

SZENT ISTVÁN UNIVERSITY

Microbiological properties of sandy soils in the Nyírség region (Hungary), affected by organic and inorganic additives

PhD THESIS

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1. Background and aims

The soil fertility is developed by the contrary effects of pedogenesis and soil erosion taking place continuously in time and in space. This permanent change is determined by natural rules, but some factors affecting can be enhanced or masked by the human's activity.

To increase the soil fertility and to improve its characteristics are known to be especially important tasks of the mankind for a long while (SOLTI 2000). Soil fertility is particularly a key issue in the Nyírség soils, where the high sand content, the shallow soil depth, the acidic pH and the wind erosion can result especially weak status of the water and nutrient regime in the soils (SZABOLCS és VÁRALLYAY, 1978). The potential improvements of sandy soil characteristics have started and developed by two excellent researchers named Sándor Egerszegi and Vilmos Westsik. Regular treatments of the low quality sandy soils with organic materials were common in their suggestions, but the way how they did it was different (WESTSIK, 1951; EGERSZEGI, 1958, 1962). Changes in our lifestyle (industry, agriculture, consumption habits) so far has resulted the drastic changes in those materials, which are appropriate for the improvement in soil fertility, such as the different wastes, by-products, inorganic materials, etc. (BERENTE et al., 2010; MAKÁDI, 2004).

Changes in soil chemical properties could create an influence on the microbial community of soils, but also the opposite tendencies (e.g. at the N_2 -fixing bacteria) could be observed. Positive, but also the negative effects of the main soil-improving materials could be detected on the quantitative, qualitative and activity changes of soil microbes.

The effects of different amendments appropriate for improving of sandy soils and its physical, chemical and microbiological soil properties were studied in this PhD work. The economic value of these amendments can be characterized by the appropriate crop yields.

The aims of this work is to establish

- the effects of different organic and inorganic materials, qualified as wastes or byproducts in their origin, on the main chemical and microbiological soil properties
- the suitability of the used microbiological methods in the notification or in the monitoring of the short- and long-term alterations in the soil quality/fertility,
- how the measured physical, chemical and microbiological properties can determine the main soil-processes.

2. Materials and methods

The effects of three different materials, as potential soil-amendments on the low quality of sandy soils of the Nyírség region were studied in this work. The bentonite is a montmorillonite type of clay mineral. The main goal of its application was to improve the water regime of sandy soil but improving of some soil chemical parameters was also presumed. The effect of bentonite was studied in a small plot experiment (Table 1).

Bentonite experiment				
Place of the experiment	Research Institute of CAAES, University of Debrecen, Nyíregyháza			
Soil type	Arenosol			
Type of experiment	Small plot experiment (10 x 10 m) in four replications, after-effect study			
Material examined	Bentonite from Sajóbábony with 40 % montmorillonite content			
Treatments	0, 5, 10 15 and 20 t*ha ⁻¹ doses of 0-5 cm fraction of bentonite were ploughed in the top (0-25 cm) soil layer in March 2002			
Sampling,	From 2002 to 2006, from 0-25 cm soil layer.			
examinations	In spring: microbiological; in autumn: physical, chemical and microbiological examinations.			
Sampling	Composite sample from five point samples in every plot			
Test plants	 2002: buckwheat (Fagopyrum esculentum Moench), 2003: mustard (Sinapis alba L.), 2004: rye (Secale cereale L.), 2005: rye with hairy wetch (Secale cereale L. és Vicia villosa L.), 2006: rape (Brassica napus oleifera). Manual harvest in every plot from 4 x 1 m². After processing, measuring 			
	the yield and conversion it into t*ha ⁻¹ unit.			

	Table 1	. Characteris	stics of the	bentonite ex	periment
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The other two studied amendments have an organic origin. The digestate is mainly used as a plant nutrition source. Its effects on the chemical and microbiological properties of soil were studied in a field-pot experiment (Table 2).

Digestate experiment					
Place of the	In the area of the Regional Biogas Plant of Nyírbátor				
experiment					
Sources of	Samples from sandy and meadow soils				
samples					
Type of	Field-pot experiment (80 L) in four replications.				
experiment					
Material	Biogas digestate				
examined					
Treatments	Absolute control, digestate, water.				
	Determination of digestate quantity: according to the plant nitrogen				
	demand (BOCZ, 1992), on the basis of the measured actual total-N content				
	of digestate. Treatments with the calculated digestate quantity: 50 % at				
	sawing, 50 % at 4-6 leaves phase of test plants, every year.				
	Water treatment: at the same quantity as digestate.				
Sampling,	In 2006-2007, from 0-20 cm soil layer.				
examinations	In spring: microbiological; in autumn: physical, chemical and				
	microbiological examinations.				
Sampling	Composite sample of 30 sticks from every pot				
Test plants	Sweet corn (Zea mays conv. saccharata).				
	Measuring of corn ears in the pots $(g^* \text{corn ears}^{-1})$				

Table 2. Characteristics of the digestate experiment
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The composted sewage sludge is suitable for plant nutrition and improving of soil structure. Its effects were studied in a small plot experiment (Table 3).

Composted sewage sludge experiment					
Place of the	Research Institute of CAAES, University of Debrecen, Nyíregyháza				
experiment					
Soil type	Lamellic Arenosol (Dystric)				
Type of	Small plot experiment (12 x 19 m) in five replications.				
experiment					
Material	Composted sewage sludge (40 %(m/m) dewatered municipal sludge,				
examined	25 %(m/m) row, $5 %$ (m/m) bentonite, $30 %$ (m/m) rhyolite.				
	Amendments: in the autumn of 2003, 2006 and 2009, ploughed of the				
	compost into the 0-30 cm soil layer.				
Treatments	$0, 9, 18 \text{ and } 27 \text{ t*ha}^{-1} \text{ doses}$				
Sampling,	In 2002 to 2006, from 0-30 cm soil layer.				
examinations	In spring: microbiological; in autumn: physical, chemical and				
	microbiological examinations.				
Sampling	Composite sample from five point samples in every plot				
Test plants	Triticale (x Triticosecale Wittmack), maize (Zea mays L.) and green pea				
	(<i>Pisum sativum</i> L.), sown in a crop rotation.				
	Manual harvest in every plot from $4 \times 1 \text{ m}^2$. After processing, measuring				
	the yield and conversion it into $t^{+}ha^{-1}$ unit.				

Table 3. Characteristics of the composted sewage sludge experiment

The measured parameters of the experiments can be seen in Table 4-5.

Table 4. The measured soil p	physical and chemical	parameters in the	three experiments
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Measured parameter	Bentonite	Digestate	Compost
Soil moisture content	+	+	+
pH, y ₁ , K _A , macro- and mesoelements	+	+	+
Water soluable salt content (%)		+	+
Organic material content	+	+	+
CEC	+		+

		Measured parameter	Bentonite	Digestate	Compost
al		r-strategist bacteria	+	+	+
gic	S	K-strategist bacteria	+	+	+
iolo	lno	l-strategist bacteria	+	+	+
hysi	50	Free-living N ₂ -fixing bacteria	+	+	+
Р		Microscopic fungi	+	+	+
	les	Invertase activity	+	+	+
Soi	zyn	Catalase activity	+	+	+
•1	en:	Dehydrogenase activity		+	
Soi	1				
resp	pira-				
tion	ı	CO ₂ production	+		
Ide	ntifi	Identification of r-strategist bacteria by			
cati	on	API-teszt	+		

Table 5. The measured soil microbiological parameters in the three experiments

Treatment effects were analyzed by one-way and multivariate analyzes of variance followed by Tukey's test for examining significant differences among the averages. Differences among the years were studied by t-probe while correlation-, factor- and discriminant analyses were done to examine the relations among the chemical and biological parameters. Statistical analyses were done at 95% probability level.

3. Results and discussions

Further I present the effects of the three examined materials on the soil physical, chemical and microbiological properties in three subdivisions.

3.1. Discussion of the bentonite effects

The mainly expected effect of the used bentonite is to improve the water regime of soils was not supported by this single application. Two reasons of this result might be: the dry period in the time of bentonite application and – by the results of MCKISSOCK et al. (2002) – the too big fraction size of bentonite used. Nevertheless, the small increase in soil pH had been found in the 10 and 15 t*ha⁻¹ bentonite treated samples in the average of the five examined years.

Significant effects of used treatments on the microbial abundance could not been found in the field plot experiment. However, considerable annual effect was noticeable on the number of r- and K-strategist bacteria. In spring of 2004 the bacterium number was higher than in spring of 2005 and 2006, while in the samples collected in autumn the changes observed were the opposite. Regarding, that those changes had been measured at all the treatments, they could not been caused by the bentonite. The multiplication and annual changes of bacterial numbers are directly determined by the precipitation and the soil temperatures.

The highest number of K-strategist bacteria had been found in the control samples which is in accordance with the observation of KOZDRÓJ (1995), who found the advantage of this microbial group in disadvantageous conditions because they slower multiplication rate needs less sources. There was no any effect of treatments found in the number of l-strategist and free-living, N₂-fixing bacteria and microscopic fungi in the spring samples. In the autumn samples, however a weak trend of treatments had been found. Measuring the abundance of r-and K-strategist bacteria seems to be more appropriate for the detection of soil processes in comparison with the other three studied bacterial groups.

In spite of the fact that no any significant effect was found in the culturable microbial abundance and in the enzymes activities, considerable differences could be recorded in the results of soil respiration and in the identification of r-strategist bacteria in control and in 10 $t^{*}ha^{-1}$ bentonite-treated samples in September, 2005 (Fig. 1). In the studied segment of bacterium community living in soil, bentonite treatment has increased the diversity of

culturable bacteria, which can show a more diverse metabolic pathway and hereby a better accommodation capacity of microbes for the changing environmental conditions.



Figure 1. CO_2 emission of bentonite treated (10 t*ha⁻¹) soil for measuring the intensity of soil respiration in September, 2005. *: significant difference from control (t-probe, p<0.05).

The intensity of soil respiration and the number of bacteria in the soil have daily fluctuation (SZABÓ, 2008). Whereby according to my results and the cited other works, the bentonite treatment and above all the 10 t*ha⁻¹ bentonite doses are able to modify the microbilogical and other biological properties of soils. Despite of the lack of significant effect on soil chemical properties, the diversity of bacteria in the studied segment of cultured bacterial community and also the soil respiration had been changed by the bentonite treatment. This fact verifies that the soil biological parameters often can respond more sensitively and faster than the soil chemical or physical parameters (DICK ÉS TABATABAI, 1992). Examining of the qualitative culturable bacterial abundance and the soil respiration can become more sensitive parameter than the quantitative culturable numbers and the measured enzyme activities. These methods can indicate significant differences between the control and the 10 t*ha⁻¹ bentonite treatments even three years after the bentonite treatment.

3.2. Discussion of the effect of digestate

Among the main effects of used digestate, it is the soil pH, which can be mentioned Despite of the slightly alkaline pH of the digestate, only a small decrease of soil pH had been measured after its usage. The decrease is assumed by the high ammonium content of digestate. The digestate is a suitable matter for the nutrient supply, due to the high quantity of the total-N, the available P and K content. The strongest effect of digestate was found on the available P content of soil.

The activities of invertase were found to be increased after the digestate treatments in both soil substances while the dehydrogenase activity decreased. The catalase activity also increased in sandy soil substance, while decreased in the meadow soil substance. The numbers of colony forming units of r-, K- and l-strategist bacteria were increased after the treatments, as well. The colony forming units of microscopic fungi decreased in 2006 and increased in 2007, in comparison with the controls.

There was a discriminant analysis used to find out the main affecting processes in the soils after the digestate treatments. Data were grouped to the following discriminate functions: <u>Function 1</u> explains 93.8 % of variations, as **pH and puffer capacity** of the soils and contains the pH, CaCO₃% and Mg. <u>Function 2</u> contains the **macroelements** (C, N, P) and the **Na**. The **catalase activity and NO₂-N** belonged to the <u>Function 3</u>, while the **salts and soil moisture** to the <u>Function 4</u>. The **microbes and the processes linking to their vital functions** can be found only in the <u>Function 5</u>. This result means that the soil microbes have only small contribution to the discrimination of soils and treatments. Results according to Function 1 and 2 can be shown in Figure 2.

The two soil substances are separated along the Function 1 while the treatments within the soils substances are separated by the Function 2, as the macronutrients and the sodium concentration. On the sandy soil substance, which contains low organic and mineral colloids, the chemical and microbiological properties had been changed by a simple irrigation (water treatment). This is indicated by the separation of H10-water and H7-digestate treatments from the H1-control one. The effect of digestate – as it was expected – was stronger than the effect of water treatment therefore its group centroid can be discriminated from the control's group centroid.

In meadow soil substance no significant effect was found of the applied doses of water treatments, the group centroids of control (R1) and water (R10) treatments could not be separated from each other. The probable reason of this result is the generally higher moisture content of meadow soils. At the same time, the high nutrient content and own microbial community of digestate has a strong effect, its group centroid therefore, is being separated from the control ones.



Canonical Discriminant Functions

Figure 2. Effect of digestate on sand and meadow soils substances assessed by discriminant analyzes, *H1: sand, control, H7: sand, digestate, H10: sand, water, R1: meadow, control, R7:meadow, digestate, R10: meadow, water.*

The results of discriminant analysis show, that the processes in soils are primarily determined by the level of soil pH, while the other parameters are able to modify these processes. Except of the invertase activity, the studied microbiological properties can contribute to these changes but only in a small portion. Most of all, these properties only can follow the soil chemical processes, the final results of the microbiological changes are influenced by the environmental conditions. But the conformity to the chemical processes results the presence of different metabolic pathways which contribute to the continuous changes of soils and the results of different treatments. The microbes are able to adapt to the environmental conditions quickly. The underground variability and diversity therefore are always higher than the aboveground diversity of plants.

3.3. Discussion of the sewage sludge compost treatments

Two-factorial analyses of variance were used in this experiment with the following factors: 1) doses of compost treatment and 2) test plants. The soil chemical properties were affected statistically only by the test plants in 2007. These properties were the total-N, NH₃-N and K₂O and soluble salt content of soil. In this year there were no any significant effect found of the compost, applied. However in 2008 significant effect of compost doses on the soil pH, hydrolytic acidity, the soluble salt, phosphorus, sodium and magnesia content and cation exchange capacity of soil were found. Test plants had significant effect on the NO₃-N, potassium content and cation exchange capacity also in this year. In cases where only the test plants had significant effect, the measured values within the plant species increased or decreased according to the applied compost doses, e.g. the organic material and potassium content increased while the hydrolytic acidity decreased.

Similarly to the chemical properties, the changes in measured microbiological parameters did not show significant compost effect, so the measured microbiological parameters were not better sensitive than the chemical ones. Nevertheless, the Colony Forming Unit (CFU) of r-strategist bacteria and the catalase activity were shown an increase with the increasing doses of the applied compost. In 2008 the CFU of K-strategist bacteria and the catalase activity was determined by the compost doses and the other measured microbiological parameters had also been increased with the increasing compost rates.

Changes in the soils are mainly determined by complex factors of the soil properties, less than by the bilateral interrelations between the single parameters. To study the groups of measured parameters (the factors), which determines the changes in soils, the factor analyzes could be a possible method. Results of this analysis showed, that the changes in soil processes are mainly influenced by the **pH** (Factor 1) in the 28.5% of variance. Factor 2 is the **soil moisture and the energy-gain processes**. Its explanatory rate is 13.7%. The **salt content and the organic material** belong to the Factor 3, with 10.6% explanatory rate of variances. The Factor 4 represents **bacteria**, the explanatory rate of variances is 7.1%. The Factor 5 is the group of **microscopic fungi** with 4.9% explanatory rate of variances. This is the factor of the **intensity of general microbes' metabolism** (Table 6).

Table 6. Rotated component matrix after analyzing the effect of sewage sludge compost treatment with factor analyzes.

	Component					
	1	2	3	4	5	6
pH H ₂ O	0,902			0,247		0,119
pH KCl	0,908	0,200		0,235		0,112
y ₁	-0,815	-0,320		-0,188	-0,119	-0,153
K _A	0,165	0,514	0,115	-0,114	0,458	
water soluable salt %	0,292	0,228	0,668			0,204
CaCO ₃ %	0,761	0,366				-0,192
humus %			0,714	0,168	0,179	
total-N %		0,891				0,114
NO ₃ -N mg*kg ⁻¹			0,735	0,126		0,106
$P_2O_5 mg^*kg^{-1}$	0,635	-0,156	0,504		0,114	0,165
$K_2O mg^*kg^{-1}$		0,124	0,497	0,143	0,486	-0,489
Na mg*kg ⁻¹	0,592	-0,170	0,561		-0,104	
Mg mg*kg ⁻¹	0,108	0,514	0,289	-0,221		0,472
CEC mgeé*100 g ⁻¹ soil	0,268	0,233	0,728		-0,100	-0,257
r-strategists lg CFU	0,322	0,110		0,733		-0,113
K-strategists lg CFU	0,392			0,675		0,190
l-strategists lg CFU	-0,115		0,177	0,546	0,181	
free-living N2-fixing lg CFU	0,142	0,181	0,170	0,515		0,369
mivroscopic fungi lg CFU					0,879	0,131
soil moisture (bacteria)		0,893	0,121		0,142	
invertase activity		0,777		0,140		0,177
soil moisture (invertase)	0,184	0,837				
catalase activity	0,209	0,316		0,151	0,182	0,646

Extraction Method: Principal Component Analysis.

a

Rotation Method: Varimax with Kaiser Normalization.

Rotation converged in 6 iterations.

Thus the changes of soil processes are mainly explained by certain soil chemical parameters, but the activity of certain microbial groups, the intensity of biochemical processes (enzymes activities) are also influenced significantly by the soil processes or rather the activity of those microorganisms can make a contribution to these processes directly and indirectly. I suppose that the more important role of microbes in soils can not been demonstrated, because they are altering components of soils but they are changing permanently along the environmental conditions. The effects of different microbial groups on the soil chemical properties are not uniform. The continuous capability of soils for changes, which are insured by the microbes, can be the bases of the soil fertility and the crop production.

4. New scientific results

- I established that the favourable effect of bentonite as an inorganic amendment was shown in the soils, determined mainly by its moisture content. A single application of bentonite in the field was found to improve the bacterial diversity far after a three-year of bentonite treatments. The bentonite use could increase the number of those bacteria, which are potentially able to produce the plant growth promoting materials. This fact can promote the plant nutrition indirectly.
- 2. I proved that the biogas digestate and composted sewage sludge as organic amendments, was found to contribute to the soil fertility and plant nutrition also by direct and indirect effects. Primary role of soil pH in acidic sandy soils was first demonstrated with the help of the discriminant and factor analysis, furthermore the effects of host plants on soil properties were also proven.
- 3. Among the studied microbiological properties the activities of invertase and catalase enzymes are in positive correlation with the soil moisture content, the pH and also with the available macroelements. The invertase enzyme has an important role in soil processes, however its activity can show a serious fluctuation seasonally in comparison with the catalase activity. The adequacy of invertase enzyme as a potential indicator of soil-fertility can be diminished by this fluctuation.
- 4. I established that the correlations among the physico-chemical and microbiological parameters are mainly determined by the chemical properties of the soils developing through the amendments. The results of multivariate statistical analysis could show, that soil microbiological properties affected directly and indirectly by the amendments, and crops studied can modify the soil processes. Microbiological effects can become as a secondary outgrowth of the environmental (primarily the pH) conditions.

5. Conclusions and suggestions

The effects of organic and inorganic materials appropriate for improving of the physical, chemical and microbiological properties of the soils in the Nyírség region of Hungary were studied in this PhD work. Besides the soil properties, the crop yields of the test plants were also measured to find out the economical impact of the above materials. Among the amendments used for nutrient supply and/or improving soil fertility, there is an increasing role of different wastes and by-products that do not contain, or contains only low concentrations of toxic components. These materials can be beneficial for soil quality and fertility, on the bases of their organic and inorganic colloid and nutrient contents. In this manner, waste materials can be re-used and re-utilized while increasing the soil fertility at the same time.

In the light of the above, the effects of inorganic bentonite and organic biogas digestate and also the composted sewage sludge products were studied in field experiments on some soil physical, chemical and microbiological parameters and crop yields.

According to the results obtained, the 10-15 t ha⁻¹ doses of bentonite are the most suitable for field application. Rainfall and irrigation enhanced the positive effects of bentonite, therefore its application is recommended mainly on irrigated fields. Similarly, positive effects of bentonite can be expected in other irrigated crops such as vegetable and fruit growing, establishing other woody plants cultures, in composting process as additive. A single application of bentonite in the field could improve the bacterial diversity even after three years of the treatments. The bentonite use increased the number of those bacteria which are potentially able to produce plant growth promoting materials. This fact can promote plant nutrition indirectly. Examination the qualitative property of culturable bacteria and measuring the soil respiration proved to be more sensitive parameters than the quantitative culturable parameters and the enzyme activities. Qualitative properties of culturable bacteria and measuring soil respiration statistically differed at the 0 and 10 t ha⁻¹ bentonite treatments even after three years of the treatments. Further examinations should be needed, however for the regular application of bentonite to determine the optimum doses on the bases of the actual plant and soil conditions.

Digestate can be an appropriate water- and nutrient source (PFUNDTNER, (2002); SIEBERT et al., 2008; ELSINGA, 2008), plants are able to utilize it and this material highly stimulates microbiological activities of the soil. The effect of digestate is greater on sandy soils due to the lower nutrient contents of those soils. Small-scale changes can cause relatively more remarkable effects on sand. The good regulation of amendments utilization guarantees the

protection of soil quality but does not hamper the farmers' work. This approach have to been adopt also in our country because quantity of digestate will increases by the increasing number of biogas plants in the near future (SOMOSNÉ ÉS SZOLNOKY, 2009).

The composted sewage sludge could increase the soil-pH, the organic material contents and nutrients of the sandy soils, by supporting highly its applications. This amendment increased also the quantity of culturable microbes, the invertase and catalase activities of the samples, treated. The application of good quality sewage sludge compost is necessary to the producers and also to the users (farmers). Replacement of decreasing organic material content of soils with sewage sludge compost seems to be an obvious solution (KÁDÁR et al., 2009). The good quality compost could be safely utilize for ages as it is presented in the reports of domestic and foreign experiments.

Examination of the relations among the variables confirmed the primary role of pH in the studied acidic sandy soils, but the effects of plants on soil processes have been proved. Among the studied microbiological parameters, the invertase and catalase activities are in positive correlations with the moisture level, the pH and the macronutrient content of soil. The invertase enzyme has important role in the soil processes but it has notable seasonal variation. In contrast, the soil catabolic activity determined as catalase enzyme has small seasonal variability. In such a way the use of catalase assays seems to be more appropriate in the further studies. The results of multivariate statistical analysis showed, that soil microbiological properties can modify the soil processes but – especially in a short-term period – they have not primary role in them.

In spite of this, it is worth to study the soil microbiological properties for examining new soil improver or nutrient supplier materials in the experimental phase, because these results can contribute to the development new products. Beyond this, the biological changes can faster indicate the effects and consequences of applications in the soil health. After any treatments of soils several (especially microbiological) properties can change as a consequence, which are having key-importance. Safe plant production and a better environmental safety can be developed by the knowledge of those effects. Effect of bentonite on the diversity of soil bacteria underlines the needs of more increased respect of soil microbiological aspects in consideration crop production and/or soil quality.

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7. Publications

Journal articles

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Vágó I., **Makádi M.**, Kátai J., Balláné Kovács A. (2008): A biogáz gyártás melléktermékének hatása a talaj néhány kémiai tulajdonságára. *Talajvédelem*, p. 555-560. (*ISSN 1216-9560*)

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Makádi M., Tomócsik A., Lengyel J. Bogdányi Zs, Márton Á (2007): Application of a digestate as a nutrient source and its effect on some selected crops and soil properties. Joint Int. Conf. on Long-term Experiments, Agricultural Research and Natural Resources. Debrecen-Nyírlugos, 31. May-1.June, pp. 102-107. (*ISBN 978-963-473-054-5*)

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