% hydrophobic chemical groups according to the amino acid sequence. Molecules of protein may be accumulated in the structure of the α-helix, β-sheet or random, unordered macrostructure. Keratin fibers in feather consist of 41% α-helix, 38% β-sheet, and 21% random structure [7, 10].

The structure of α-helix are intramolecular hydrogen bonds between the carbonyl group of one amino acid and the amino group of another amino acid. The structure β-sheet is characterized by the interchain hydrogen bonds between the amino and carbonyl groups. A hydrogen bond may be combined with the water bound in the structure of the protein. Keratin is characterized by a high stability, because of intermolecular bonds that occur between the polar and non-polar amino acids, and poor solubility caused by the presence of S-S bonds between cysteine amino acids [7, 10].

Keratin is a popular product in the pharmaceutical, medical, cosmetic, and biotechnological industry. Materials obtained from wool keratin may be converted into porous foam of different shapes, sponges, mats, coatings, gels, microfibers and materials of high molecular weight. Non-antigenic keratin has a positive effect on wound healing and tissue reconstruction. Implantation layer, construction or keratin biomaterial may be absorbed by the surrounding tissue [7, 11].

Keratin was used in a narrow range, because of the insolubility in water and a limited number of available methods for the extraction and processing. In the past it was used as a biomaterial in regenerative medicine. The interest in keratin and its modifications has increased in the past few years, especially popular has become keratin obtained from wool. Yamauchi and Tachibana described the increasing of degree of fibroblasts distribution on the keratin and keratin-chitosan coating [11]. Fujii studied the effect of proteins derived from human hair on the rat mast cells. Verma described the preparation of the protein structure of a human hair to its use in tissue engineering. Van Dyke research group conducted the study of hydrogels and porous structure based on keratin from hair and their interactions with cells and tissues. Recently tests based on the use of keratin biomaterials for biomedical applications were carried out. The literature also attempts to use the keratin coating on ocular surface reconstruction [7, 12].

Fibers of chicken feather are composed of a hydrophobic keratin, which is a protein having similar strength to nylon one, and a smaller diameter than the wood fiber. A surprising feature of the chicken feather fibers are semi-crystallinity and cross-linked structure, which enhances the resistance of composites based on polymers to mechanical stress and causes a relatively high modulus of elasticity (GPa 3.4-5). In addition, fibers of chicken feather have a high elongation coefficient. These mentioned properties suggest that there are possibilities of successful application of chicken feather fiber as a polymeric reinforcement [7, 13].

Non-woven composites formed by combination between synthetic and chicken feathers (PP, PES) have also a good sorption and mechanical properties. The wide range of excellent sorption of hydrophobic hydrocarbon and selectivity (absorb liquids immiscible with water) is typical for that kind of materials. Non-woven is cheap, lightweight, easy to store and may be used in a variety of weather conditions and surfaces (on the surface of water, concrete or asphalt). Non-woven composites are prepared by papermaking method and in the presence of water [14].

The selection and optimization of process parameters of alkaline hydrolysis of waste feathers was the aim of the study. Method of chemical hydrolysis of feathers proposed in the project reduces the temperature, pressure and shortens the time of process. Moreover, it leads to a decrease in energy consumption of the process, what saves a considerable amount of its cost and the resulting product has a high nutritional value.

Experimental part

Waste feathers provided from poultry plant were the object of the research.

During the study, the moisture content was determined using the analyzer RADWAG WPS 210s at 105°C and 5 s sampling time.

In order to provide greater surface area for heat exchange hydrolysis was carried out in a laboratory autoclave that was set in a horizontal position. Alkaline hydrolysis with calcium hydroxide was carried out in two stages. The first step was to sterilize the material agreement with the law that determined the parameters of the hydrolysis process. According to the law, animal by-products must be heated during the sterilization to a temperature above 133°C for at least 20 min at a pressure (absolute) of at least 3 bars produced by saturated steam [15]. In the second stage, the reactor temperature was lowered to 90-110°C and the pressure was lowered to ambient pressure. The removal of moisture from the material followed under these conditions. Digestible protein and total protein was determined in the product (dry up in dryer in 40°C) by the Kjeldahl method (PN-75/A-04018+Az3:2002+I-01/PN-75/A-04018:1975).

The response surface was determined using the central-compositional plan and it allowed for the optimization of the hydrolysis process and the application of created model to predict the dependent variable (total protein content and digestible in the product).

Results and discussion

A set of parameters characterizing the object of study was determined (qualitative model of research object) in order to select of the test plan and these parameters were reviewed and classified into value of input, output, and constant interference.

Weight of calcium hydroxide added to the feathers (0.05-0.75 g), the mass of water (2.0-5.0 g), the temperature of the second stage of the process (90-110°C), its duration (60-240 min) were input quantities that describe the test object (the process of alkaline hydrolysis feathers). The feather mass of 15 g and its humidity of 60% were adopted. Input values determine the composition of feather meal - content of protein and digestible protein [%]. The optimal test plan was central compositional plan with twice-repeated of the experiment at a central point.

The parameters of process and results of product's analysis are summarized in Table 1. Decomposition has been an object of research and it involved the creation of two objects of researches characterized with one the value of the output (the percentage of total protein and digestible protein).

Table 1

Summary of input variables (independent) and the content of total and digestible protein in feather meal

	Mass $Ca(OH)_2$ [g]	**Mass H_2O [g]**	**Temperature 2nd stage [°C]**	**Time 2nd stage [min]**	**Total protein [%]**	**Digestible protein [%]**
1	0.05	3.5	100	150	88.5	70.2
2	0.05	5.0	110	240	91.1	78.3
3	0.75	2.0	90	240	84	89.0
4	0.75	3.5	100	150	76.5	71.5
5	0.75	2.0	110	240	75.5	65.3
6	0.40	3.5	100	60	83.7	69.9
7	0.40	3.5	100	150	86.1	78.5
8	0.75	5.0	90	60	78.7	57.6
9	0.40	5.0	100	150	83.3	74.1
10	0.40	3.5	100	150	83.5	42.3
11	0.40	3.5	90	150	82.6	70.7
12	0.05	2.0	90	60	89.5	67.5
13	0.40	3.5	110	150	82.8	67.9
14	0.40	3.5	100	240	83.9	46.1
15	0.40	2.0	100	150	82.8	76.7
16	0.40	3.5	100	150	84.2	63.7
17	0.75	5.0	110	60	76.3	60.0
18	0.05	2.0	110	60	89.4	62.9
19	0.05	5.0	90	240	89.1	72.1

Feather hydrolysis tests performed on various parameters of the second stage of the process has shown the variability of output parameters of the process. The content of total protein is in the range 75-91%, and 42-89% of digestible protein. The lowest content of total protein (75.5%) was obtained for the parameters of process: temperature 110°C, duration of process 240 minutes and the addition of 2 g of water and 0.75 g of calcium hydroxide. It is the highest concentration (91.1%) reported for the test conducted at the same temperature and for the same period of time, but with 0.05 g of $Ca(OH)_2$ and 5 g of H_2O. The amount of calcium hydroxide added during the hydrolysis process was the main factor contributing to content of the total protein of the meal. This is due to its content in the final product after the reaction because $Ca(OH)_2$ remains in the system and enters into the composition of the product. The greater number of calcium hydroxide makes the lower content of total protein in the meal. In the case of digestible protein a greater difference between the extreme results is observed. The lowest content of this protein (42.3%) is noticed in the test carried out during 150 min and at 100°C with the addition of 0.4 g of calcium hydroxide and 3.5 g of H_2O. The highest amount (89%) is for a process carried out at a temperature of 90°C and during 240 minutes with 2 g of H_2O and 0.75 g of $Ca(OH)_2$. The amount of digestible protein in the final product depends on the degree of degradation of the keratin. The environment of the process (pH, temperature, time) influences the degree of degradation of that biopolymer. An increasing of keratin solubility in the bases is caused by peptide and disulfide bonds cleavage. The solubility is dependent on the concentration of base in the system, temperature and process time.

Tests performed during the waste feathers conversion into feather meal showed that the content of total and digestible protein depends on many parameters of the process. The mass of calcium hydroxide (0.05 g), water (5.0 g), the temperature of the second stage of the process (110°C) and duration (200 min) which were admitted as optimal parameters of the hydrolysis process.

Conclusions

Elaborated method of hydrolysis of feathers allows to obtain feather meal, which may be used as an additive to animal feed. The content of total and digestible protein in a received product was higher than those in commonly used animal feed. It is a result of high content of amino acids significant for food.

This method of hydrolysis is environmentally and economically advantageous because it is carried out under mild conditions of temperature and pressure, and the shorter the duration.

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HYDROLIZA MATERIAŁÓW KERATYNOWYCH POCHODZĄCYCH Z PRZEMYSŁU DROBIARSKIEGO

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Abstrakt: W 2011 roku w Unii Europejskiej wyprodukowano około 45 mln ton mięsa. W tym czasie Polska zajmowała czwarte miejsce pod względem produkcji mięsa drobiowego oraz wieprzowego, była również siódmym producentem wołowiny. Spożycie mięsa drobiowego wzrasta z roku na rok, co skutkuje zwiększeniem jego produkcji. Pióra zbudowane są w 90% z keratyny i stanowią 5-7% całkowitej masy dorosłych kurczaków, wskutek czego są głównym źródłem odpadów keratynowych. W trakcie procesu przetwórstwa drobiu pióra są odrzucane, a jako produkt odpadowy przyczyniają się do zanieczyszczenia środowiska. Rozwój przemysłu drobiarskiego na świecie doprowadził do generowania ponad 4 milionów ton odpadowych piór rocznie. Średniej wielkości ferma w Polsce generuje około 7 ton pierza kurzego w ciągu doby, a w skali kraju rocznie wytwarzane jest 77 000 ton tego odpadu. Keratyna posiada wysoką odporność na czynniki fizyczne oraz chemiczne, co jest powodem poszukiwania nowych metod przetwarzania odpadów keratynowych. Pomoże to uniknąć problemów ze składowaniem odpadowego pierza. Zastosowanie hydrolizy zasadowej jest jednym ze sposobów utylizacji piór. Podczas tej hydrolizy keratyna pęcznieje i ulega degradacji. Rozerwanie wiązań peptydowych i disiarczkowych powoduje wzrost rozpuszczalności keratyn w zasadach oraz prowadzi do zwiększenia plastyczności keratyny i spadku jej wytrzymałości. Celem badań było określenie wpływu parametrów procesu na stopień degradacji keratyny oraz optymalizacja procesu w celu uzyskania jego maksymalnej wartości.

Słowa kluczowe: keratyna, pióra, hydroliza

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CHEMICAL OXYGEN DEMAND FRACTIONATION OF REJECT WATER FROM MUNICIPAL WASTEWATER TREATMENT PLANT

FRAKCJE CHEMICZNEGO ZAPOTRZEBOWANIA NA TLEN WÓD OSADOWYCH Z MIEJSKIEJ OCZYSZCZALNI ŚCIEKÓW

Abstract: This study presents results of chemical oxygen demand fractionation measurement of reject water from municipal wastewater treatment plant in Malaga (Spain). Analyzed reject water is a high-strength wastewater, internally generated at WWTP during sewage sludge treatment. Additionally, in many cases the reject water is recycled to the main stream of WWTPs without any treatment. Because of high concentrations of nitrogen and phosphorus as well as organic matter, those sludge liquors could significantly effect on WWTPs operation. In this research, the samples of reject water were obtained from following installations: centrifuge dewatering, centrifuge thickening, gravity thickener and sludge dryer. The respirometric method was applied to determine biodegradable COD fractions. In case of reject water form sludge dryer, gravity thickener as well as centrifuge dewatering the obtained results of COD fractionation indicated that the organic matter of wastewater is mostly biodegradable, this fraction was ranged from 63.6 to 88.6 of total COD. Whereas, the reject water from centrifuge thickening was characterized by significant share of inert fractions, 86.9 of total COD.

Keywords: COD fraction, organic matter, reject water, side-stream wastewater, respirometry

Introduction

Currently, the chemical oxygen demand (COD) fraction has become an indispensable tool for the characterization of wastewater and expression of model components [1, 2]. The COD fractionation often replaced biological oxygen demand (BOD), volatile solids (VS) and total organic carbon (TOC) as the parameter to describe organic matter presented in wastewater [3-5]. The total COD is divided due to rate of degradation, in ASM1 (Activated Sludge Model no. 1) is distinguished following fractions: readily biodegradable (S_S) and slowly biodegradable (X_S) (biodegradable organic matter) as well as soluble inert COD (S_I) and particulate inert COD (X_I) (non-biodegradable organic matter) [5, 6]. The biodegradable fractions (S_S and X_S) could be absorbed by organisms and metabolized for energy and synthesis. However, the slowly biodegradable substrate firstly should be hydrolyzed by exocellular enzymes of bacteria [2, 7]. The soluble inert COD passes through the system without influencing the biochemical processes in the reactor. Whereas, the particulate inert fraction (X_I) is mostly accumulated in the activated sludge and removed from the system with waste sludge [4, 8]. Generally, the COD fraction has been applied in modeling of the microbial processes in activated sludge as well as control purposes [2].

This study presents results of chemical oxygen demand fractionation measurements of reject water from municipal wastewater treatment plant in Malaga (Spain). Recently, more attention has been focused on the issue of return flows. Those streams mostly include

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removed liquid phase from processes such a thickening, anaerobic digestion, chemical conditioning as well as dewatering of sewage sludge. This high-strength wastewater also called as side-stream may contain significant concentrations of nitrogen, phosphorus and organic matter [9, 10]. Additionally, in many cases the reject water is recycled into the main stream of WWTPs without any treatment, which could cause several problems on WWTP operation. Most significant of those issues are biological process disturbances connected with substrate unequilibrium, overloading of bioreactor which can led to increase of operational as well as maintenance costs [11, 12].

Materials and methods

As a materials in presented research was used reject water obtained from following installations: centrifuge dewatering, centrifuge thickening, gravity thickener and sludge dryer of the MWWTP situated in Malaga (Spain). To determine COD fractions the freshly collected samples were used, maximally 2 hours after sampling.

The tests were conducted using respirometer BM-T (Surcis). The equipment consisted of three parts: batch reactor (volume 1.0 dm^3) thermostatic unit and computer with software. The respirometer's chamber was equipped with a stirring and aeration systems as well as dissolved oxygen probe and the pH electrode. It recorded directly two fractions: total biodegradable (C_S) and readily biodegradable (S_S).

The respirometer was inoculated with activated sludge from biological reactor. This sample was aerated over 24 hours before the measure to reach the endogenous respiration. For all measurements the temperature was maintained at 20.0 ± 0.1ºC. In order to eliminate oxygen consumption due to nitrification about 10 min before the analysis 10 cm^3 solution of allylthiourea (ATU) was added to respirometer's chamber. The measurement was carried out in accordance with the procedure given by Surcis.

The inert as well as the slowly biodegradable fractions were based on following calculations:

$$X_S = C_S - S_S \ [\mathrm{mg \cdot dm^{-3}}] \tag{1}$$

$$S_I = S_T - S_S \ [\mathrm{mg \cdot dm^{-3}}] \tag{2}$$

$$X_I = C_I - S_I \ [\mathrm{mg \cdot dm^{-3}}] \tag{3}$$

where: S_I - soluble inert COD substrate [mg·dm^{-3}], S_T - total soluble COD [mg·dm^{-3}], X_I - particulate inert substrate [mg·dm^{-3}], X_S - slowly biodegradable organic matter [mg·dm^{-3}].

In order to estimate (S_T) the total soluble COD, the samples of wastewaters were filtered through 0.45 μm filter, then the chemical oxygen demand was measured. All chemical oxygen demand analyses were performed with Hach Lange UV - VIS DR 5000 using Hach analytical methods.

Results and discussion

The results of presented study are shown in Table 1. The concentrations of total and soluble COD were differed over a wide range. The average value of total COD was

2110.0 mg·dm^{-3} and varied within a range of 86.8 to 8660.0 mg·dm^{-3}. The highest value was observed in case of sludge dryer, whereas the lowest result in centrifuge thickening. The soluble COD concentrations were differed in the range of 39.1 to 7850.0 mg·dm^{-3}, with a mean value of 1618 mg·dm^{-3}.

Table 1

Estimated chemical oxygen demand fractions of reject water from different facilities

		C_T	**C_S**	**S_S**	**X_S**	**S_I**	**X_I**
		[mg·dm^{-3}]		**[%]**			
centrifuge dewatering	average	1406.8	660.5	46.0	17.5	8.7	27.8
	min	991.0	371.0	21.2	5.5	0.1	10.0
	max	1869.0	905.0	71.1	31.5	23.3	43.5
	SD	460.0	251.0	20.8	10.9	11.1	17.1
centrifuge thickening	average	114.2	59.8	11.1	1.9	42.3	44.5
	min	86.8	39.1	8.3	0.1	21.5	32.5
	max	151.0	94.9	12.4	4.0	53.0	65.4
	SD	32.1	24.4	1.9	1.8	14.4	14.7
gravity thickener	average	978.8	483.0	51.7	11.9	3.8	32.5
	min	543.0	215.0	40.2	7.5	0.2	21.4
	max	1355.0	693.0	68.3	18.0	8.7	41.4
	SD	333.7	201.8	13.0	4.5	4.5	10.3
sludge dryer	average	5940.0	5270.0	83.9	4.8	17.4	5.4
	min	3220.0	2690.0	79.8	3.7	2.7	5.7
	max	8660.0	7850.0	87.9	5.9	3.8	10.6
	SD	3846.7	3648.7	5.8	1.6	29.6	7.5

In case of reject water from sludge dryer, gravity thickener as well as centrifuge dewatering the obtained results of COD fractionation indicated that the organic matter of wastewater is mostly biodegradable, this fraction was ranged from 63.6 to 88.6 of total COD.

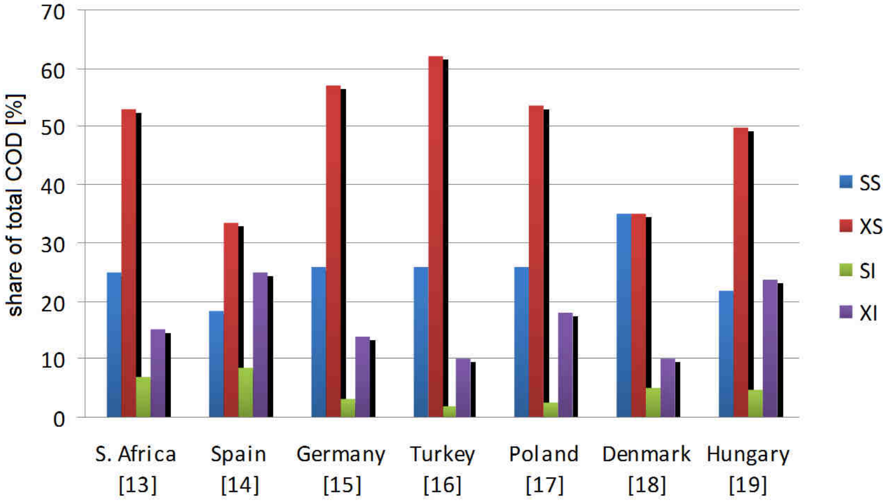

Fig. 1. The summary of COD fractions in raw wastewater from various countries

The presented results for reject water from centrifuge dewatering and gravity thickener were comparable. Those samples were characterized by significant percentage of readily biodegradable faction, 46% for centrifuge dewatering and 51.7% for gravity thickener. The similar data was also observed in case of slowly biodegradable substrate, 17.5 and 11.9% of total COD, respectively. The highest value of biodegradable fractions was obtained in wastewater from sludge dryer 88.7%, of which 83.9% was a readily biodegradable component. The results show that those samples contain a relatively low fraction of slowly biodegradable COD, with a mean value of 11.4 of total COD, compared to data reported at the literature and depictured at Figure 1 for raw wastewater (X_S between 33-62 of C_T) [13-19]. Whereas, the non-biodegradable fractions were dominated in case of sample from centrifuge thickening, the average value was 42.3% for soluble inert fraction and 44.5% for the particulate inert fraction. In raw wastewater inert fractions (soluble and particulate) might contain up to 33 of total COD [14]. A significant share of non-biodegradable fraction in the influent could interfere the biological treatment.

Additionally, the obtained results of COD fractionation were characterized by significant standard deviation, which could be attributed to changing influent quality as well as the process efficiency.

Summary and conclusions

Although flow of reject water is relatively small, it could affected on wastewater treatment plant efficiency. Furthermore, this high-strength wastewater is returned to influent of wastewater treatment plants. In this study, the results of chemical oxygen demand fractionation of reject water from municipal wastewater treatment plant in Malaga (Spain) were presented.

In case of reject water form sludge dryer, gravity thickener as well as centrifuge dewatering the obtained data of COD fractionation indicated that the organic matter of wastewater is mostly biodegradable, this fraction was ranged from 63.6 to 88.6 of total COD. Whereas, the reject water from centrifuge thickening was characterized by significant share of inert fractions, 86.9 of total COD.

The result of this research could be applied in modelling activated sludge systems as a part of influent characterization as well as for designing separate treatment of those returned streams.

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FRAKCJE CHEMICZNEGO ZAPOTRZEBOWANIA NA TLEN WÓD OSADOWYCH Z MIEJSKIEJ OCZYSZCZALNI ŚCIEKÓW

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Abstrakt: Artykuł przedstawia wyniki badań udziału poszczególnych frakcji chemicznego zapotrzebowania na tlen w wodach odprowadzanych z urządzeń przeróbki osadów w miejskiej oczyszczalni ścieków w Maladze (Hiszpania). Analizowane wody osadowe można scharakteryzować jako silnie zatężone ścieki generowane wewnątrz oczyszczalni, powstałe w efekcie prowadzonych procesów technologicznych - głównie obróbki osadów. W wielu stosowanych obecnie układach technologicznych tego typu wody zawracane są na początek głównego ciągu technologicznego oczyszczalni. Jednakże ze względu na bardzo wysokie stężenia związków zawierających azot, fosfor oraz węgiel organiczny odcieki te mogą znacząco wpływać na realizowane procesy oczyszczania. Podczas badań prowadzonych w ramach niniejszego opracowania próbki analizowanych wód procesowych pobierane były z następujących urządzeń gospodarki osadowej: wirówki odwadniającej, wirówki zagęszczającej, zagęszczacza grawitacyjnego oraz suszarni osadów. Za pomocą metody respirometrycznej określony został udział frakcji biodegradowalnych w całkowitym chemicznym zapotrzebowaniu na tlen analizowanych próbek. W przypadku wód osadowych po suszarni osadów, zagęszczaczu grawitacyjnym oraz wirówki odwadniającej uzyskane wyniki analizy frakcyjnej ChZT wskazują na znaczny udział substancji biodegradowalnych - zakres od 63,6 do 88,6% całkowitego ChZT. Natomiast odcieki z wirówki zagęszczającej charakteryzują się znacznym udziałem substancji niebiodegradowalnych - udział frakcji inertnych wynosił 86,9% ChZT całkowitego.

Słowa kluczowe: frakcje ChZT, materia organiczna, wody odpadowe z przeróbki osadów, respirometria

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MAGNETIC SEPARATION OF ELECTRONIC WASTE AFTER THE COMBUSTION PROCESS IN THE FLUIDIZED BED

SEPARACJA MAGNETYCZNA ODPADÓW ELEKTRONICZNYCH PO PROCESIE SPALANIA W ZŁOŻU FLUIDALNYM

Abstract: The paper presents the results of magnetic separation of materials received after the thermal utilization process of mobile phones in a laboratory fluidized bed reactor. The starting material constituted ten mobile phones which were subjected to the combustion process receiving brittle, solid products. Next, the received materials were grinded to 1 and to 0.5 mm and after the magnetic separation was conducted using neodymium magnet, plate separator (three-phase) and disk (belt) separator. The received waste fractions were subjected to the analyze of content phases (XRD) and chosen chemical elements (ICP).

Keywords: WEEE, printed circuit board, magnetic separation

Introduction

Both in Europe and the world, there is a constant upward trend in the amounts of waste electrical and electronic equipment (WEEE). Each year in the European Union alone, approximately 8 million tons of electronic waste is produced, and the rate of such production increases annually by 3-5%. Every year, 20-50 million tons of this hazardous waste appear worldwide [1].

The most increasing tendency among WEEE constitute the information and telecommunication equipment such as mobile phones and personal computers due to the much shorter liveliness. These kind of equipment is exchanged almost every 2-5 years. At present it is estimated that every year about 17 mln of computers are thrown away because of improper work of these equipment, old technology or willing to possess equipment that is more functional. It is predicted that in 2020, the quantity of waste cell phones will increase sevenfold [2, 3].

Mobile phones, especially those of older generation, pose a significant risk to the environment due to the fact that they contain such elements as lead, cadmium or mercury. A printed circuit board with its electronic components alone contains numerous harmful elements, including noble metals (Au, Ag, Pt, Cu), coloured metals (Sn, Ni, Cr, Zn) as well as iron and silicon [4]. Therefore, unprocessed electronic waste which is stored together with domestic waste in landfills is a serious threat to human health and the environment, as it may lead to water and soil contamination and consequently to the contamination of agricultural produce as well [5].

In order to properly manage hazardous waste like electrical and electronic equipment, the European Union introduced the Directive 2002/96/EC of the European Parliament and of the Council of 27 January 2003, whose main purpose is to reduce the quantity of WEEE by proper reusing and recycling [6].

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In this paper the magnetic separation process of solid products received after combustion of mobile phones fragments in laboratory fluidized bed reactor were conducted. The received solid products were subjected to the grinding process to 1 and 0.5 mm. The neodymium magnet, plate separator, and disk separator were used for magnetic separation process. The received waste fractions were analyzed for particular phases by X-ray diffraction powder (XRD) and chosen chemical elements (ICP).

Experimental

In order to obtain material for the magnetic separation tests combustion process of mobile phones was conducted in the laboratory fluidized bed reactor which was previously used for the studies on selected aspects of fuel and waste burning [7, 8]. The process was proceeded in a hot fluidized bed, which constituted of glass sand with the grain size of 0.3-0.385 mm and weight of 413.6 g. The fluidized bed formed the environment with a homogeneous high temperature and considerable turbulence of the solid phase and gas phase, providing constant access of the oxidizer to the surface of the introduced samples. This access was necessary for the proper course of thermal treatment of the combustible components contained within the samples. Combustion was conducted with the use of oxygen from the air and propane-butane as auxiliary fuel assuring constant, high temperature.

As the starting material ten mobile phones were used. Initially, the phones were mechanically disassembled, the batteries were separated and the resulting material was divided into smaller parts with a guillotine. Next, the combustion process was conducted leading to receive brittle, solid products which were used to the magnetic separation. Solid products after combustion in the fluidized bed were crumbed and grinded. This way two materials with the grain size below 1 mm and more than 1 mm were received. Both materials were conducted the separation process, this over 1 mm was separated using the neodymium magnet. The sample below 1 mm was given to two-stage separation using plate and belt separator. After the separation processes of material with grain size below 1 mm was divided as two magnetic and one non-magnetic fractions. The last one was grinded to about 0.5 mm and the separation process on the belt separator was conducted again.

The first device used for the magnetic enrichment was plate separator with magnetic pole generated by alternating electric current. It is built with the flat plate made of transformer metal plates. In the trenches of plate there are windings connected to the three-phase electrical source (AC) producing three independent magnetic streams (similar to the stator of three-phase engine). Magnetic field which changes in time and space has waving character and moves along the plate with definite speed. The advantage of this separator is the opportunity of separating grids with strong magnetic properties due to the changing magnetic field.

The second used device was disk separator to which the amount of raw material is given through the hopper on the conveyor belt rolled on the drum and it leads material into the slit under the plate that is turning around. The magnetic ingredient is caught by the turning around plate and is drained outside the operation range of magnetic field where it mechanically comes off on the sharp edge putted to the plate. The non-magnetic ingredients come under the surface of plate and it is drained on the moving conveyor belt to the container of non-magnetic product. The operating slit of separator constitutes magnetic system wedge-surface. Inside the plate there are installed magnetic pole pieces producing magnetic field [9].

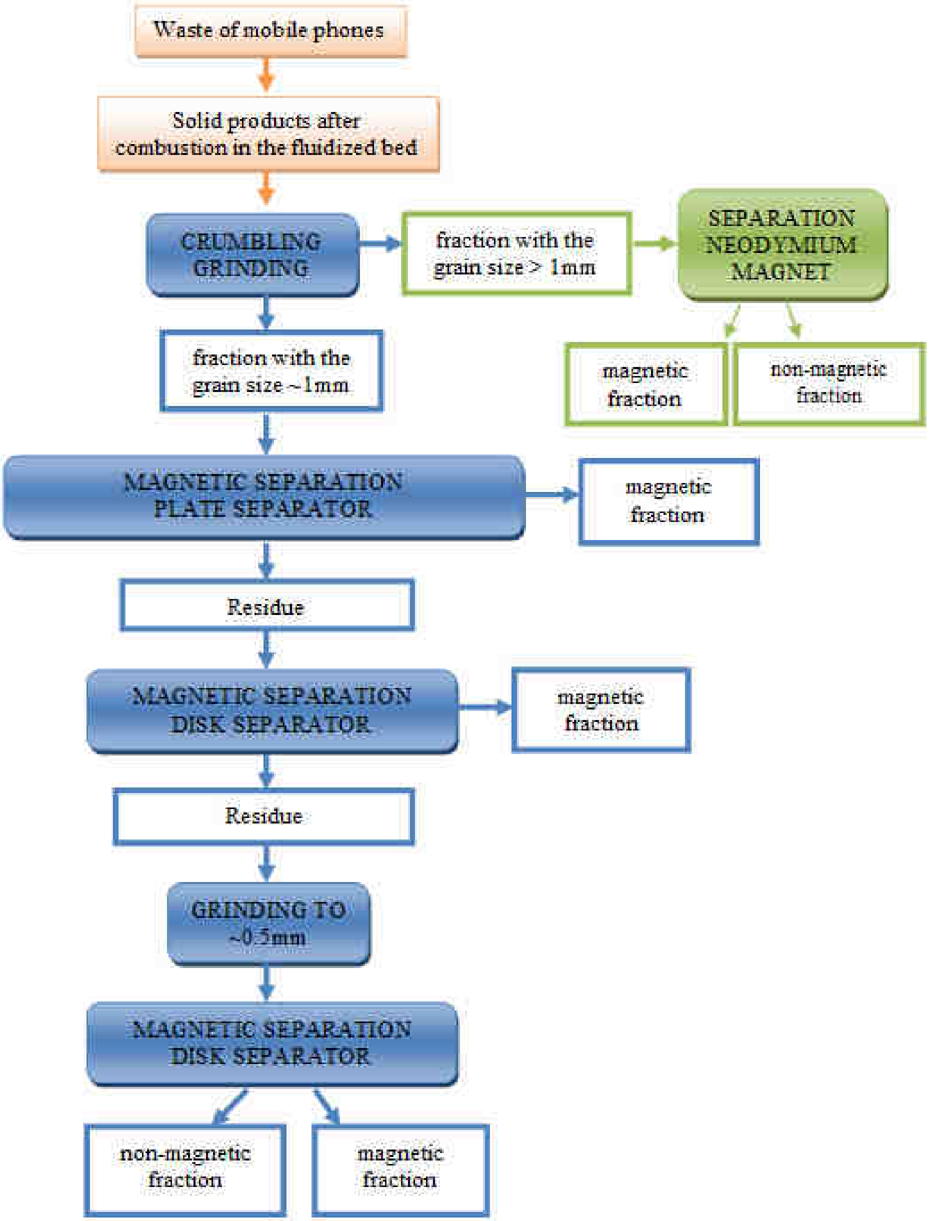

Fig. 1. The scheme of magnetic enrichment process of solid products after the combustion of used mobile phones

In Figure 1 there is presented the whole scheme of magnetic enrichment of solid products after the combustion process of used mobile phones.

Results and discussion

Few-stages process of magnetic enrichment allowed to receive particular fraction with various masses as presented in the Table 1. From the material with the grain size of < 1 mm after separation process on the plate and belt separator received three magnetic fractions with the weight of 44.9 g and non-magnetic fraction with the weight of 207.7 g.

Table 1

Received fractions after magnetic enrichment processes

No.	Separation	Fraction	Grain size [mm]	Mass [g]	Picture
1	The sample after crumbling and separating on sieves		> 1	70.4	
1a	Neodymium magnet	Magnetic		27.2	
1b		Non-magnetic		43.2	
2	The sample after crumbling and separating on sieves		~ 1	263.2	
2a	Plate separator	Magnetic		2.5	
2b	Disk separator	Magnetic		19.8	
2c	Disk separator	Magnetic	< 0.5	22.6	
		Non-magnetic		207.7	

Moreover, despite of repetition of crumbling and grinding processes of the initial material the fraction with the grain size > 1 mm was also received which mass was 70.4 g. This coarse fraction after the separation process with the use of neodymium magnet divided to the magnetic material with the mass of 27.2 g and non-magnetic material with the mass 43.2 g.

Table 2

The contents of selected chemical elements and their separation degree in each fraction

No.	Element	Element content in fraction [mg]					Separation degree in fraction [%]			
		0	I	II	III	IV	I	II	III	IV
1	Cu	44846	77.6	3010	4048	37710	0.17	6.71	9.03	84.1
2	P	13022	18.1	851	1146	11008	0.14	6.53	8.80	84.5
3	Fe	3139	1425	1416	135	164	45.4	45.1	4.29	5.23
5	Ni	2215	285	992	179	759	12.9	44.8	8.09	34.2
6	Ba	2021	75.7	1025	173	747	3.75	50.7	8.54	37.0
7	Al	1719	0.001	230	256	1233	0.00	13.4	14.9	71.7
8	Pb	1190	6.96	115	139	929	0.59	9.70	11.7	78.0
9	Sn	876	3.23	32.2	1.18	839	0.37	3.68	0.13	95.8
10	Zn	686	74.0	134	84.2	394	10.8	19.5	12.3	57.4
11	Nd	506	338	159	5.22	3.99	66.8	31.4	1.03	0.79
12	Ir	447	77.8	244	32.8	92.4	17.4	54.5	7.35	20.7
13	B	344	13.0	25.1	41.8	264	3.78	7.30	12.2	76.8
14	Au	188	0.876	15.3	7.19	164	0.47	8.16	3.83	87.5
15	Sr	82.3	3.63	35.9	6.28	36.57	4.41	43.5	7.62	44.4
16	Cr	81.4	20.9	49.1	3.25	8.09	25.7	60.4	3.99	9.94
17	Mn	67.5	4.19	51.8	4.45	7.05	6.22	76.7	6.59	10.4
18	Ti	45.7	7.94	8.15	3.82	25.8	17.4	17.8	8.37	56.4
19	La	26.8	0.001	22.4	0.732	3.72	0.00	83.4	2.73	13.9
20	Co	26.7	16.9	4.71	1.29	3.80	63.3	17.6	4.82	14.2
21	Ag	11.6	0.179	1.51	0.470	9.47	1.54	13.0	4.04	81.5

0 - initial material received after combustion of mobile phones
I - magnetic fraction (1 mm) after enrichment plate separator
II, III - magnetic fraction 1 mm (II), 0.5 mm (III) after enrichment on the disk separator
IV - non-magnetic fraction (0.5 mm) after enrichment on the disk separator

Table 3

Crystalline phases in particular fractions determined by the x-ray diffraction

Fraction	Crystalline phase identified				
	Si	Cu	Fe	Sn	Others
I Magnetic 1 mm Plate separator	-	-	$Fe_{14}Nd_2B$ - 31.4% Fe_3O_4 - 25.7%	SnO_2 - 5.7%	Ni - 37.2%
II Magnetic 1 mm Disk separator	SiO_2 - 62.3%	Cu_2O - 8.9% CuO - 4.8% Cu - 5.6%	CuFe - 3%	SnO_3Sr - 2.7% SnO_2 - 2.2%	$BaTiO_3$ - 10.6%
III Magnetic 0.5 mm Disk separator	SiO_2 - 61%	Cu_2O - 25.3% $Tl_{0.01}Cu_{3.99}$ - 6.7% CuO - 3.6% $CuGaTe_2$ - 1.4%	-	SnO_2 - 2.2%	-
IV Non-magnetic 0.5 mm Disk separator	SiO_2 - 59.8%	Cu_2O - 29.4% $Tl_{0.01}Cu_{3.99}$ - 5.1% CuO - 2.1%	-	SnO_2 - 3.5%	-

Next, to determine the chemical and elementary composition and the separation degree of chosen elements for each fraction the analysis using the method of spectrometry mass with the inductively coupled plasma and x-ray diffraction were conducted. The results are presented in the Tables 2 and 3.

Elementary analysis showed that the plate magnet separated the most effectively valuable rare earth element, which is neodymium. The results showed in this magnetic fraction the separation degree of 66.8% neodymium but also 63.3% cobalt and 45.4% iron.

As a result of second stage magnetic enrichment on the disk separator received the separation degree in the fraction for iron 45.1% and neodymium 31.4%. Total degree of two-stage magnetic enrichment of sample with the grain size of 1 mm was 80.9% for Co, 90.5% for Fe and 98.2% for Nd. In addition, the analyses of powder x-ray diffraction (XRD) showed total separation of neodymium as an alloy $Fe_{14}Nd_2B$, whose content in the magnetic fraction after the plate separation is 31.4% and also iron as Fe_3O_4 (25.7%) and CuFe (3%). The received results allow to conclude that the separation of such kind of materials with the grain size of 1 mm is sufficient and additional grinding to 0.5 mm is not necessary.

The received crystalline phases indicate the presence of cassiterite SnO_2 in each fraction which content is in the range from 2.2% (magnetic fractions after disk separator) to 5.7% (magnetic fraction after plate separator). Also in each fraction, apart from the magnetic after the plate separator, was shown the presence of quartz SiO_2 in the range from 59.8 to 62.3%.

The copper which was identified in the obtained fractions occurred in the metallic form Cu (5.6% in the fraction II), as oxide (cuprite Cu_2O from 8.9% in the fraction II to 29.4% in the fraction IV, tenorite CuO from 2.1% in the fraction IV to 4.8% in the fraction II), $Tl_{0.01}Cu_{3.99}$ in the range from 5.1% (fraction IV) to 6.7% (fraction III) and $CuGaTe_2$ in the magnetic fraction III (1.4%).

Non-magnetic fraction received after three-stage magnetic enrichment showed the highest separation degrees for tin 95.8%, gold 87.5%, copper 84.1% and silver 81.5%.

Conclusions

The combustion process of used mobile phones in the fluidized bed reactor allow to receive a starting material for magnetic enrichment which was a brittle material, so it was possible to grind it to the intended grain size. However, about 21% of subjected mass was not possible to grind to the expected size what was due to presence of the larger metal fragments coming out from cases of mobile phones.

Magnetic enrichment of material with the grain size with about 1 mm by plate separator (three-phase) and by disk separator allowed to receive in the magnetic fractions total separation degree 80.9% for cobalt, 90.5% for iron and 98.2% for neodymium. It can be conclude that to the achieve an effective magnetic enrichment of such type of materials the grain size of 1 mm is sufficient. As a result neodymium is separated in the form of alloy $Fe_{14}Nd_2B$ which constitutes one of the best materials used for producing permanent magnets.

Non-magnetic fraction, received as a result of magnetic enrichment, contain tin (95.8%), iron (87.5%), copper (84.1%), silver (81.5%) compared to the initial material.

The magnetic separation with the use of plate separator (three-phase) and disk separator allows the effective separation of ferromagnetic elements (Co, Fe, Nd) from non-magnetic (Cu, Sn, Au, Ag).

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SEPARACJA MAGNETYCZNA ODPADÓW ELEKTRONICZNYCH PO PROCESIE SPALANIA W ZŁOŻU FLUIDALNYM

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Abstrakt: W artykule przedstawiono wyniki badań separacji magnetycznej materiałów uzyskanych po procesie termicznej utylizacji telefonów komórkowych w laboratoryjnym reaktorze fluidyzacyjnym. Materiał wyjściowy stanowiło 10 telefonów komórkowych, które poddano procesowi spalania, uzyskując kruche produkty stałe. Następnie otrzymane materiały zmielono do uziarnienia 1 oraz 0,5 mm oraz przeprowadzono separację magnetyczną, wykorzystując magnes neodymowy, separator płytowy (trójfazowy) oraz separator talerzowy (taśmowy). Uzyskane frakcje odpadów poddano analizie na zawartość faz (XRD) oraz wybranych pierwiastków (ICP).

Słowa kluczowe: ZSEE, obwód drukowany, separacja magnetyczna