Chapter 8

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Successive Effect of Fluidal Ashes on the Number of Microorganisms Participating in Transformations of Carbon and Nitrogen in Soil

1. Fluidal ashes and their characteristics

Polish energy sector (public and industrial) is based mainly on combustion of hard coal and lignite.

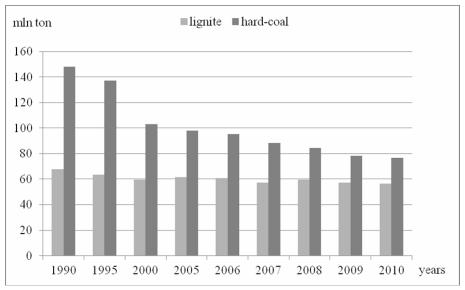


Fig. 1. Production of hard coal and lignite in million tonnes (Central Statistical Office of Poland 2011)

Hard coal mining in the first decade of the twenty-first century decreased nearly 2-fold in relation to the 90s of the twentieth century, and lignite mining declined only by a few percent (Fig. 1). A decline in the production of these minerals has also been observed for the last 5 years. As stated in the Directive (2009/28/EC) adopted by the European Parliament and the European Council, the share of energy from renewable sources in brutto energy consumption should reach the level of 15% in Poland, in 2020 (Fig. 2).

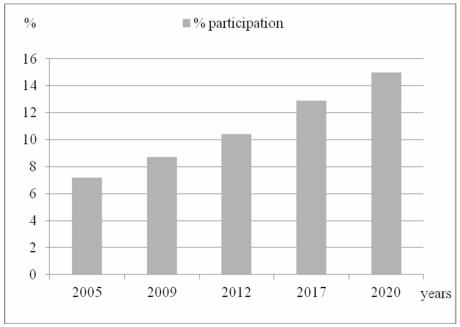


Fig. 2. National goals for the participation of energy from renewable sources in the final brutto energy consumption (Berent-Kowalska *et al.* 2010)

Nevertheless, significant reduction of the amount of coal combustion by-products (CCB) from technological processes in energy industry is not expected, due to the persistent high level of energy production in recent years, in Poland (Fig. 3). The main components of combustion wastes include: fly ash, boiler slag, bottom ash and flue gas desulfurization products. Storing and utilizing the CCB is a problem in the field of natural environmental protection. The amount of produced and used CCB is significantly different in each country (Hycnar, 2009). In 2006 Poland produced 15.627 thousand tons of combustion waste, 79.4% of which was used. Polish results in the field of using combustion waste are positively outstanding against the world's largest economies (USA, Japan, EU, Germany).

In Poland, the largest quantities of these wastes in the balance of their use concern production of clinker and cement (1.1 million tonnes), concrete (1.9 million tonnes), road construction and engineering works (0.6 million tonnes)

and mining (4.6 million tons). Economic utilization of fly ashes helps to reduce their negative impact on the environment at their place of storage.

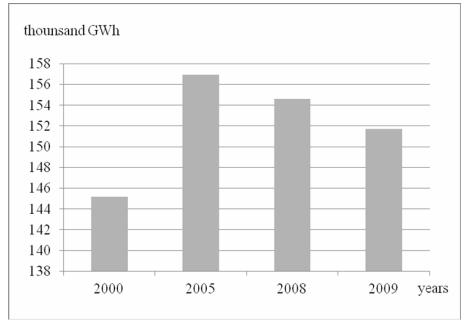


Fig. 3. Production of electric energy in Poland (Central Statistical Office of Poland, 2010)

Physicochemical properties of ashes depend on the kind of combusted coal and the type of power boiler used in the process (Table 1). The ashes from conventional boilers consist of spherical, glassy particles, containing $65 \div 88\%$ of glass, $1 \div 17\%$ of mullite, $1 \div 9\%$ of quartz, $1 \div 18\%$ of hematite, $1 \div 8\%$ of magnetite, $1 \div 3\%$ of anhydrite and $1 \div 8\%$ of CaO (Giergiczny 2006).

Fluid bed boilers came into use in the Polish professional energy industry in the mid-nineties. Unlike the ashes from conventional power boilers, the ashes from fluid (Table 1) bed boilers consist mainly of grains of clay minerals, characterized by a higher content of calcium compounds (Piotrowski, Eliasz-Bocheńczyk 2008). In the fluidal ashes, there is no glass and mullite, and the content of other ingredients is as follows: quartz $2\div12\%$, less than 50% of illite, hematite $4\div15\%$, less than 1% of magnetite, $10\div20\%$ of anhydrite and $5\div30\%$ of CaO (Brandštetr *et al.* 1997, Giergiczny 2006).

Diverse properties of the ashes determine their economic usage. They can be used in various industry branches, such as: production of cement, concrete, mortar and sorbents, in engineering and road works, in the production of aggregates and neutralization of waste, for example: sewage sludge, or in underground mining and agriculture. In the cultivation of plants, the soil environment determines the size of the obtained crop. The soil is like an organism, in which different groups of microbes produce enzymes and catalyze the transformations of organic and mineral components. The measure of biological soil activity may be the amount of assimilable organic matter, which under the influence of microorganisms decomposes in given time. This is accompanied by a strong multiplication of heterotrophic microflora, enhancement of its enzymatic activity and creation of synthesis and mineralization products (e.g. CO_2 , NH_3 , H_2S).

Table 1

Example chemical	composition	of	Polish	fluidized	and	conventional	ash	(Piotrowski,
Uliasz-Bocheńczyk	2008)							

Origin of ash from hard coal		Content %									
	loss on ignition	SiO ₂	Fe_2S_3	Al_2O_3	CaO	SO ₃	Na ₂ O	K ₂ O	CaO free		
			Flui	dized asl	1						
Power station from Tychy	13.79	31.39	3.35	14.70	19.75	4.68	0.74	2.08	6.67		
Power station from Siersza	5.55	40.43	6.82	21.52	11.52	6.13	1.58	1.96	1.48		
Conventional ash											
Power station from Jaworzno	2.91	47.86	7.65	28.75	4.94	0.58	1.28	2.33	0.0		
Power station from Lublin- Wrotków	10.75	58.46	3.99	14.00	5.31	0.77	0.59	1.24	1.25		

It determines the fertility of soil – the state of the environment, which enables good plant growth. Supplementing the soil with ash, especially on degraded areas, could improve soil fertility and its biological activity. Fluidal ash, as a byproduct of CHP industry, is long-term accumulated within the plant, which leads to creation of huge heaps of troublesome waste. The utilization of ash in agriculture seems to be a good solution, which also brings a beneficial effect in protecting the environment in the proximity of the power plant. The goal of using the fluidized bed ash in agriculture is to:

- improve the migration of water in the soil,
- reduce washing out of fertilizers and small fractions of the soil,
- neutralize the acidic components of the soil,
- fertilize the soil and deactivate the contaminated soil (Stout *et al.* 1997, Ritchey *et al.* 1999, Hycnar 2006).

Fly ash, directly used for fertilization, acts as a magnesium and calcium fertilizer. Lignite ashes can be characterized by slightly less alkalizing properties, than the basic calcium fertilizers (Piotrowski, Eliasz-Bocheńczyk 2008). In addition to large amounts of calcium, magnesium and potassium, the ashes contain also micronutrients (Maciak, Liwski 1981, Bogacz *et al.* 1995, Hermann 1996, Kalembkiewicz *et al.* 2007, Wojcieszczuk *et al.* 2009).

Roszyk et al. (2004) demonstrated that the phytotoxicity of solutions of ash extracts used for foliar fertilizing cauliflowers depended on the concentration,

pH and type of acid used for extraction. Water and 20% citric acid extracts proved to be non-toxic. Właśniewski (2009) reports that fly ash used in the pot experiment improved the sandy soil's richness in magnesium, phosphorus and potassium. The optimal dose of fly ash, which does not cause excessive burden, because of heavy metals, which it contains (lead and cadmium), was the dose of 67.2 t-ha^{-1} calculated according to double hydrolytic acidity. It should be borne in mind, that by adding the ashes to the soil, we introduce heavy metals, too (Rosik-Dulewska, Dulewski 1989), which may negatively affect the count of soil microorganisms such as bacteria (*Azotobacter* spp.), actinomycetes and sometimes also affect the yield of arable crops (Stevens, Dunn 2004, Antonkiewicz 2007).

Bielińska *et al.* (2009b) have demonstrated a beneficial effect of using fluidized bed ashes from hard coal on the activity of enzymes catalyzing the most important processes of soil transformation of organic matter and the circulation of essential nutrients in the soil environment, irrespective of the dose of ash and kind of cultivated plant species. The authors stress that the ashes may be an alternative to the classical liming, without damaging important metabolic processes that determine soil fertility. The fluidized bed ashes themselves, due to their physicochemical properties, such as very high pH and lack of organic matter, are not an optimal environment for the growth of microorganisms. Therefore, due to the low biological activity, they are being enriched in various types of organic materials (e.g. digested sewage sludge, straw, effective microorganisms – ceramic EM-X and EM-1), which, as a source of microflora, indirectly may also be a soil forming factor (Bielińska *et al.* 2009b, Jasiewicz *et al.* 2007, Krzywy, Wołoszyk 1996, Mazur 1996).

The use of fluidized bed ash in forestry, same as in agriculture, aims to raise soil pH and revitalization of the forest soils (Hycnar 2009).

2. Preparation of effective microorganisms

The soil along with the organisms which inhabit it, especially microbes, is a key element, shaping the properties of an ecosystem. Physicochemical, structural and biological properties of agricultural soils are formed by conducting agrotechnical treatments and cultivation of selected plants. Therefore, arable soil microorganisms are characterized by:

- specific properties,
- favoring the creation of lumpy structure,
- mineralization of organic matter,
- vegetation of artificial plant communities, usually monocultural agrocenosis.

Microbiologists for many years have made attempts to use beneficial soil microorganisms for vaccinating the soil in order to prevent the harmful effects of phytopathogenic organisms, including bacteria, fungi and nematodes. These tests, carried out generally by the use of single pure cultures of microorganisms, have failed for many reasons. Because micro-organisms in ecosystems are on one hand involved in soil formation, and on the other they create symbiotic relationships with plants, as in the creation of ryzosphere and mycorrhizae, their biodiversity is probably the key element to the functioning of the system.

Many researchers think that the culturing and implanting beneficial microorganisms into the soil can not give expected good results. The reason is their small number, in relation to the microorganisms already inhabiting the soil. Native microorganisms relatively quickly inactivate the implanted microorganisms. Thus, if a dose of beneficial microbes causes the expected effect in limited circumstances (e.g. in the laboratory), it is impossible to achieve comparable results in natural conditions. This way of thinking can be seen to this day. It should be emphasized, that the majority of microorganisms present in every soil is harmless to plants, except of those very few, which act as plant pathogens or as potential pathogens. Harmful microorganisms can be dominant, if the prevailing conditions are optimal to their growth, activity and reproduction.

Under such conditions, harmful microorganisms multiply very quickly, making significant damages in the crop. If conditions change, the population of pathogens will decrease as quickly as it rose, returning to its average size.

The idea of vaccinating soil with beneficial microorganisms was often rejected by conservationists and proponents of alternative agriculture. They claim, that the number of beneficial microorganisms in the soil increases naturally. The effect of soil quality improvement can be achieved by providing the soil with organic improvers in the form of carbon-containing organic material and nutrients. This assumption may be correct mainly in small farms, where no organic waste is discharged to the industrial facilities, where it is utilized. In most cases, the large amounts of beneficial soil microorganisms were found in those places, where in the agricultural areas, the crops were kept in accordance with good agricultural practice and without the use of pesticides. This fact explains why scientists have been interested in vaccinating the soil with microorganisms in order to keep it in balance, improve soil quality and the amount and quality of the crops for so long. Most would agree with the statement, that the basic principle of agricultural practice is to grow crops adapted to climate and environment. In many cases, for the economical reasons, the soil is used on the border of its total sterilization. Presently, there are many experiments being carried out, aimed at enabling the crops to grow in the places, where it was not possible yet.

Effective Microorganisms (EM) is a naturally occurring biological vaccine, that does not include any genetically modified organisms in its composition, but only those, which exist in the natural environment worldwide. Microbiological EM preparations are used in agriculture, horticulture, fruit growing, animal husbandry, water and sewage treatment, soil, waste piles and landfill remediation, etc., and also in medicine and chemical industry. Effective microorganisms are very carefully selected and meet the strict criteria for the harmlessness to humans, animals and plants. The concept of effective microorganisms has been developed in the eighties of the twentieth century by a Japanese microbiologist Teruo Higa (1994). Currently it is believed, that properly selected composition of microorganisms has a beneficial impact on the environment. Effective microorganism's preparation is a specific mixture of genetically unchanged microorganisms, aerobic and anaerobic. Such microbial preparations consist mainly of:

- lactic acid bacteria,
- photosynthetic bacteria,
- actinomycetes,
- fermenting fungi,
- yeast.

Each of these groups of microorganisms plays a completely different role in biochemical processes.

Lactic acid bacteria are a strong sterilizer, and thanks to that they delay and inhibit the growth of harmful microorganisms (e.g. fungi of the genus *Fusarium sp.*). These bacteria produce lactic acid from sugars and other carbohydrates, produced in metabolic processes of photosynthetic bacteria and yeast. They support the fermentation of all kinds of organic matter, e.g. cellulose. The decomposition of this element occurs without the harmful effects that arise in the process of decay, giving plants more nutrients in an easily digestible form.

Photosynthetic bacteria are independent organisms. In their metabolism they use carbon dioxide from the atmosphere, sunlight and warmth of the soil. They participate in the synthesis of useful substances from the secretions of the root crops, organic matter and harmful gases such as hydrogen sulfide. After processing these products, they are formed into amino acids, nucleic acids, bioactive substances and sugars. These metabolites are directly absorbed by the plants' roots, acting as a base for the growth of other bacteria and fungi, at the same time.

In such conditions vesicular mycorrhiza VA develops well, which is essential for the proper run of biochemical processes in the soil. An example of its influence is the increase of solubility of phosphates in the soil, making easier the access to natural resources of phosphorus and other nutrients for plants. VA mycorrhiza may perfectly interact with the family of Azotobacter bacteria (binding nitrogen from the air) by increasing the natural ability of leguminous plants to bind nitrogen. The axis of their actions are photosynthetic bacteria, stimulating simultaneously the activity of other microorganisms. This phenomenon can be called coexistence and common development or the principle of synergy. Photosynthetic bacteria that out incomplete anaerobic photosynthesis, are the most useful carry microorganisms, because they have the ability to transform the soil by converting putrescent substances such as sulfuric acid (VI). This helps to achieve efficient use of organic material and ensures soil fertility. Photosynthesis consists of photolysis of water, with molecular oxygen as a by-product. Therefore, these microorganisms help plants in meeting their demand for oxygen, which is very important for their vital functions.

Unwanted substances, such as methane and sulfuric acid (VI) are formed when organic matter decomposes without oxygen. These substances are toxic, and can

significantly inhibit the activity of microbial nitrogen retention. But introducing synthetic microorganisms to the soil might prevent this phenomenon from happening. Then the nitrogen binding microorganisms, living in soil together with photosynthetic bacteria, can operate effectively even in the absence of oxygen. Photosynthetic bacteria can not only carry out photosynthesis, but also retain nitrogen. It was even determined, that their ability to bind nitrogen increases when they are present along with the bacteria of *Azotobacter* type.

The structure of **actinomycetes** (Actinomycetales), places them between bacteria and fungi. They produce substances from amino acids, secreted by photosynthetic bacteria and from organic matter. Thanks to their actions, nitrogen is better bounded by bacteria of the genus *Azotobacter*. They can perfectly coexist with photosynthetic bacteria. Both of these species improve the quality of the soil environment by increasing plant resistance to pathogens, e.g. soil pathogens.

Fermenting fungi, which include *Aspergillus* and *Penicillinum* types, cause rapid decomposition of organic matter which produces alcohol, esters and substances which reduce the number of harmful microbes. They also inhibit decay processes by reducing the amount of evolved gases and prevent the development of pests.

Yeasts synthesize antibiotic and useful substances from amino acids and sugars, which contributes to the reduction of the number of microorganisms, e.g. in food. These microorganisms produce also other useful substances favoring the growth of plants. They produce hormones and enzymes, which activate cell division. Yeast secretions are useful substrates for lactic acid bacteria and actinomycetes.

In relation to the soil, the preparation of effective microorganisms improves soil fertility and health, which is reflected in the increase of the enzymatic and biological activity of soil and causes beneficial changes in its chemical and physicochemical properties. Moreover, the effective microorganisms formulation accelerates decomposition of soil organic matter and makes nutrients from the hard soluble phosphate and potassium rocks more accessible for plants. With these changes the N:P:K and C:N ratio is maintained at a good level, the humus content in the soil increases. This ensures high quality of the produced food.

The use of EM-1 in agriculture, horticulture and fruit growing eliminates decay processes, enhances the effect of photosynthesis in plants, dissolves the minerals not available for plants, binds atmospheric nitrogen, increases the amount of humus improving the soil fertility and structure, raises the temperature of the soil and increases its water capacity, accelerates the conversion of organic matter, enables conventional tillage and direct seeding, has a beneficial effect on the process of recultivation of contaminated soils and accelerates germination, flowering and ripening of plants, increases the biological value and the cost-yield of the crops.

However, the use of effective microorganisms preparation in agricultural practice raises many controversies. The supporters of this preparation stress that it

significantly improves the plants' health and as the outcome increases the crop yield and improves its quality (Boligłowa, Gleń 2008, Górski, Kleiber 2010).

Moreover, there has been a positive influence of effective microorganisms on the rapid regeneration of arable soils, exposed to particularly harmful effects of various kinds of ksenobiotics. Addition of the effective microorganisms preparation to the soil contributes to its greater resistance to water stress, more rapid mineralization of organic carbon, increased release of nutrients, as well as more rapid decomposition of residues of chemical compounds (Woodwort 2003). Opponents of the effective microorganisms formulation point out, that the research took too short time and its coverage was local, which does not allow to obtain reliable results. In addition, the positive role of the effective microorganisms preparation in composting organic waste and wastewater treatment is being undermined. The positive impact of the preparation on the chemical and biochemical properties of soil and on the increase of crop yields and increased nutrient uptake also arises doubt (Bielińska *et al.* 2009, Vukobratovic *et al.* 2008, Condor *et al.* 2007, Jakubus *et al.* 2010).

3. Experimental conditions

The studies were undertaken to analyze the impact of the fluidal ashes, introduced to the light soil, on the number of microorganisms involved in the metabolism of protein and fat compounds and starch. The impact of the introduction of effective microorganisms preparation along with the fluidal ashes into the soil was also examined.

The field experiment was established by the Department of General Chemistry of The Agricultural University in Szczecin in 2007. The field for the experiment was located near Gorzów Wielkopolski in Małyszyn, on the soil of granulometric composition of a weakly clay sand with pH_{H20} 5.13, characteristic for acidic reaction. Due to the soil requirements of the tested plant species, dolomitic lime and fluidal ash were used to raise the soil's pH.

The experiment was conducted using the method of completely randomized blocks in four replications. The size of each field was 10 m². The experimental consisted of seven variants of fertilization (control, dolomitic lime, fluidal ash and fluidal ash combined with microbiological EM-1 preparation). Dolomitic lime (CaCO₃ · MgCO₃) and ash were used in two doses, 1.0 and 1.5 corresponding with the hydrolytic acidity of the soil, expressed in mmol H⁺ kg⁻¹ of soil. The doses of calcium fertilizer and fluidal ash were based on the content of calcium and magnesium in them. The high calcium fly ashes from CHP Żerań, classified as very active (with a content of CaO≥14%) according to the current Polish Norm PN-S-96035, were used in the experiment.

The applied microbiological EM-1 preparation contains in its composition a mixture of about 80 modified microorganisms such as lactic acid bacteria, photosynthetic bacteria, yeast, fungi and actinomycetes. The EM-1 preparation is produced in Poland by Greenland Company, the effective microorganisms technology is on the EMRO License – EM Research Organization, Japan. The effective microorganisms technology products were many times awarded with prizes, received certificates and were legally permitted for use in ecological farming. According to the manufacturer, the preparation has a positive effect on the decomposition of organic matter in the soil and causes faster access of nutrients to the plants. The cultivated plants, in the three-year rotation, starting from 2007, were: spring barley, beans and spring wheat.

Table 2

Number	Object
1	control-spring wheat
2	control-spring barley
3	control-beans
4	1 hydrolytic acidity of soil CaCO ₃ MgCO ₃ - spring wheat
5	1 hydrolytic acidity of soil CaCO ₃ MgCO ₃ - spring barley
6	1 hydrolytic acidity of soil CaCO ₃ MgCO ₃ - beans
7	1,5 hydrolytic acidity of soil CaCO ₃ MgCO ₃ - spring wheat
8	1.5 hydrolytic acidity of soil CaCO ₃ MgCO ₃ - spring barley
9	1,5 hydrolytic acidity of soil CaCO ₃ MgCO ₃ - beans
10	1 hydrolytic acidity of soil fluidal ash - spring wheat
11	1 hydrolytic acidity of soil fluidal ash - spring barley
12	1 hydrolytic acidity of soil fluidal ash – beans
13	1.5 hydrolytic acidity of soil fluidal ash - spring wheat
14	1.5 hydrolytic acidity of soil fluidal ash - spring barley
15	1.5 hydrolytic acidity of soil fluidal ash – beans
16	1 hydrolytic acidity of soil fluidal ash + microbial EM-1 preparation -spring wheat
17	1 hydrolytic acidity of soil fluidal ash + microbial EM-1 preparation -spring barley
18	1 hydrolytic acidity of soil fluidal ash + microbial EM-1 preparation - beans
19	1.5 hydrolytic acidity of soil fluidal ash + microbial EM-1 preparation - spring wheat
20	1.5 hydrolytic acidity of soil fluidal ash + microbial EM-1 preparation - spring barley
21	1.5 hydrolytic acidity of soil fluidal ash + microbial EM-1 preparation - beans

List of the reaserch objects

The samples for microbiological testing were taken from the topsoil layer of soil after harvesting the crops. Microbiological analysis was performed using a common method of plating soil dilutions on selective bases, appropriate for each group of microorganisms. The cultures were incubated in room temperature (20°C) for 3-14 days. The number of test organisms is presented in CFU (colony forming units) in 1 g of dry soil mass.

The results of the research of the number of groups of soil microorganisms were studied with a two-way analysis of variance. Afterwards the values of NIR_{0,05} were calculated with Tukey's test and homogeneous groups were formed at the significance level of P = 0.05. Calculations were performed using *Statistica* 9.

4. Effect of fluidal ashes on the number microorganisms in soil

Higa (2002) points out that controlling the soil microflora, in order to strengthen the advantage of beneficial and effective microorganisms can help to improve and sustain the chemical and physical properties of the soil. If the culture of beneficial microorganisms after seeding into the soil is to operate efficiently, its initial population must reach a certain critical level. If these conditions are not fulfilled, the microbial vaccination, despite of its beneficial character, will not only fail to bring the desired effect, but there might even be no effect at all.

The doses of the EM-1 preparation applied to the soil were optimal, because the results obtained in 2009, indicated a significant increase in the number of physiological groups of microorganisms in all experimental objects, in comparison with the first year of the study.

Table 3

Object (I)	Year							
		2007		2009				
	Plant (II)							
	wheat	beans	barley	wheat	beans	barley		
С	321518,4	340155,4	427843,5	614738	636161	825617		
1w	407543,9	517845,2	267271,6	789674	993884	496532		
1,5w	259390,7	391583,8	354200,1	470370	729761	661315		
1a	286611,3	430495,8	536689,2	543520	766994	986667		
1,5 a	260251,0	590129,9	491405,1	393731	1114929	646487		
1a+EM-1	442308,1	569788,3	469551,0	548293	802119	601644		
1,5a+EM-1	379826,2	488724,4	525870,3	652174	705706	708098		
LSD _{0,05} for I*II		i.d.		177064,0				

The numbers of proteolytic microorganisms in 1 g of dry mass of soil depending on the test object and cultivated plant

i.d. - insignificant difference

<u>Signs of the objects</u>: $c - control, 1w - 1 CaCO_3 MgCO_3, 1,5w - 1,5 CaCO_3 MgCO_3, 1 a - 1 fluidal ash, 1,5 a - 1,5 fluidal ash, 1a+EM-1 - 1 fluidal ash+microbiological EM-1 preparation, 1,5 a+EM-1 - 1,5 fluidal ash+ microbiological EM-1 preparation$

In case of proteolytic microorganisms, in 2007 there were no significant statistical differences for interactions (object x plant) and the main factors (Fig. 4, 5, Table 3). But in the last year of the studies statistical significance of the analyzed factors and their interactions, had the effect on the number of proteolytic microorganisms. The smallest number of these microorganisms was found in the object 1.5 w, and the highest number was marked in object 1w and 1a (Fig. 4).

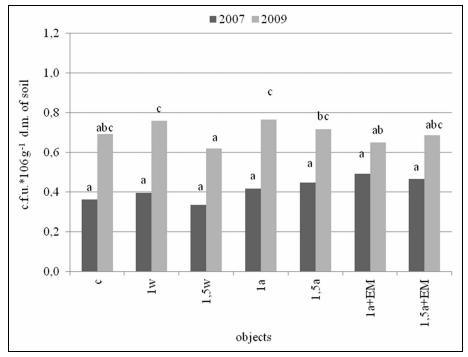


Fig. 4. The numbers of proteolytic microorganisms in the soil, depending on the analyzed object

In case of the cultivated plants, the highest number of proteolytic microorganisms was found in 2009, in the soil where beans were grown, and the smallest in the soil, where spring wheat was grown (Fig. 5).

In case of amylolytic microorganisms associated with the transformations of starch, in the first year of study (2007) statistically significant differences in the abundance of this group of microorganisms for the main factors and their interactions were found (Fig. 6, 7, Table 4).

The smallest number of these microorganisms was recorded in object 1, and the highest in object 1.5 a+EM-1 (Fig. 6).

Depending on the cultivated plant, the number of amylolytic microorganisms was the smallest in the soil under cultivation of wheat, then – barley. The highest number of these microorganisms was found in the soil under cultivation of beans (Fig. 7). In 2009, the smallest number of amylolytic microorganisms was found in soil in the object 1.5 a, and the largest in the soil of object 1 a. When it comes to the tested plant, the effect was the same as in 2007.

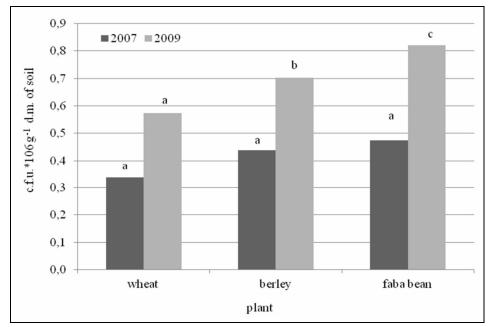


Fig. 5. The numbers of proteolytic microorganisms in the soil, depending on the species of the cultivated plants

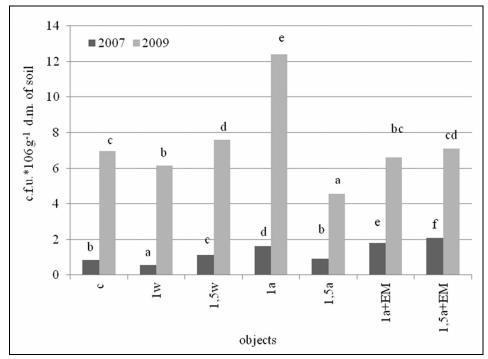
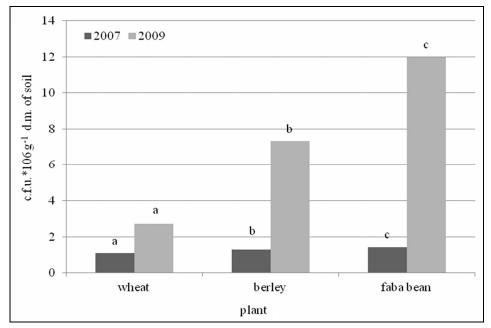


Fig. 6. The numbers of amylolytic microorganisms in the soil, depending on the analyzed object



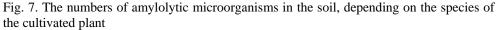


Table 4

The numbers of amylolytic microorganisms in 1 g of dry mass of soil, depending on the test object and cultivated plants

	Lear								
Object (I)		2007		2009					
	Plant (II)								
	wheat	beans	barley	wheat	Beans	Barley			
С	1256205	892593	372058	1529052	2069717	2280502			
1w	672748	279593	737768	2370370	2429765	2672776			
1,5w	1323417	1266886	752315	4782767	4857997	4976852			
1a	1272936	1786248	1794286	5720824	6129398	7193974			
1,5 a	1061286	766369	941620	7216495	8445946	8571429			
1a+EM-1	925076	2181025	2280502	9250765	11212467	11276758			
1,5a+EM-1	1183658	2815315	2266364	11621824	13244048	26354053			
LSD _{0,05} for I*II		188846,6		1349716,1					

i.d. - insignificant difference

<u>Signs of the objects</u>: c - control, 1w - 1 CaCO₃ MgCO₃, 1,5w - 1,5 CaCO₃ MgCO₃, 1 a - 1 fluidal ash, 1,5 a - 1,5 fluidal ash, 1a+EM-1 - 1 fluidal ash+microbiological EM-1 preparation, 1,5 a+EM-1 - 1,5 fluidal ash+microbiological EM-1 preparation

Like in the previously discussed groups, lipolytical microorganisms, digesting fatty compounds, increased their numbers in 2009, in comparison to their number in the first year of the study (Fig. 8). In 2007, statistically significant differences in the lipolytical microorganisms content were found for the main factors and their interactions (Fig. 8, 9, Table 5). The smallest number of these microorganisms in the soil was recorded in object 1 w, and the biggest one in objects 1 a and 1.5 a. In case of analyzed plants, the quantity of lipolytical microorganisms was the smallest in the soil under cultivation of beans, and the largest in the soil under cultivation of beans, and the largest number of lipolytical microorganisms was found in the soil from object 1.5 a, while the highest number of these microorganisms was found in the soil with a single dose of dolomitic lime. In the same year a different structure of abundance of these microorganisms for the tested plants was recorded. The smallest number of microorganisms was determined in the soil in which wheat and beans were grown, and the biggest, where barley was grown.

The obtained results of microbiological tests indicate, that the addition of dolomitic lime to the soil, caused no significant increase in the number of proteolytic and amylolytic microorganisms in relation to the amount of those organisms measured in the control soil. Only in case of lipolytic microorganisms, in 2009, there was a significant increase of their abundance in the soil fertilized with a single dose of dolomitic lime.

Table 5

Object (I)	Lear							
		2007		2009				
Object (1)	Plant (II)							
	wheat	beans	Barley	wheat	Beans	Barley		
С	20618,6	49629,6	21640,1	882016	598958	910494		
1w	14063,1	7988,4	28669,7	444191	997706	1818182		
1,5w	42334,1	36874,8	14274,7	677778	600532	860092		
1a	47018,3	15321,4	342857,1	703155	712468	910476		
1,5 a	336322,9	14136,9	66290,0	626911	733104	328849		
1a+EM-1	252293,6	10541,6	34207,5	911402	760499	728700		
1,5 a+EM-1	20236,7	277777,8	16269,4	629291	608108	821092		
LSD _{0,05} for I*II	25080,5 301874,9							

The numbers of lipolytic microorganisms in 1 g of dry mass of soil, depending on the test objects and cultivated plants

i.d. - insignificant difference

<u>Signs of the objects</u>: $c - control, 1w - 1 CaCO_3 MgCO_3, 1,5w - 1,5 CaCO_3 MgCO_3, 1 a - 1 fluidal ash, 1,5 a - 1,5 fluidal ash, 1a+EM-1 - 1 fluidal ash+microbiological EM-1 preparation, 1,5 a+EM-1 - 1,5 fluidal ash+ microbiological EM-1 preparation$

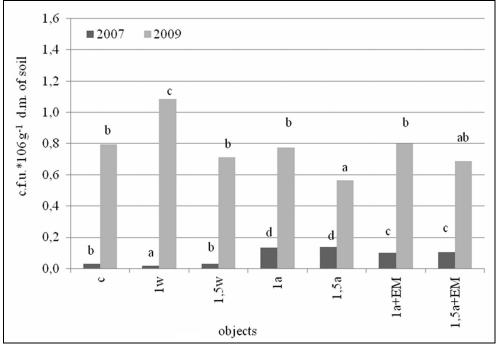


Fig. 8. The numbers of lipolytic microorganisms in the soil, depending on the analyzed object

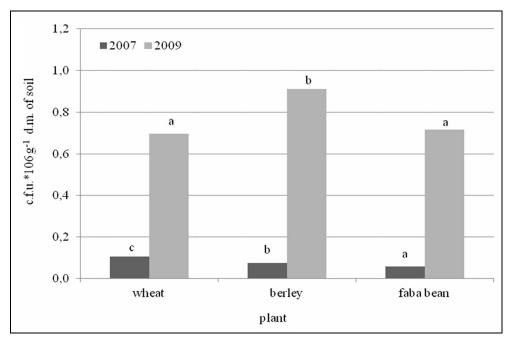


Fig. 9. The numbers of lipolytic microorganisms in the soil, depending on the plant species

As some authors (Bielińska *et al.* 2009a, Hajnos *et al.* 1998, Kurek 2002) point out, liming soil changes its physicochemical properties and may lead to the reduction of organic matter in the soil. Michalcewicz *et al.* (2008) emphasize, that the addition of dolomitic lime to the soil, especially at higher doses, causes an increase in biomass content of live microorganisms, increasing the number of bacteria and actinomycetes, which was associated with the deacidifying properties of lime. A similar effect for the soil can also be caused by fluidal ash.

Ashes may be an alternative to popular liming, without making harm to the most important metabolic processes that affect soil fertility (Bielińska et al. 2009). Significant increase in the number of studied microorganisms was recorded in the first year of study in the soil, which had the addition of fluidal ash with microbiological EM-1 preparation. The earlier reports by other authors, that the enrichment of the soil, containing fluidal ash, in the EM-1 microbial preparation, causes an increase in the content of microorganisms belonging to different taxonomic, physiological and biochemical units, were confirmed during the study. It has beneficial influence on the functioning of the soil ecosystem, as well as on the intensity of plant growth and development. The results of this study indicate that the addition of the formulation of Effective Microorganisms to the soil, especially in the first year of the experiment, increased the number of microbes, leading to the transformation of protein compounds, and of starch and fat, and hence could affect the intensification of metabolic processes and biochemical changes in the soil, as well as soil formating properties of microorganisms.

Enriching the soil in the fluidal ashes and in the microbiological preparation accelerated mineralization of soil organic compounds, and thus caused an increase in biological activity of the soil. Almost 10-fold increase in the number of all examined physiological groups of microorganisms was recorded, regardless of the type of object in the final year of research, which can be explained by the positive impact of cultivated plants. They secrete root exudates (that is a watery juice seeped from the root section), which may be an additional source of carbon, nitrogen and energy for heterotrophic microorganisms, contributing to their growth and development.

The obtained results of the addition of EM-1 preparation to the ash indicate no statistically significant differences in the number of lipolytic microorganisms. In case of the remaining physiological groups of microorganisms the differences were significant. The study was conducted over a three years period. Many researchers emphasizes, that it was a too short experimental period, which does not allow to obtain clear results.

In conclusion, it should be stated, that after the enrichment of soil in microbial EM-1 preparation, containing fluidal ash, in the following years of research, there has been an increase of the number of proteolytic microorganisms involved in hydrolysis of starch, in comparison with the first year. In the first year of the study, the number of microorganisms, metabolizing fatty compounds increased in the soil, which contained fluidal ash and microbiological preparation. However,

in 2009 the results were not as clear. Although, there was no clear impact of the microbiological EM-1 preparation on the number of microbes metabolizing carbon and nitrogen compounds in the soil. The study showed that the type of the cultivated plant had a significant impact on the number of proteolytic soil microorganisms.

On the base of the conducted experiments, it can be stated that adding the fluidal ashes to the soil had an influence on the increase of the content of basic physiological groups of microorganisms, associated with the transformations of carbon and nitrogen. This resulted in more intensive metabolic processes in the soil, causing an increase of its biological activity. So there is a possibility of using fluidal ashes from fluidized bed coal as a fertilizer. However, there was no significant effect of microbiological EM-1 preparation on the microbiological properties of the tested soil.

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