EFFECT OF WASTEWATER SLUDGE ON MICROBIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION IN SECALE CEREALE L. RHIZOSPHERE

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Abstract
Pot experiment was conducted to study the effects of wastewater sludge treatment on biological properties of rye rhizosphere after 9 weeks cultivation. Soil was kovárvány brown forest soil collected from Nyíregyháza and treated with different doses of the sludge. Different microbial properties and enzymatic activities were studied. Results indicated that the enzymatic activities in soil samples treated with the sludge were increased 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 was 2.367. Finally, soil amended with the sludge stimulates the biochemical and microbial properties of the rye 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.

Introduction
Application of sewage sludge compost as a fertilizer on landscaping provides a potential way for the effective disposal of sludge. Monitoring of microbiological and biochemical parameters is one of the most essential properties to qualify soil health when treated with wastewater sludge. Today, one of the most pressing environmental problems is the increasing volume of waste, including wastewater sludge treatment, utilization and disposal. The possible way to use wastewater sludge as organic fertilization is utmost importance because it improves soil structure and induces useful microbiological processes. Wastewater sludge is environmentally polluting and on the other hand, suitable for organic farming as organic fertilizer. As a result of wastewater sludge treatment, the amount of microorganisms 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. The organic matter of wastewater sludge is decomposed by heterotrophic nutrition prokaryotes and fungi. Biodegradation is basically the result of microbial and biochemical processes, therefore all factors that have an impact on the structure, function and enzymatic activity of microorganisms affect the rate of degradation. Microbial mineralization products of organic constituents differ in aerobic and anaerobic conditions. During the present work, we investigated the effects of wastewater sludge on the biological properties of the amended soils in the rhizosphere of rye plant.

Experimental
The origin of soil samples were: 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 wastewater sludge from the municipal wastewater treatment plants (Nyíregyháza) and soil samples are presented in Table 1. The air dried soil was

thoroughly mixed with wastewater sludge so, the final mixture contained wastewater sludge in the soil sample was as following percentages: 0% (wastewater -free control soil), 20, 40, 60 and 100% (wastewater sludge only, without soil). Rye (*Secale cereale* L.) seeds were sterilized and planted in plastic containers of 3 kg of tested soil as prepared above. After 10 days of germination, young plants were reduced to be 10 plants/pot.

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

Parameters	Soil type: KBET	Wastewater sludge: NySzv
$pH_{(KCl)}$	5.78	6.71
a) Dry matter content, %	na	53
b) Organic matter, %	na	21.7
c) Humus content, %	2.54	na
d) Total-N, $mg \cdot kg^{-1}$	na	7470
NO_3-N, $mg \cdot kg^{-1}$	23	na
NH_4-N, $mg \cdot kg^{-1}$	5.6	na
Mg, $mg \cdot kg^{-1}$	214	2507
Na, $mg \cdot kg^{-1}$	64	994
P_2O_5, $mg \cdot kg^{-1}$	318	28720
K_2O, $mg \cdot kg^{-1}$	412	3171
Zn, $mg \cdot kg^{-1}$	1.7	537
Cu, $mg \cdot kg^{-1}$	1.4	110.4
Mn, $mg \cdot kg^{-1}$	55	421
Fe, $mg \cdot kg^{-1}$	945	11308
Cd, $mg \cdot kg^{-1}$	1.7	2.3
Pb, $mg \cdot kg^{-1}$	1.3	66.9

na: no data available

Heterotrophic and aerobic soil microorganisms

The total plate count of aerobic bacteria, aerobic endospore-forming bacteria, filamentous fungi, yeasts, cellulose decomposers and phosphate-solubiliers 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. Ten grams of the washed roots were cut and placed in 90 ml of sterile saline. 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 [1] 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 [2] amended with 0.05 g/l cyclohexamide. Examination of phosphate solubilisation was done in the medium described by Goldstein [3] 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. [4]. 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 identification 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.

Monitoring the enzymatic activity

Dehydrogenase activity (µg INTF/g^{-1} dry soil) was measured according to García et al. [5]. Phosphatase activity (µmol p-nitrophenol (PNP)/g^{-1} dry soil/h^{-1}) regard to the method of Tabatabai and Bremner [6]; β-glucosidase activity (µmol p-nitrophenol/g^{-1} dry soil/h^{-1}) was determined by the method described by Masciandaro et al. [7]. Invertase activity was measured by Siegenthaler [8] 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 (µmol nitrophenol g^{-1} dry soil/h^{-1}) was determined according to Tabatabai and Bremner [6] (absorption of p-phenol at 400 nm after incubation with PNP sulphate).

Results and discussion

Composition of microbial population: It was found that population of the different microbial groups increased by the addition of sludge to soil. This suggests that microbial populations are able to utilize large quantities of organic matter and use wastewater sludge as energy sources (Figure 1).

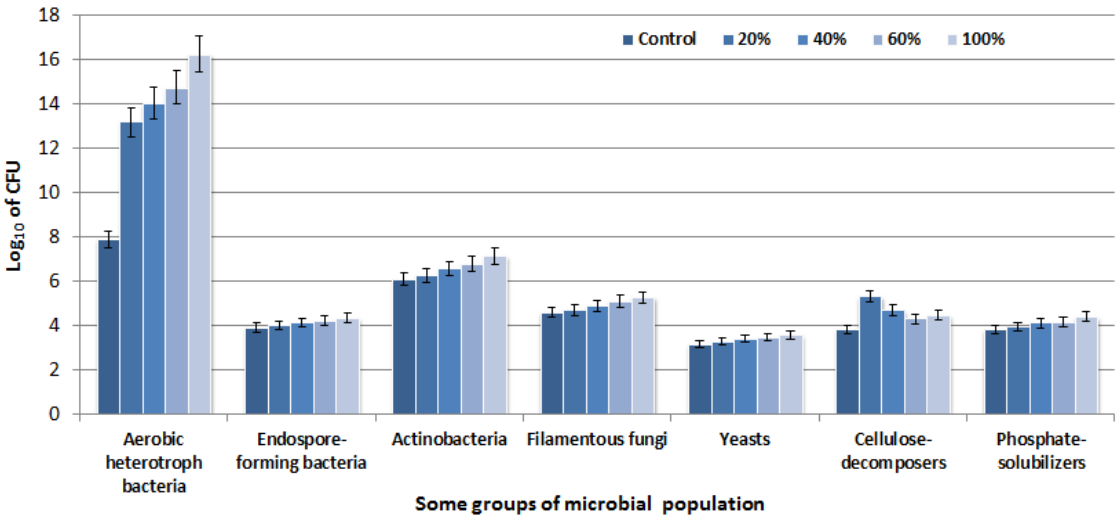

Figure 1. Effect of sludge application on microbial structure in soil of Nyíregyháza

The most common isolates were belong to genera of *Achromobacter, Acinetobacter, Aeromonas, Alcaligenes, Arthrobacter, Azotobacter, Bacillus, Brevundimonas, Burkholderia, Cellulomonas, Chromobacterium, Chryseobacterium, Corynebacterium, Enterobacter, Escherichia, Flavobacterium, Klebsiella, Microbacterium, Micrococcus, Pseudomonas, Rhodococcus, Serratia, Stenotrophomonas, Staphyllococcus, Streptococcus, Streptomyces* and *Zooglea*. The number of filamentous fungi was greater than in the control and 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 (Figure 1).

Bacterial communities: It was found that Gram negative bacteria were dominated in the rye rhizosphere treated with the sludge. The proportion of Gram-negative to Gram-positive bacteria was 3.24.

Enzymatic activities: The results of measuring dehydrogenase activity confirm the microbial population. Also, the enzymatic activity exceeded in higher values than the control

samples. Soil dehydrogenase activity refers to the total oxidative activity of the soil microbial activities and can therefore be a good indicator of the degree of microbial activity. Sludge addition increased dehydrogenase activities for each treatment (Figure 2).

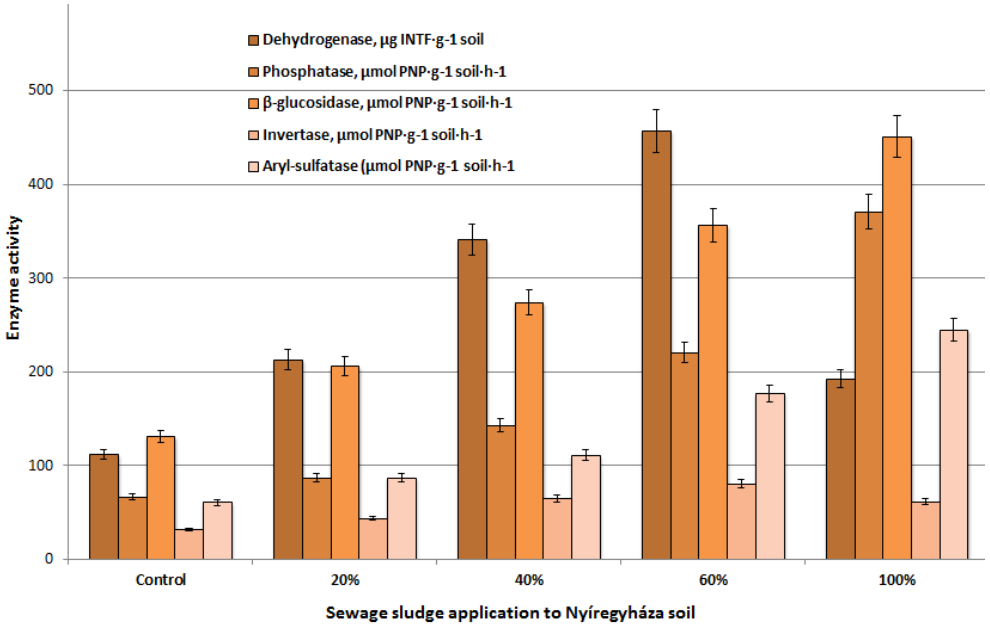

Figure 2. Effect of sludge application on the activities of some enzymes in soil

The highest enzymatic activities of urease, protease, phosphatase, β-glucosidase and aryl sulfatase (Figure 2) were observed. The higher enzyme activity can explained the increases of microbial activity that is caused by the high nutrient and organic content of sludge. Protease activity increased significantly after 9 weeks of growth time. During the experiment, the plant grown in sludge treated soil had a positive effect on the microbial growth and increases the β-glucosidase.

Evaluation of results: Investigation of the data showed that there is more and more evidence that such parameters are also sensitive indicators of the composition and function of the stressed soil caused by the use of sludge because microbiological activity directly affects the stability of agro-ecological systems and fertility. There is also 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 sludge. In fact, microorganisms capable of utilizing the organic material of sludge are rapidly propagating.

According to Stadelmann and Furrer [9], the number of aerobic bacteria and beetles increased due to sludge addition. The sludge application causes an increase in the microbial populations. Environmental factors also affect microbial activity and mineralization of sludge. Wastewater 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. [6] 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. [10] 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 sludge. 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 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 sludge is applied in agriculture is to avoid toxicity effects on soil, plants and man [11]. 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 Mondala et al. [12]. The greater soil urease and invertase activities in spring soil amended with sludge provided evidence of increased soil microbial population [12]. Soil microorganisms excrete a variety of enzymes such as urease, invertase, dehydrogenases, cellulases, amylases and phosphatases that have long been recognized as a primary means of degrading xenobiotics in soil and water ecosystems [13]. Therefore, in municipal solid waste composts use over agricultural lands, heavy metal contents should always be taken into consideration and excessive uses should be avoided [14].

Conclusion

Findings showed that microbial activity in the soil depends on the C and N content; soil enzymatic activity increase with the addition of sludge. Therefore, the wastewater sludge utilized in the present study could be used as a valuable organic fertilizer in rye cultivation land and could also act as an eco-friendly method for the recycling of wastewater sludge.

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