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ECOLOGICAL MONITORING OF MICROBIOLOGICAL, BIOCHEMICAL CHARACTERIZATION AND GROWTH OF *SECALE CEREALE* L. PLANT IN SOILS AMENDED WITH SEWAGE SLUDGE

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Abstract: The objectives of the model pot experiment were to determine the effects of sewage sludge application on biological (Different microbial and activities of enzymatic properties, carbon dioxide production) and physicochemical (pH, soil moisture) properties as well as rye plant productivity for 9 weeks were studied. Two soil types [kovárvány brown forest soil (Nyíregyháza), and meadow chernozem soil (Szeged)] were used and treated with various rates of sewage sludge [Control soil (0), 20, 40, 60, 100 (sludge) %] originated from Nyíregyháza sewage water purification.. Results indicated that the soil retained its moisture content for a longer period than the free wastewater sludge control soil. Also, soil pH was maintained to be favourable for plant growth more than the control soil. In addition to exhibiting healthy growth and development, the plants also produced the greatest dry matter mass on soils with the largest proportion of sewage sludge (60–100%). Also, enzyme activities in the soil samples treated with sewage sludge were increased in soil with higher sludge doses. There was an increase in the density of the microbial population in the rye rhizosphere as the sludge dose increased. Results demonstrated that Gram negative bacteria were dominant in the rye plant rhizosphere and the ratio between Gram-negative and Gram-positive bacteria in the Kovárvány brown forest soil and meadow chernozem soil treated with Nyíregyháza sludges were 2.367 and 2.35, respectively. Finally, soil treatment with the sludge stimulated rye plant growth, the biochemical and microbial properties of the rye plant rhizosphere. For maintaining the soil quality, the authors recommend to treat the acidic soil with a ratio of 40–60% of this sludge to improve the fertility of the soil.

Keywords: monitoring, rhizosphere properties, microbiological, biochemical, rye plant growth, sewage sludge

INTRODUCTION

Monitoring of soil properties, microbiological and biochemical parameters is one of the most essential characterizations to qualify soil health when amended with sewage sludge. The biological activities of the soils are determined by several microbiological and soil enzyme activities together. Demographic growth and western economic model seriously menace these vital resources: soil scarcity, soil losses through erosion, natural disasters and contamination; water scarcity and contamination; and limitation and exhaustion of mineral resources and fossil energies.

Today, one of the most pressing environmental and environmental problems of is the increasing volume of waste, including wastewater amendment, sewage sludge application, utilization and disposal.

Sewage sludge is used as possible way of organic fertilization and importance because it is not only nutrient input into the soil, but it improves soil structure and induces useful microbiological processes. Sewage sludge is environmentally polluting if the concentrations of heavy metals level is high. And, on the other hand, is suitable for farming as organic fertilizer. From an environmental and soil protection point of view, it is important that the sewage sludge used does not restrict the production of safe food raw materials.

The organic matter of sewage sludge is decomposed by heterotrophic nutrition prokaryotes and fungi. Biodegradation of organic matter in soil is basically

the result of microbial and biochemical processes, therefore all factors that have an effect on the structure, function and enzymatic activity of microorganisms impact the rate of degradation. In the case of agricultural and forestry utilization it contributes to increasing the content of organic matter of the soil and has a favorable influence on the physical and chemical properties of the soils. Sewage sludge increases the water absorbing ability of soils, promote aggregation of the medium on sandy soils and increase the cation exchange ability. As a result of sewage sludge application, the amount of microbotas in the soil usually increases. The nutrient flow for the soil is determined by the activity of enzymes, in addition to the physical, chemical parameters, plant cover and microbial activity.

Generally, most growth parameters, as well as the biomass of treated wheat, were significantly increased with the amendment of SS, up to the addition rate of 40 g kg⁻¹. The content of all heavy metals (except Cr in grains and Pb in spikes) significantly increased in different tissues of treated wheat with the increasing rate of SS application [1].

Reutilization of putrescible municipal solid wastes in agriculture can provide valuable plant nutrients. However, it may pose serious non-carcinogenic health risks for a human when contaminants, especially the heavy metals in MSW, end up in plants through the waste-soil-plant continuum [2]. Results indicated that for the most oxidative processes, the

released organic matter was probably mineralized by the hydroxyl radicals produced during the treatments. It is interesting to remark that even if the biochemical methane potential was barely enhanced by the different methods applied, all the methods demonstrated to enhance the overall kinetics of the biomethanation processes, increasing the rapidly biodegradable fraction of the sludge.

During the present work, author investigated the effects of sewage sludge on the some soil properties such as soil pH, moisture content, CO₂-production, FDA activity and the rye plant growth at different levels of sewage sludge treated in two soil types [3]. Results showed that MSWC doses over 10 t ha⁻¹ may create a heavy metal risk in long term for soils with pH ≥ 7. Therefore, in MSWC use over agricultural lands, heavy metal contents should always be taken into consideration and excessive uses should be avoided [4].

From the literature covered it can be concluded that sludge deposition induces two detrimental effects on the environment:

- 1) raising of the levels of persistent toxins in soil, vegetation and wild life and
- 2) slow and long-termed biodiversity-reduction through the fertilizing nutrient pollution operating on the vegetation.

Since recent studies show that eutrophication of the environment is a major threat to global biodiversity supplying additional nutrients through sludge-based fertilization seems imprudent. Toxins that accumulate in the vegetation are transferred to feeding herbivores and their predators, resulting in a reduced long-term survival chance of exposed species. We briefly review current legislation for sludge deposition and suggest alternative routes to handling this difficult class of waste [5]. Although guidelines limit the addition of toxic elements in sludges and soils, thus reducing the quantities of these elements accumulated by plants, total concentrations of toxic elements in soil provide no indication of their availability to plants.

The procedures applied to the determination of the forms of elements in sludges and soils and attempts to relate forms extractable in a variety of reagents to their availability to plants have been discussed. The factors which influence the forms of nutrient and toxic elements, their long term availability and hence their accumulation by crops are also reviewed [6]. It appeared that improving the structure to enhance the contact efficiency between the wastewater and the soil in soil mixture blocks was important for enhancing treatment performances.

The combined use of existing wastewater treatment systems with the MSL system was effective for preventing environmental pollution over a long period [7].

MATERIAL AND METHODS

The origin of soil samples were: Meadow chernozem soil (RCST) of the Szeged and Kovárvány brown forest soil (KBET) was from the Center for Agricultural and Technical Sciences at the Nyíregyháza Research Institute of the University of Debrecen.

Soil samples were collected from the upper layer of 0-25 cm. Some chemical properties of communal sewage sludge from the municipal wastewater treatment plants (Nyíregyháza) and soil samples are presented in Table 1.

The air dry soil was thoroughly mixed with sewage sludge so that the final mixture contained sewage sludge in the soil sample was as following percentages: 0% (sewage-free control soil), 20, 40, 60 and 100% (sewage sludge only, without soil). Rye (*Secale cereale* L.) seeds were sterilized [8] and planted in plastic containers of 3 kg of tested soil as prepared above. After ten days of germinating, young plants were reduced for 10 plant densities/pot.

Soils pH was measured by Pérez De Mora et al. [9] at various sewage sludge doses (after 63 days of incubation). The pH of the untreated and treated soil was tested in a 1: 2.5 (soil: 1 mole KCl) g ml⁻¹ ratio after shaking for 60 minutes. The moisture content of the treated and untreated soil samples was modified by the method of Brzezinska et al. [10] (measured at 48 hours at 28°C, incubation). Initial soil moisture was of the soil samples was 60%. The relative dry weight of the rye plants samples was determined after 9 weeks of cultivation (with a constant moisture content of about 60%) (at 75°C, drying cabinet, to constant weight).

Table 1. Properties of the soils and sludge used in the model experiment

Parameters	Soil types		Wastewater sludge: NySzv
	KBET	RCST	
pH _(KCl)	5.78	6.02	6.71
Dry matter content, %	na	na	53
Organic matter, %	na	na	21.7
Humus content, %	2.54	3.55	na
Total-N, mg·kg ⁻¹	na	na	7470
NO ₃ -N, mg·kg ⁻¹	23	39	na
NH ₄ -N, mg·kg ⁻¹	5.6	4.5	na
Mg, mg·kg ⁻¹	214	257	2507
Na, mg·kg ⁻¹	64	53	994
P ₂ O ₅ , mg·kg ⁻¹	318	378	28720
K ₂ O, mg·kg ⁻¹	412	428	3171
Zn, mg·kg ⁻¹	1.7	1.1	537
Cu, mg·kg ⁻¹	1.4	2.4	110.4
Mn, mg·kg ⁻¹	55	61	421
Fe, mg·kg ⁻¹	945	1094	11308
Cd, mg·kg ⁻¹	1.7	1.02	2.3
Pb, mg·kg ⁻¹	1.3	0.96	66.9

na: no data available

Determination of CO₂ production: For the measurement of CO₂ emissions, 0.5 kg of sewage

sludge treated soil was poured into 2 l glass containers and in the middle of the soil were placed a plastic tube containing 50 mL of 1.0 mol of NaOH solution to bind the developing CO₂, then the containers are tightly closed. The NaOH solution was titrated with 1 mol of HCl solution and calculated the volume of CO₂ released during the soil respiration [11, 12].

— **Characterization of soil microorganisms:**

The total plate count of aerobic bacteria, aerobic endospore-forming bacteria, filamentous fungi, yeasts, cellulose decomposers and phosphate-solubilizers in the rye rhizosphere was determined by means of a soil suspension. The roots separated from the plants were washed in sterile tap water to remove sticky soil particles followed by washing with a sterile 0.85% NaCl solution again. 10 g of the washed roots were cut and placed in 90 ml of sterile saline. A suspension of sterile tap water was prepared from the suspension. The total numbers of colony forming units (CFU) of culturable microorganisms were determined by serial dilution and plating on selective media. Plate counts of culturally viable bacteria and endospore-forming bacteria were made on Tryptone Soya Agar (TSA; Oxoid, Basingstone, Hampshire, England) amended with 0.1 g/l cyclohexamide. For fungi the Martin's medium for fungi [13] was Rose Bengal Agar (RB; Oxoid) amended with 30 mg/l streptomycin sulphate. Yeasts were cultivated on Malt Extract Agar, actinobacteria were counted on Glycerol Casein Agar [14, 15] amended with 0.05 g/l cyclohexamide. Examination of phosphate solubilisation was done in the medium described by Goldstein [16] for the selection of phosphate solvents. Dicalcium phosphate agar plates were inoculated, so that pure ring-producing strains around their cells are phosphate-free. Cellulose agar plates were seeded using two types of media (PDA: fungi and Nutrient agar: bacteria), which included the carboxymethylcellulose Congo red (CMC-Congo red) substrate as determined by Hendricks et al. [17].

All plates were inoculated with 0.1 ml of soil suspension and cultured at 25°C for 4 to 7 days for fungi, 30°C for 2 days for heterotrophic and endospore-forming bacteria and for 10 days for actinobacteria. Isolation and classification of microorganisms were done according to their morphological characteristics (colour, shape, appearance, cell size). Cultivable aerobic heterotrophic bacterial isolates belonging to different genes were studied by colony and cell morphology, Gram staining, spore staining, oxidase and catalase reactions, oxidation and fermentation of glucose, and motion and pigmentation.

— **Enzymatic Activity**

Dehydrogenase activity ($\mu\text{g INTF/g}^{-1}$ dry soil) was measured according to García et al. [18]. The catalase

activity ($\mu\text{mol O}_2/\text{min}^{-1}/\text{g}^{-1}$ dry soil) was measured by potassium permanganate O₂ consumption following the addition of H₂O₂ [19]. Urease and protease activity ($\mu\text{mol NH}_4^+\text{-N/g}^{-1}$ dry soil/h⁻¹) according to Nannipieri et al. [20]; phosphatase activity ($\mu\text{mol p-nitrophenol (PNP)/g}^{-1}$ dry soil/h⁻¹) regard to the method of Tabatabai and Bremner [21]; β -glucosidase activity ($\mu\text{mol p-nitrophenol/g}^{-1}$ dry soil/h⁻¹) was determined by the method described by Masciandaro et al. [22]. Invertase activity was measured by Siegenthaler [23] using p-nitrophenyl α -D-glucopyranoside (Fluka, Buchs, Switzerland). After adding a solution containing p-nitrophenol, tris buffer (pH 9.5), it is converted to nitrophenolate anion which can be measured by a spectrophotometer due to the pH effect. The extinction value at 400 nm is multiplied by 21.64 in an invertase number. The aryl sulfatase activity ($\mu\text{mol nitrophenol g}^{-1}$ dry soil/h⁻¹) was determined according to Tabatabai and Bremner [21] (absorption of p-phenol at 400 nm after incubation with PNP sulphate).

RESULTS AND DISCUSSION

The results of the present studies showed an increase in the observed parameters (pH and moisture content) after treatment of soil samples with sewage sludge. For acid soils, the pH value (Figure 1) was increased in sewage treated samples and the moisture content remained longer than in the control (Figure 2).

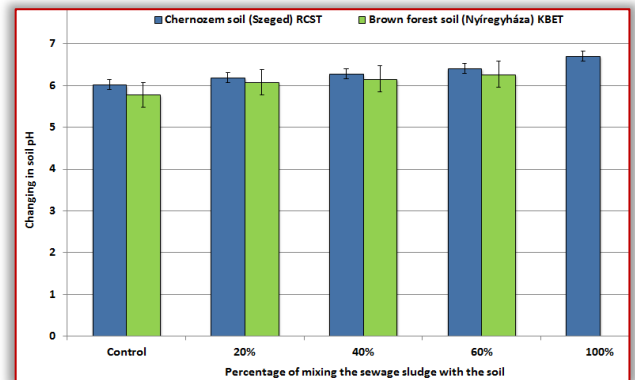


Figure 1. Impacts of sewage sludge on pH of the two soil samples

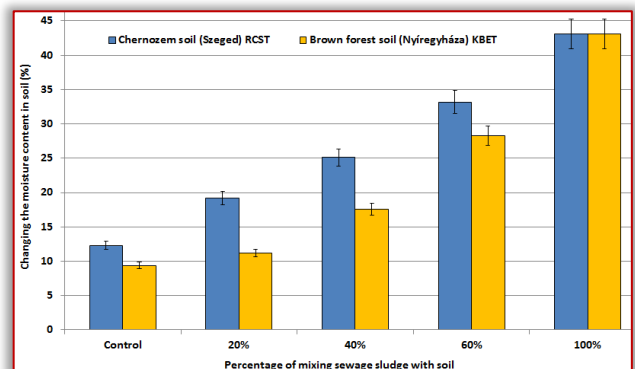


Figure 2. Impacts of sewage sludge on moisture content of the two soil samples

The addition of sewage sludge to soil significantly increased rye plant dry matter content (Figure 3) for each soil sample. Growth and development of plants were faster and healthier, in particular the clay abrasion of 60% sewage sludge in the case of brown forest soil and chernozem soil treated with sewage sludge from Nyíregyháza.

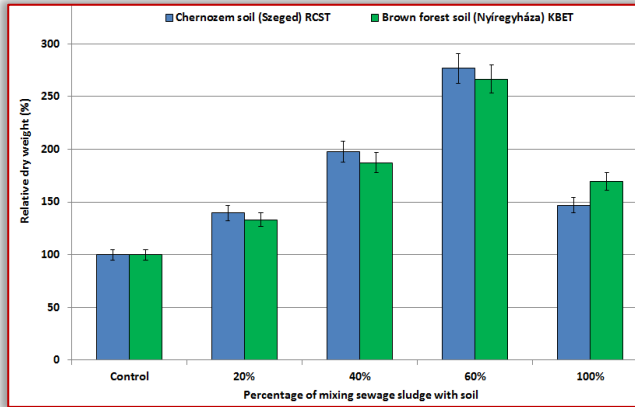


Figure 3. Effect of sewage sludge on rye plant dry weight in two soil samples

The total biomass mass of the plants increased proportionally with the increase in the amount of sewage sludge added to soil. Growth and nutritional needs were uniform on the basis of morphological characteristics during the vegetation period. Adverse symptoms were not observed on either control plants or plants derived from sewage sludge treated soil. The morphological characters of all plants (leaves, shape, colour and size) were normal and healthy. The maximum rye plants dry matter mass was observed with a mixture of sewage sludge 60: 40%. No significant statistical difference was observed in the relative dry weight of the rye in the brown forest soil treated with the sewage sludge from Nyíregyháza. According to the results, alkaline pH sewage sludge increases the pH of acidic soils, which favours the growth of rye plants and reduces or inhibits the harmful effects of heavy metals.

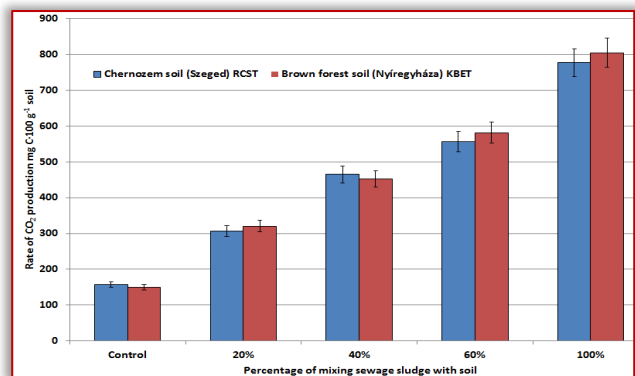


Figure 4. Effect of sewage sludge on the amount of CO₂ released in soil samples

The degree of microbial activity can be determined by the amount of CO₂ released from the two soil samples. In this study, the value of soil respiration compared

with the control increased significantly by increasing the sewage sludge dose. Figure 4 shows that the amount of CO₂ released from the metabolism of microorganisms, root respiration in the soil of the meadow chernozem and in the brown forest soil from Nyíregyháza.

Such rate of respiration can provide valuable information on the increased metabolism activity of the soil microorganisms. The highest CO₂ emissions were observed in the soil of the brown forest soil from Nyíregyháza.

— Enzymatic activities

The results of measuring dehydrogenase activity also confirm the microbial population. In our experiments, the enzymatic activity measured in sewage sludge treated soils exceeded double the values of the control samples. The enzyme activity of FDA hydrolysis was determined by spectrophotometric measurement, and the results of which are shown in Figure 5. The largest FDA activity was registered in RCST soil. In samples of sewage sludge: soil mixture 60: 40% to 100: 0% FDA activities showed positive significance for each treatment. The results show that the amount of fluorescein produced by FDA hydrolysis (spectrophotometric measurement) is in direct proportion with microbial growth, and the hydrolytic activity of FDA shows a close correlation with soil respiration.

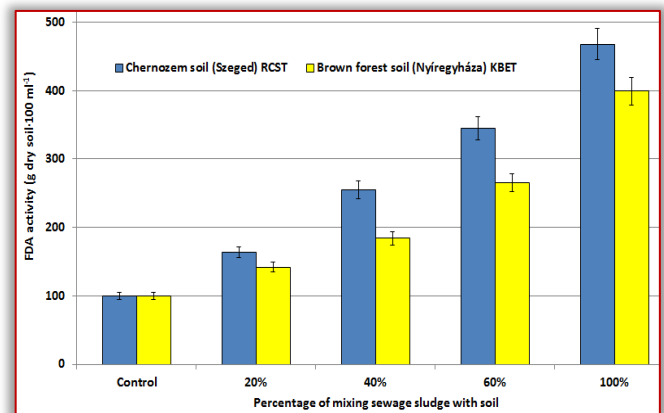


Figure 5. Effect of sewage sludge application on the activity of FDA enzyme in two soil samples

Accordingly, the organic material turnover of the examined model experiment is influenced and determined by the overall microbial activity. There was also a growing tendency for FDA activity after increasing the amount of sewage sludge mixed with soil. The equilibrium effects of sewage sludge did not only significantly increase the soil microbial population, but also the activity of the soil enzymes investigated, soil respiration and FDA activity.

Soil dehydrogenase activity refers to the total oxidative activity of the soil microbial and can therefore be a good indicator of the degree of microbial activity. Sewage sludge addition increased

dehydrogenase activity and catalase activity for each treatment (Figures 6 and 8).

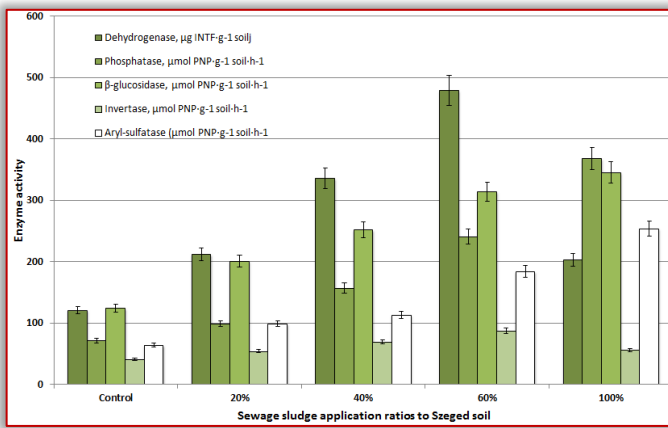


Figure 6. Effect of sewage sludge on the activities of some enzymes in soil of Szeged

The highest enzyme activities of urease, protease (Figures 7 and 9), phosphatase, β-glucosidase and aryl sulfatase (Figures 6 and 8) were found in the presence of sewage sludge only. The higher enzyme activity can be explained by the increased microbial activity that is caused by the high nutrient and organic content of sewage sludge. Protease activity increased significantly after 9 weeks of growth time. The more organic matter the sewage sludge in question is, the more enzyme activity is due to its complexity with the resistance to organic matter. The enzyme activity was also higher in the rhizosphere of brown forest soil mixed with the sewage sludge in Nyíregyháza.

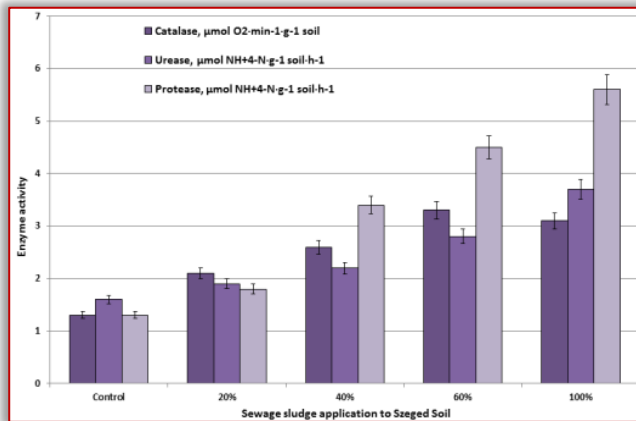


Figure 7. Effect of sewage sludge application on the activities of catalase, urease and protease enzymes in soil of Szeged

During the experiment, the plant grown on sewage sludge treated soil had a positive effect on the synthesis of the β-glucosidase enzyme. The highest values were measured in soil treated with sewage sludge, apparently due to the degradation of organic matter. The values of aryl sulfatase activity in the rhizosphere of rye treated in different soil types are similar to that of protease and urease activity.

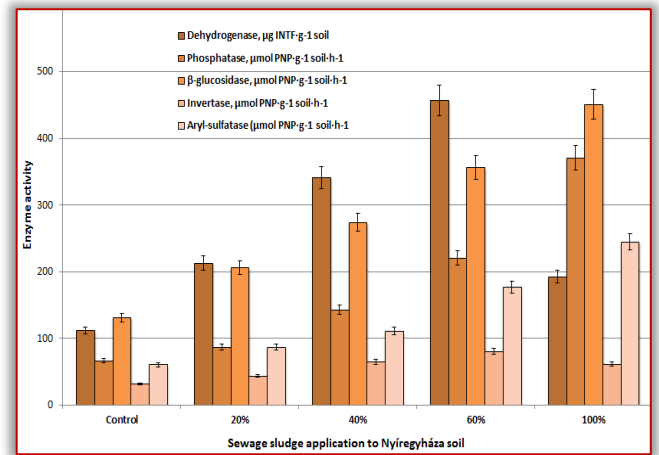


Figure 8. Effect of sewage sludge on the activities of some enzymes in soil of Nyíregyháza

— Composition of microbe population

The number of aerobic heterotrophic bacteria, aerobic spore-forming bacteria, actinobacteria and fungi was determined after 9 weeks of plant growth in the two soils mixed with different doses of sewage sludge are presented in the Figures 10 to 12.

It was found that population of the different microbial groups significantly increased with the addition of 20% sludge to soil. This suggests that the increased microbial populations are able to utilize large quantities of organic matter and use sewage sludge as energy sources. Consequently, sewage sludge has a beneficial effect on the general microbial activity of the soil and on some specific microbial portions.

A 1254 bacterial strain was isolated from the rye-soil sewage sludge model experiment. It can be concluded that the bacterial populations increased significantly with sewage sludge treated soils by increasing the amount of sewage sludge used (Figures 10 and 12). The bacterial number of bacteria was 4 to 14 times greater than the control depending on the sewage sludge-soil-plant system. The largest bacterial number was found in brown forest soil mixed with the sewage sludge in Nyíregyháza.

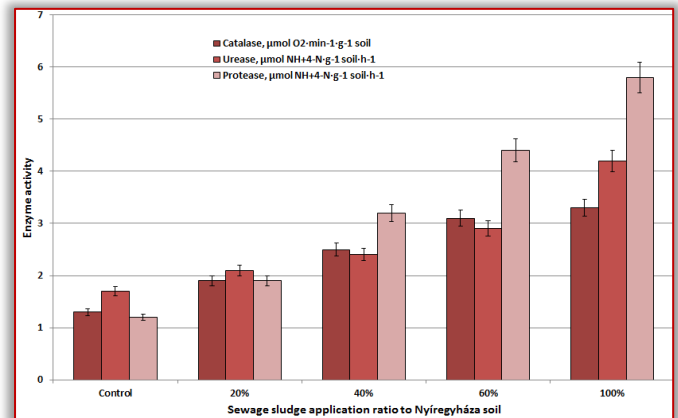


Figure 9. Effect of sewage sludge on the activities of catalase, urease and protease enzymes in soil of Nyíregyháza

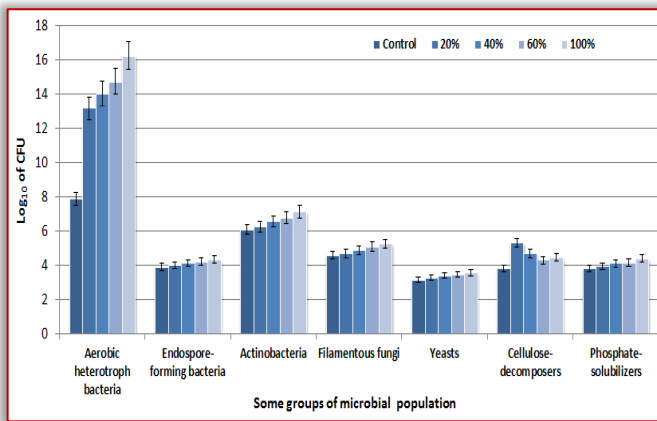


Figure 10. Effect of sewage sludge application on microbial structure in soil of Nyíregyháza

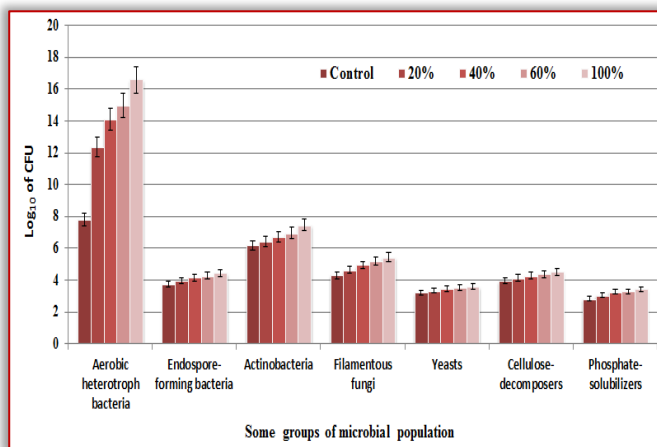


Figure 11. Effect of sewage sludge application on microbial structure in soil of Szeged

The most common isolates were the following genera: *Achromobacter*, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Brevundimonas*, *Burkholderia*, *Cellulomonas*, *Chromobacterium*, *Chryseobacterium*, *Corynebacterium*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Klebsiella*, *Microbacterium*, *Micrococcus*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Stenotrophomonas*, *Staphylococcus*, *Streptococcus*, *Streptomyces* and *Zooglea*. We have not found representatives of *Aeromonas*, *Citrobacter*, *Listeria*, *Salmonella*, *Shigella*, *Vibrio* and *Yersinia*. The bacterial number of aerobic spore-forming bacteria in sewage sludge-soil-plant model experiment was 3 to 7 times greater than control. Sewage sludge treatments increased the population of actinobacteria compared to untreated soils. The number of actinobacteria in the sewage sludge-soil-plant model experiment was 2-22 times higher than the control. The highest number of actinobacteria was observed in brown forest soil of Nyíregyháza. Domestic isolates in different sewage sludge/soil mixtures belonged to the *Streptomyces* genus. The fungal populations in each sewage sludge/soil mixture were largely different from the control. The number of filamentous fungi

according to the sludge-soil-soil ecosystem was 2-13 times greater than that of the control.

In a model experiment composed of various sewage sludge/soil mixtures, more than 350 representative fungal strains were isolated. These isolates belong to the following genera: *Alternaria*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma*. In addition, there are many strains belonging to the *Saccharomyces* genus, which were only isolated from soil of Nyíregyháza. Most of the filamentous fungi were found in brown forest soil, especially when the soil was treated with the sewage sludge in Nyíregyháza (Figures 10 and 11).

—Bacterial communities

In our experiments gram negative bacteria dominated the rhizosphere of the rye. The proportion of gram-negative and gram-positive bacteria (Figure 12) is proportional to the brown forest soil and the Chernozem soil in the meadow mixed with Nyíregyháza sewage sludge 2,367; and 3.24 respectively. These data suggest that there is no statistical difference between the bacterial communities of rye rhizosphere in the brown forest soil treated with the sewage sludge in Nyíregyháza, while in the other soil there was a significant difference. The soil treated with the sewage sludge in Nyíregyháza was higher in gram-negative bacteria than in soil from Szeged.

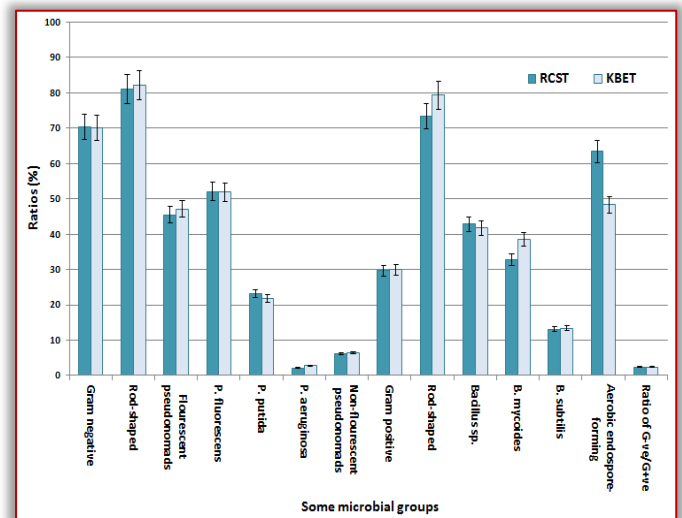


Figure 12. Effect of sewage sludge application on microbial communities in soil samples of Nyíregyháza and Szeged

Sustainable agriculture will pay attention primarily to water and soil protection and conservation, avoiding soil erosion and degradation, and also care for the use of resources (water, energy, machinery, fertilizers and amendments and soil labours) in a frame of environmental respect. Sludge and sustainability, when proper sludge products quality attained and recycling is feasible many objectives connected with sustainability are accomplished: Soil economy and

recovery eliminating landfill disposal and contributing to rangelands restoration; The use of resources through promotion of soft technologies; Mineral nutrients savings through recycling and mineral fertilizers replacement; Fossil fuel energy savings through mineral fertilizers replacement; Nature's protection through reduction of fertilizers production; and Erosion control and increase of soil fertility and productivity.

Investigation of the data showed that there is more evidence that such parameters are sensitive indicators of the composition and function of the stressed soil caused by the use of sewage sludge because microbiological activity directly affects the stability of agro-ecological systems and fertility. There is a growing interest in the use of soil enzymes as indicators of soil fertility, because the activity of soil enzymes is sensitive to many factors. Since the enzyme activity is substrate-specific, it is difficult to predict the nutrient supply of the soil from the activity of an enzyme, and the parallel measurement of several properties can better describe the microbiological activity of the soil.

The number of heterotrophic microorganisms in the soil usually increases following the addition of sewage sludge. In fact, microorganisms capable of utilizing the organic material of sludge are rapidly propagating.

In the pot experiment of two soil samples and one sample of sewage sludge to form plant-soil systems, whose application possibilities can favourably contribute to plant nutrition. We found that besides rye growth, the plant health was better than control. So the plant utilized the micro and macro nutrients necessary and easy to apply from the immediate environment.

According to Stadelmann and Furrer [20], the number of aerobic bacteria and beetles increased due to sewage sludge addition.

In summary, the treatment of soil samples with sewage sludge stimulates the plants development, improves the physical, biochemical and microbial properties of the rhizosphere, helps to maintain soil moisture and increases soil pH, which is also favourable for plant growth. To improve soil fertility, the authors propose an alkaline sewage sludge treatment for acidic soils.

The sewage sludge application causes an increase in the microbial populations. Environmental factors also affect microbial activity and mineralization of sewage sludge. Sewage sludge and its management affect the quality of the organic material, its degradation speed, the time required for it, and the amount of nutrient released. Our results confirm the statement by Garcia et al. [18] that microbial and dehydrogenase activity is directly related to each other and depends on the metabolic state of microbial populations in soil.

Crecchio et al. [25] observed that with increasing use of communal waste compost the organic C, N, dehydrogenase, β -glucosidase, urease, nitrate reductase and phosphatase activity of the soil increased with the composition of the bacterial communities living in soil did not change significantly. However, in our case, the activity of soil enzymes and the density of microbial populations increased with the addition of sewage sludge.

Sewage sludges, as products obtained by wastewater treatment, contain organic matter, micro and macronutrients and are potentially useful for any agriculture use. They may contain undesirable harmful materials. For these reasons, the use of sewage sludges in agriculture, at European Union (EU) level, is regulated by the EU Sludge Directive 86/278/EEC. One of the current Council Directive of 12 June 1986 aims on the protection of soil environment, when sewage sludge is applied in agriculture is to avoid toxicity effects on soil, plants and man [26, 27]. Considerable improvement in dehydrogenase activity and aggregate associated organic matter was observed particularly when higher amount of sludge was applied our results are confirmed with the observation of Mondal et al. [28]. The greater soil urease and invertase activities in spring soil amended with sewage sludge provided evidence of increased soil microbial population [29]. Soil microorganisms excrete a variety of enzymes such as ureases, invertases, dehydrogenases, cellulases, amylases and phosphatases that have long been recognized as a primary means of degrading xenobiotics in soil and water ecosystems [29]. According to Pierzynski et al. [30], soil quality is determined mainly by physical (structure, water retaining capacity, etc.), chemical (organic and inorganic substances concentration) properties which can strongly affect fertility, biological activity, or other important soil factor. Our results are in agreement with the Pierzynski et al. [30] in regards to the issues of plant growth and soil quality. The degradation of the organic material of the sewage sludge can be monitored well in the soil based on the measured amounts of the releasing of CO₂. Previous studies [31] have shown that soil respiration has increased due to addition of sewage sludge.

CONCLUSION

The presence and activity of soil microorganisms is essential for the fertility of agricultural soils. The beneficial effect of fertilization on soils' productivity has long been known. The results of our experiments support the above mentioned works that the density of microbial populations is related to the amount of sewage sludge mixed with soil. Our results are in accordance with the above mentioned work, which increases the fertility rate and the density of microbial populations as well as soil enzyme activity by

increasing the proportion of sewage sludge mixed with soil. Sewage sludge extraction can be partially replaced by organic fertilization and fertilization, as well as the treatment of soil physico-chemical properties. At the same time, due to the aspects of food safety, the use of continuous state monitoring methods is recommended.

Note:

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