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EFFECTS OF SEWAGE SLUDGE APPLICATION ON THE SOME BIOLOGICAL ACTIVITIES IN THE RHIZOSPHERES OF COMMON BEAN AND CORN

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Abstract: *Significant population growth has generated several problems over the last half century. Due to the increased land use, the amount of fertile land is constantly decreasing. Minerals released from the soil by crop production and extraction needs to be replenished, as its self-sustaining mechanism cannot reverse such damage. The utilization of sewage sludge is playing an increasingly important role in the field of environmental protection due to the expanding sewerage network. The further storage, treatment and disposal of sewage sludge generated as a by-product during wastewater treatment are a significant problem for wastewater treatment plants, mainly due to the increasing volume. However, its special feature is that, despite being a waste, it is an important source of nutrients that, when returned to the soil, improves its quality. The aobjective of the study was to measure the effect of sewage sludge on soil biochemical properties and its remedial effect. In the course of the research, soil samples were treated with different proportions of sewage sludge for the biological activities measurements. The study was carried out in the rhizospheres of two plant species (common bean, maize), to measure the enzyme potencial activity of urease, ß-glucosasease, dehydrogenase, and phosphatase based on the content of sewage sludge. Based on the results of the study, it can be concluded that the application of organic waste sludge improves the quality of the soil in all cases, however, after 15% treatment, a significant change can be observed; there was a spectacular increase in soil enzymatic potencial activity of the investigated enzymes. Finally, the study covers the situation of domestic agriculture and the legal framework of sewage sludge disposal.*

Keywords *Sewages sludge, enzymatic activity, Risk assessment, soil, plant rhizosphere*

INTRODUCTION

Significant population growth has generated several problems over the last half century. Increased land use induced by feeding people. It causes a steady decline in the amount of fertile land even today. Minerals extracted from the soil by crop production and extraction must be replenished, as its self-sustaining mechanism cannot reverse such rapid and large-scale damage. Another burning problem is the growing amount of waste, with wastewater occupying a special place as it grows in proportion to the population, there

is no way to reduce it, but due to the expanding sewerage network, an increasing amount has to be addressed. The further storage, treatment and disposal of sewage sludge generated as a by-product during wastewater treatment is a significant problem for wastewater treatment plants, as it is a very time- and energy-intensive operation. However, its special feature is that, despite being a waste, it is an important source of nutrients and organic matter, which improves its quality when returned to the soil.
In the European Union, there are almost 12 directives related to waste water policy, which has, among other things, contributed to the ban on the dumping of sewage sludge at sea. The gradual introduction of Directive 91/271 / EEC on urban waste water treatment will increase the amount of sewage sludge that Member States are required to dispose of. A wide range of sewage sludge treatment technologies are used in EU countries. The predominant choice is the direct application of agriculture followed by composting, but significant quantities are burned and deposited. However, the rate of use of sludge in agriculture varies greatly from country to country. [1] The agricultural use of sludge from urban waste water is regulated by Directive 86/278 / EEC, which is transposed by the Member States. The main purpose of the directive is to encourage agricultural use and to regulate activities so as not to contaminate the soil with harmful components in sewage sludge. For this reason, the regulation mainly covers the limit values for heavy metals, organic pollutants and pathogens.
In wastewater treatment plants (WWTP), various physicochemical and biological processes take place in order to treat efficiently wastewater. These processes result in the creation of primary and secondary sewage sludge as a by-product. Consequently, in order to put circular economy into practice, it is necessary to assess and promote alternative long-term solutions for sludge utilization and materials' recovery. [2] There are several potential uses of biosolids (i.e., treated sludges) including the application in agricultural and nonagricultural land as well as energy valorization. Agricultural land application of biosolids for fertilization purposes is a viable solution that presents both financial and environmental benefits. Properly treated domestic sewage sludge can provide essential plant nutrients, such as N and P; replenish soil organic matter; and stimulate plant growth. [3]
In a recent European consultation, it has been found thatthere is a significant gap in information on emerging pollutants in biosolids and that there should be harmonization on sampling and analysis on metals, pathogens, and organic pollutants [4]. Hence, it is imperative that biosolids are fully characterized in order not to cause harm instead of benefit when released to the environment. [5] An effective way of assessing the environmental footprint of biosolids released into the soil or near aquatic receptors is the use of ecotoxicity tests. Ecotoxicological analyses, in combination with chemical analyses, highlight costeffectively toxic substances and evaluate their potential effects on organisms.
In Hungary, sewage sludge plays a significant role in agricultural utilization. In 2013, about 38% of the sludge generated was used for agricultural purposes, preceded only by use for soil remediation, mainly due to mining activities. In Hungary, two decrees cover agricultural posting, 50/2001. (IV. 3.), which regulates the agricultural use and treatment of wastewater and sewage sludge, and 36/2006. (V. 18.) of the Ministry of Agriculture and Rural Development, which deals with the licensing, storage, distribution and use of crop-increasing substances. [6]
The use of sewage sludge as a soil improver is becoming more widespread, especially for soils with low organic matter content. The composition and proportion of organic matter in sludges varies, but by examining a general composition, the effect of each substance on soil microorganisms can be determined. With the introduction of organic matter, the microorganisms become activated, their enzyme production begins, the change of which can be measured, thus allowing conclusions to be drawn. This amount of enzyme is the enzyme activity, which is actually a measure of reaction rate. [7] Enzyme production is also affected by the physical, chemical and biological properties of the soil, so the response to nutrient uptake gives an idea not only of the number and composition of microbes but also of the physico-chemical state of the studied soil. [8]
During the research work, soil mixtures treated with different proportions of sewage sludge are examined, with different application rates. The study is based on the rhizosphere enzyme activity of two plant species (common bean and maize) such as phosphatase, urease, *ß*-glucosasease, and dehydrogenase, based on the content of sewage sludge.

MATERIALS AND METHODS

The basis of the research work was a soil sample from the area of Szent István University in Gödöllő, which

is a brown forest soil. Its altitude is around 200m, and the average annual rainfall is between 540-580mm. The soil sample was taken from the top 25 cm layer of the ground cover. [9]
During the experiment the used sludge was collected from the Nyíregyháza wastewater treatment plant, which is domestic aerobically digested sewage sludge. Table 1 contains some physicochemical characteristics of the Gödöllő soil sample and the Nyíregyháza municipal sewage sludge sample.

Table (1) Physico-chemical characteristics of the tested soil and sewage sludge samples [10]

Parameters	Soil sample (Gödöllő)	Sewage sludge sample (Nyíregyháza)
$pH_{(KCl)}$	4.72	6.71
Dry matter content (%)	-	53
Organic matter (%)	-	21.7
Humus content (%)	1.24	-
Total-N (mg/kg)	-	7470
NO_3-N (mg/kg)	33	-
NH_4-N (mg/kg)	1.77	-
Mg (mg/kg)	201	2507
Na (mg/kg)	21	994
P_2O_5 (mg/kg)	69.1	28720
K_2O (mg/kg)	135	3171
Zn (mg/kg)	7.1	537
Cu (mg/kg)	2.3	110.4
Mn (mg/kg)	53	421
Fe (mg/kg)	1187	11308
Cd (mg/kg)	1.6	2.3
Pb (mg/kg)	1.1	66.9

Two test plants. Corn (Zea mays) and Common beans (Phaseolus vulgaris L.) were used and greminated in soil sample and soil samples mixed with different sewage sludge rations for 21 days (see Figures 1 and 2)

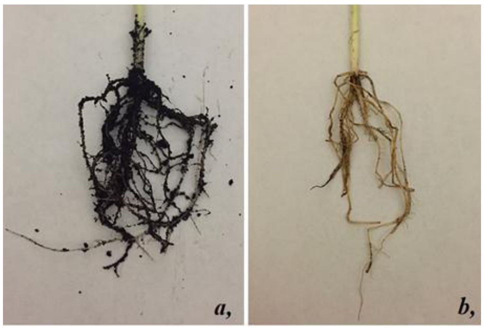

Figure (1) Vegetable bean root, before cleaning (a,) and after cleaning (b,)

Figure (2) Corn root before cleaning (a,) and after cleaning (b,)

Dehydrogenase activity was measured according to the method of GARCÍA et al. [11]. I exposed 1 g of soil to 0.2 ml of a 0.4% INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride) solution as an electron acceptor for 20 hours, 22°C, in the dark. The iodonitrotetrazolium formazan (INTF) formed was extracted with 10 ml of methanol by vigorous shaking for 1 min on a filter paper. INTF was measured spectrophotometrically at 490 nm. The results were expressed in µg INTF/g^{-1} soil. Urease activity was determined according to the method of Nannipieri et al. [12] in 0.1 M phosphate buffer at pH 7. One M urea as a substrate was used. Then, 2 ml of buffer and 0.5 ml of substrate were added to 0.5 g of soil sample, which was incubated at 30°C for 90 min. The enzyme activity was defined as NH_4^+ released in the hydrolysis reaction. The results were expressed in µmol NH_4^+ -N/ g^{-1} soil/h^{-1}. Phosphatase and β-glucosidase activities were performed according to the method of Tabatabai and Bremner [13].
P-nitrophenyl phosphate disodium (PNPD, 0.115 M) and p-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) were added spectrophotometrically at 400 nm after incubation. Data were expressed in µmol PNP/g^{-1} soil/h^{-1}. The experiment was set up in a randomized block design, in three parallel studies, with three replicates. The results were represented the avarage of the replicated/treatment/ plant/sewage sludge dose. Then a simple linear regression analysis was used and the coefficients of determination (R^2) were calculated for all the examined parameters. The statistically significant difference between the treatments was calculated at the $P < 0.05$ level.

RESULTS AND DISCUSSION

The biochemical activity of clay loam brown forest soil was investigated on the basis of the potencial activities of dehydrogenase, urease, β-glucosidase and phosphatase. Based on the results obtained, it can be determined, that in all cases, regardless of the degree of treatment, there was an increase in enzyme activity compared to the control. Even with 10% treatment, there is a visible increase in the enzyme activity.
Dehydrogenase activity (Figure 3) indicates oxidative processes of all soil microorganisms. Due to 10% treatment there is a small, however not a significant increase in activity, but a remarkable increase ($P<0.05$) above 15% treatment could be observed, compared to the control sample. The highest dehydrogenase enzyme activity was measured at the highest treatment (30%) and the lowest dehydrogenase enzyme activity was measured at the control. In the root-soil of corn, the values doubled as a result of 15% treatment, while a similar increase was achieved in case of common bean under the influence of a 20% sludge dose.

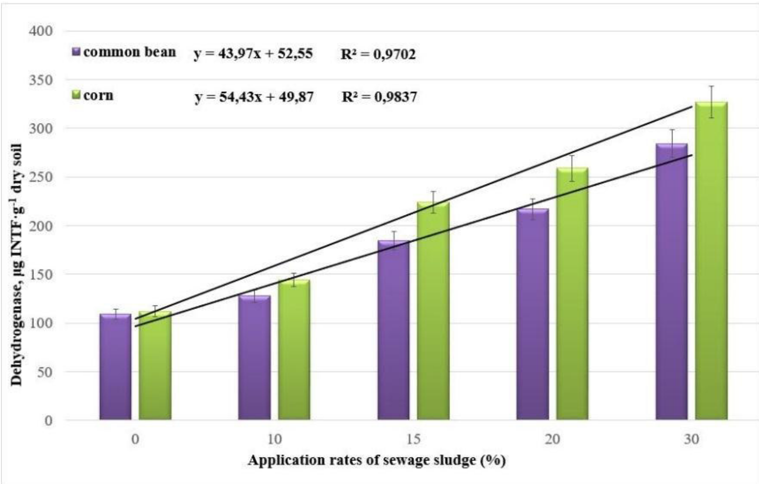

Figure (3) Changes in dehydrogenase activity in clay loam brown forest soil due to sewage sludge treatment

The urease enzyme is responsible for the hydrolysis of urease in the soil, which is included in the wastewater by human urine. Here the values were also lowest at the control sample. (Figure 4) The increase here was greater with 10% treatment than with dehydrogenase. A higher increase in maize values can be observed as a result of sludge treatment than in beans. The 30% treatment in the root-soil of corn nearly tripled the degree of urease activity compared to the control sample, while the growth of the values of common bean was just over double.

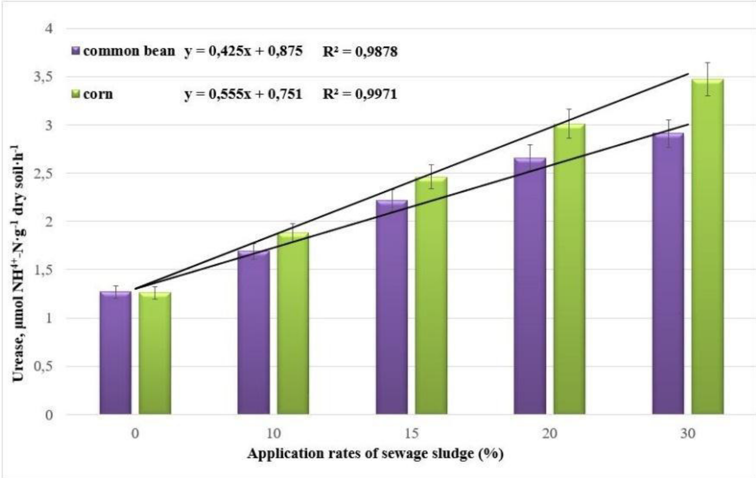

Figure (4) Changes in urease activity in clay loam brown forest soil due to sewage sludge treatment

β-glucosidase activity values (Figure 5) were similar to those of urease enzyme activity as a result of sewage sludge treatment. I experienced a slowing increase in values, as a result of an increase sludge dose. The increase in values is below 10% with 30% treatment compared to 20% treatment.

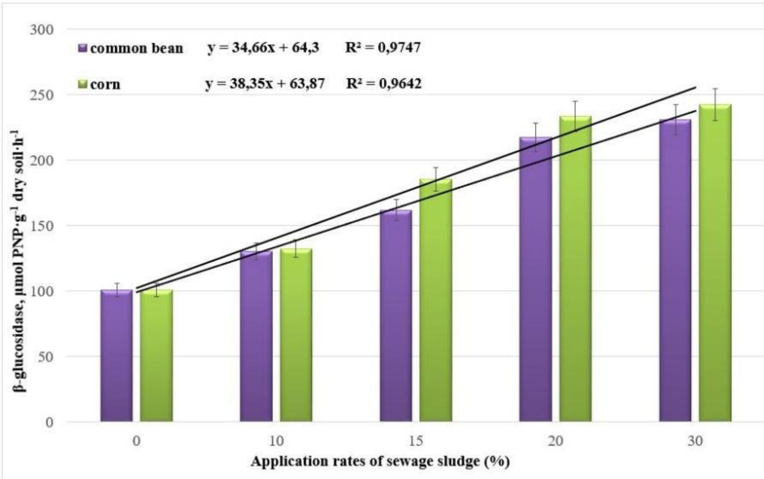

Figure (5) Changes in β-glucosidase activity in clay loam brown forest soil due to sewage sludge treatment

The application of sewage sludge significantly affected ($P <0.05$) the activity of the enzyme phosphatase in the soil. (Figure 6) The values of the enzyme responsible for dismantling the various insoluble phosphate compounds increased significantly compared to the control sample, even under the influence of 10% sludge treatment. As a result of the 20% treatment, both plants experienced an increase of one and half times compared to the 15% treatment values. 30% sludge treatment caused the measured values to exceed four times the values of the control sample.

Based on the results, it can be seen that the evolution of enzyme activity is an important indicator of the functioning of microorganisms, which illustrates their reaction well to the nutrients introduced. The use of municipal sewage sludge for soil improvement will improve the biological and biochemical properties of the soil. The reason for the increase in enzyme activity detected in the soil is an increase in the number of microorganisms and the activation of their metabolism.

Considering my measurement results, it can be stated with absolute certainty that the introduced sludge had a beneficial effect on the soil properties in any amount. Soil respiration clearly increased with nutrient uptake compared to control soil, indicating that microorganisms were activated, their metabolic processes started, which is due not only to the amount of nutrients ingested but also to the heavy metal content of the sewage sludge used in the experiment.

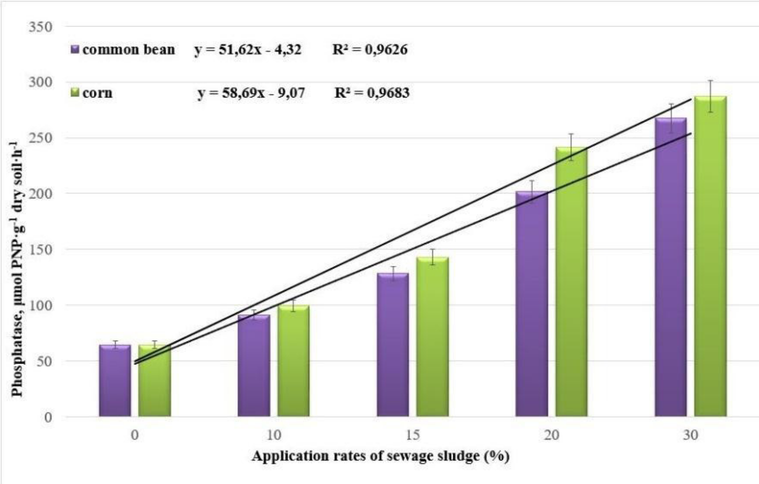

Figure (6) Changes in phosphatase activity in clay loam brown forest soil due to sewage sludge treatment

Sludge treatment increased the amount of aerobic heterotrophic bacteria, filamentous fungi, cellulose breakers and phosphate solvents in plant rhizospheres. As a result, the dehydrogenase, β-glucosidase, urease and phosphatase activities of the treated samples also increased. The activity of these enzymes shows a linearly increasing correlation with the soil organic matter content due to the sewage sludge added to the soils. [26]
According to EU data, municipal sludge production for the period of 2003–2006 is on the rise. [4], [14] Sludge contains significant quantities of N and P, and therefore, the biosolids may be used for crop fertilization. However, the possible presence of metals of toxicological interest in biosolids poses a risk for the humans and the environment. So, the concentration limits, which are laid out in Council Directive 86/278/EEC (1986) [15], should be respected.

CONCLUSIONS

It was found that the results of the study research are extremely positive, but to accurately determine the effects, field experiments are required under different conditions, and it is justified to examine the interaction between these conditions and sewage sludge in advance. Based on the results, it can be stated that the treatment increased with increasing sludge doses, and although the difference of 20% to 30% did not bring as much increase in the measured parameters as, say, the difference of 0% to 15%, it would be worthwhile to apply the effects of higher application rates to examine. In the long run, it can consider this to be the only option for soil improvement, as maintaining a cycle is an essential condition for the existence of the worldwide. However, the fact that sewage sludge also contains recovery inhibitors such as heavy metals, pathogens and organic micropollutants cannot be neglected. A significant proportion of these come from industrial effluents, so it would consider the separate collection of industrial effluents and the treatment of their sludge and their recovery in other ways (eg incineration energy recovery) to be a costly but long-term effective solution. This should minimize the release of dangerous substances by humans into the soil. In addition, further studies are needed, mainly on the effects of heavy metals on the agriecosystem productivity and on humans and animals health, as their effects do not have an immediate effect, and their accumulation in the body poses hitherto unknown dangers in the long-term. The legislation in Hungary is very strict regarding the disposal of agricultural sewage sludge, which is essential in order to avoid dangers.

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