INFLUENCE OF SLUDGE ON MICROBIAL CONTENTS AND ENZYMATIC ACTIVITIES IN THE RHIZOSPHERE OF SPRING WHEAT (*TRITICUM VULGARE*)

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Abstract
In greenhouse, plants were grown for 50 days in pots containing meadow chernozem soil were treated with different sludge rates 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% sludge (w/w). The results show that the max. plant relative dry weight was laid between 40 and 80%, and for S/R ratio was max. at 70%. The microbial populations and the activities of enzymes at all concentrations increased significantly with increasing the treatments of soil by sludge. Although differences from control (only soil) were statistically significant ($P < 0.05$) by increasing the level of application over 30%. High significant differences were found between 70 and 80% sludge contained-soil, where microbiologically, maximum enumerations were increased significantly by increasing the dose of sludge. The ratio between the Gram negative and Gram positive bacteria in the rhizosphere of spring wheat was 2.3. It is concluded that the positive effects of sludge when used as organic fertilizer are more than the its negative effects. However, one of the main problem remains is the final recommendation and model to used such environmental waste materials in the agriculture avoiding the overgrowth of heavy metals in soil and crops.

Introduction

With the increasingly growing global production of sewage sludge, evaluation of its applicability in agriculture and land restoration is essential. The sludge providing essential nutrients for crop needs and organic matter for improving soil tilth, and beneficial microorganisms. Recycling municipal sewage sludge through the soil-plant system has been widely recognized as a promising method of disposal. Sludge loading rates are usually calculated according to the nitrogen content of the sludge. Zhang *et al* (2002) mentioned that the chemical characteristics of municipal waste ashes from various locations in Japan were examined with the aim of evaluating their suitability for use in agriculture. Average phosphorus concentrations for the five types of wastes ranged from 10 to 29 g kg^{-1} and average potassium concentrations ranged from 14 to 63 g kg^{-1}, respectively. Benitez *et al.* (2001) mentioned that the investigated biosolids were denatured, anaerobically digested biosolids, composted biosolids and biosolids-ash. The columns were planted with wheat. The addition of digested biosolids decreased the dry matter yield of wheat. Treatments including organic biosolids increased Cu and Zn concentrations in wheat roots, straw and grain, whereas the addition of biosolids-ash did not affect the levels of these metals in wheat. The objective was to determine the effect of sludge-treated soil on the growth and the biological activities in the rhizosphere of wheat.

Materials and Methods

***Soil and sludge*:** The meadow chernozem soil used in the experiment was obtained from Szeged, Hungary. It is characterized by total salts 0.1%, $CaCO_3$ 4%, humus 3.55%, NO_3-N 39 mg/kg, NH_4-N 4.4 mg/kg, P_2O_5 378 mg/kg, K_2O 428 mg/kg, Mg 257 mg/kg, Na 53 mg/kg, Zn 1.1 mg/kg, Cu 2.4 mg/kg and Mn 61 mg/kg. The aerobically digested sewage sludge sample was collected from domestic wastewater treatment plant in Hódmezővásárhely. The sludge was characterized by (mg/kg dry matter) soluble organic extracted material (17860), Ag (1.315), As (35.6), B (49.7), Ca (45610), Cd (4.168), Co (20.35), total Cr (37.03), Cu (182.3), Fe (13610), Hg (4.16), K (2908), Mg (11860), Mn (351.2), Mo (4.933), total N (43311), Na (1441), Ni (56.3), total P (20104), Pb (540.7), Se (less than 0.5), Zn (1068) and the pH of the sludge was 7.99.

Weight by weight, air dried soil samples were mixed well with sewage sludge to give a final mixing rates as following 0 (control only soil without adding sewage sludge) 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% (only sludge without soil).

***Effect of sludge application on plant growth*:** 10 surface sterilized seeds of spring wheat (*Triticum vulgare*) were cultivated in 2 kg plastic pot containing one of the above-mentioned preparation. The seedlings were thinned to 5 seedlings/pot after one week of emergence. Relative plant dry weight (RPDW) was calculated after 50 days of emergence and drying in oven for constant weight at 75°C.

***Estimation of some soil microorganisms in plant rhizosphere treated with sludge*:** Plants were carefully uprooted and thoroughly washed with light tap water to remove all loosely adhering soil particles followed by washing with sterile 0.85% saline Milli Q water. The roots were macerated in 0.85% saline Milli Q water. Serial dilution technique was carried out for all macerated-root samples in sterile distilled water. Plate count technique was done to estimate the total number bacteria, spore-forming bacteria, actinomycetes and fungi in the rhizosphere. From each of homogenate sample, one ml was plated on the following media. King-B agar, Nutrient agar, Tryptone-glucose-yeast-extract agar, Martin agar, Malt agar, Selective medium and Jensen agar and Küsten Williams's agar media used for isolation of *Pseudomonas*, *Bacillus*, spore-forming, fungi and yeasts, *Trichoderma* and actinomycetes respectively. The plates were incubated at 37°C for bacterial cultures, 28°C for fungi and yeasts and 30°C for the cultivation of actinomycetes. Bacteria, fungi and actinomycetes were counted after 2, 5 and 7 days of incubation, respectively. Different microbiological and biochemical tests were carried out to help in the identification of isolated bacteria. The common microflora associated with roots of the rhizosphere was identified according Nautiyal & Dion (1990).

Effect of application of sludge on soil enzymatic activities: Dehydrogenase activity was assessed by García *et al*. (1993) method and is expressed as μg INTF/g dry soil. ***Catalase activity*** was estimated by measuring the amount of O_2 consumed by $KMnO_4$ after addition of H_2O_2 to the soil samples (Tabatabai & Bermner 1970) and is expressed as μ mol O_2 /min/g dry soil. ***Urease activity*** was measured according to the method of Nanniperi *et al*. (1980). It is expressed as μmol of NH^+_4-N released/g soil/h. ***Protease activity*** was estimated using the method of Nanniperi *et al*. (1980). It is expressed as μmol of NH^+_4-N released/g dry soil/h. ***Phosphatase activity*** was measured according to the method of Tabatabai & Bermner (1969) and is expressed as μmol of P-nitrophenol (PNP)/g dry soil/time (h). ***β-Glucosidase activity*** was measured using the method of Masciandaro *et al*. (1994). It is expressed as μmol of P-nitrophenol (PNP)/g dry soil/time (h).

Statistical analysis: The experiments were conducted in a complete randomized block design in triplicates. Data are shown as percentage of control in term of relative plant growth (%). ANOVA was used to determine the statistical differences among treatments and LSD at $P < 0.05$ was calculated.

Results

Effect of sludge application on the plant growth and microbial populations: Fig. (1) demonstrates the highest significant difference of RPDW of the plant grown at 70% sludge treated-soil. Therefore, the increases in the sludge doses did not reduce the RPDW of the plants compared with the controls. But, the effect on S/R ratio was not highly differentiated between 10 and 60%. Maximum ratio was obtained at 70% sludge amended soil (Fig. 2).

Below and higher concentration than 70 % sludge, the ratios were not significant. Result in Table (1) illustrates the population densities of total bacteria, spore-forming bacteria, actinomycetes and fungi in the rhizosphere. The population densities of microbiots were increased significantly at P < 0.05 when the rhizospheres contained at least 50% soil and 50% sludge as well as the followed combination upto 100% sludge contained-cultivated medium.

Table 1. Changing the number of some microbial contents in the rhizosphere of wheat according to sewage sludge application

Treatment	Mean per gram soil			
	Bacteria ($x10^6$)	Spore-forming ($x10^3$)	Actinomycetes ($x10^3$)	Fungi ($x10^4$)
100% Soil	71	8.4	1.2	3.4
90%	108	12.8	1.7	3.7
80%	144	17.6	2.2	3.9
70%	160	28.5*	2.8*	5.1
60%	216*	28.6*	3.4*	6.2
50%	225*	30.3*	4.1*	8.5*
40%	401*	34.7*	4.4*	9.3*
30%	427*	35.2*	4.7*	12.1*
20%	463*	37.9*	5.2*	13.3*
10%	514*	46.4*	5.3*	16.3*
LSD	**125.64**	**9.52**	**1.05**	**3.36**

The number labelled with (*) within the column is significant difference with control at $P < 0.05$.

Increasing the additional ratio of sludges in cultivated soil mixture increased the changes in the populations occupied in each plant rhizosphere. These increases in the numbers of microbial population densities in the short term application of sludge suggests that the nutritive values present in these cultivated mixtures especially phosphorus and nitrogen were potentially contained in high amount. The impact of sludge containing heavy metals on the soil microbial populations was studied. The unicellular yeasts (mostly *Saccharomyces* sp.) were the most probable fungi in all concentrations, but it increased by increasing the dose of sludge. *Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus* and *Trichoderma* were the common macrofungi found in soil treated with sludge and the population densities of fungi were more in high soil treated with sludge doses. More organic matter applied to the soil through the application of sludge more fungal population. Few actinomycetes were isolated from moderate and high soil applied with sludge especially *Streptomycetes* species. Table (2) shows the percentages of rhizobacteria in the rhizosphere.

Table 2. Percentage of rhizobacterial populations in the wheat rhizosphere treated with sewage sludge.

Microbial population	(%)
Gram negative	69.7
Rod shape	87.2
Fluorescent pseudomonads	19.4
Gram positive	30.3
Rod shape	91.4
Bacillus sp.	56.1
Spore-forming	96.7

It was found that fluorescent pseudomonads occupied 19.4% of the total rod shape Gram negative bacteria while the spore-forming bacteria of *Bacillus* sp. that was the most common Gram positive bacteria isolated from all soil treated with sludge occupied 96.7%. Commonly, it was found that the ratio between Gram negative and Gram positive bacteria in the studied rhizospheres was mainly 2.3.

Effect of sludge application on the enzymatic activities in the rhizosphere: Table (3) shows the activities of enzymes that are increased when the rates of sludge in the rhizosphere reach 70%. Also, the results obtained show that the values between 30% and 90% sludge added to soil were completely significant at $P < 0.05$ compared with the control (100% soil), and in some cases it reached to be significant at 100% sludge.

In every case the activities of tested enzymes at 100% sludge were more than the activities at 0% sludge (control soil). The results show that dehydrogenase activity was positively affected (compared with non-treated soil control) when sludge was applied to the soil at different rates. So, the great enzyme activity was observed at 80% doses of sludge materials. The catalase activity was positively significant between 20% and 90%. Maximum protease and urease activities in the rhizosphere were at 70% soil-containing sludge. The results indicated that the activities of phosphatase and β-glucosidase were more than the controls. The most significant values of both enzymes were at 80% soil amended with sludge.

Table 3. Some enzymatic activities of the wheat rhizosphere of soil treated with different levels of sewage sludge

Sludge (%)	Enzyme Activities					
	μg INTF/g soil	μmol O_2/min/ g soil	μmol NH_4-N/g soil/h		μmol PNP/g soil/h	
	Dehydrogenase	Catalase	Protease	Urease	Phosphatase	β-Glucosidase
0	132	1.7	1.9	1.8	77	126
10	151	2.1	2.2	2.3*	93	147
20	178*	2.5*	2.6*	2.5*	119	172
30	193*	2.9*	3.1*	2.8*	141*	197*
40	201*	3.3*	3.6*	3.2*	167*	223*
50	237*	3.6*	3.8*	3.4*	182*	241*
60	251*	4.1*	4.3*	3.8*	216*	177*
70	282*	4.4*	4.6*	4.2*	255*	331*
80	314*	3.5*	3.7*	3.6*	314*	372*
90	217*	2.8*	2.8*	2.7*	247*	281*
100	148	2.2	2.5*	2.1	118	173
LSD	**38.49**	**0.57**	**0.58**	**0.5**	**58.46**	**51.96**

The number labelled with (*) within the column is significant different with control at $P < 0.05$

Discussion

Tsadilas *et al*. (1995) studied the influence of sludge application on some soil properties and on the growth of wheat. The S/R ratios throughout the various mixing ratios of soil and sludge were increased with the increasing rate of the sludge added to the soil, even at 100% sludge. The S/R ratios of plants cultivated in sludged-soil were higher than controls, which could give an idea that the shoot length was more than the root length. The improvement of plant growth and health was more than controls. It can suppose that the plant takes up what it needs from micro- and macronutrients easily from the surrounding environment. Similarly, the RPDWs was also improved by the increasing rate of the sludge added to the soil. The maximal S/R ratio and RPDWs (%) were found at the 30% soil amended with 70% sludge.

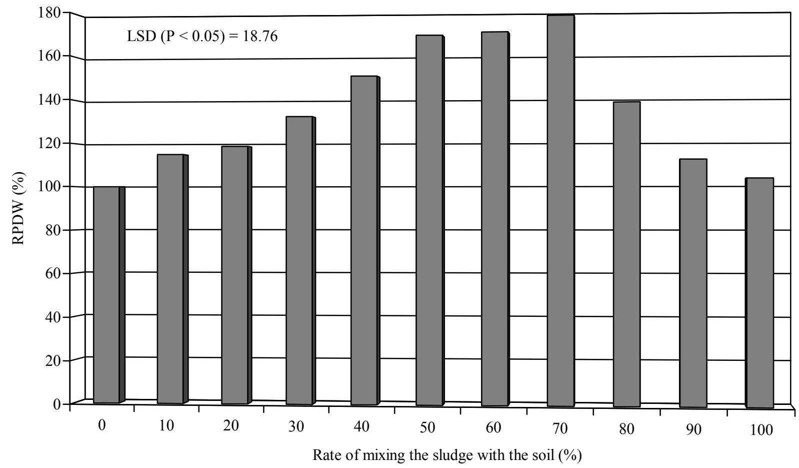

Fig. 1. Effect of sewege sludge obtained from Hódmezővásárhely on relative wheat plant dry weight (RPDW %) cultuivated in meadow chernozem soil of Szeged for 50 days

Our results are in agreement with Krauss & Diez (1997) who mentioned that the morphological characters of spring wheat that cultivated in sandy soil contaminated with heavy metal originated in sludge were more increased than the control. Microorganisms in the soil, including fungi, bacteria and actinomycetes mediate many of the processes that influence soil fertility. Sludge application to soil generally raises the activity of soil microorganisms by increasing soil organic matter content. Our results are supported by the above mentioned works, where the microbial population were increased by increasing the rate of sludge mixed with soil. Sludge applied to the soil may reduce the activity of dehydrogenase (Reddy & Faza 1989), urease and phosphatase (Reddy *et al*. 1987). Our results are in agreement with Aichberger & Öhlinger (1988) who determined that dehydrogenase and protease activity increased in soil supplied with metal contaminated sewage sludge. There was no effect of sludge application on glucosidase and urease activity, whereas phosphatase declined only at large rates of applied sludge. Brookes et al. (1984) also reported that phosphatase activity was not affected by elevated soil metal concentrations due to sludge application. It is concluded that the use of sludge in agriculture as a partial substitute of fertilizers and as an amendment for soil physical properties is a common method and seems to be the only environmentally sound practice.

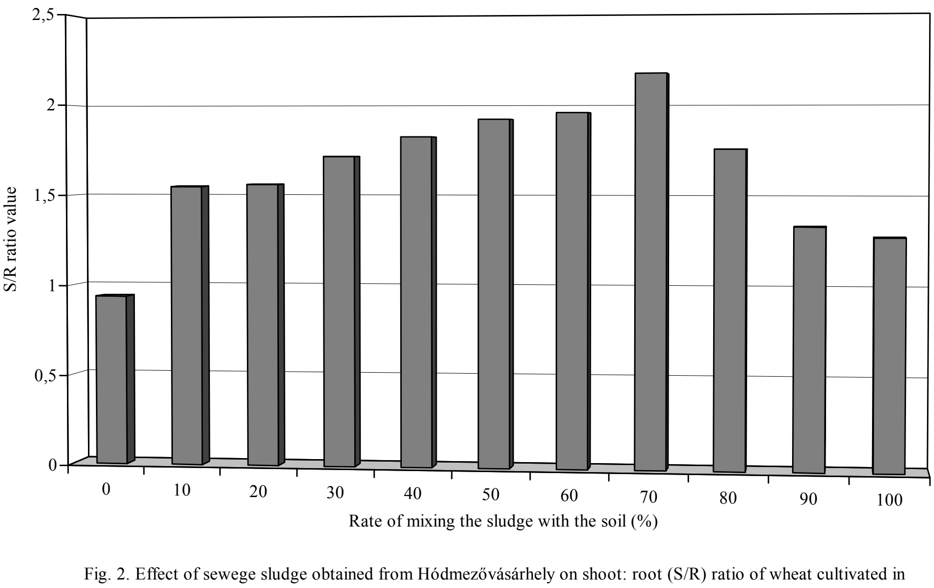

Fig. 2. Effect of sewege sludge obtained from Hódmezővásárhely on shoot: root (S/R) ratio of wheat cultivated in meadow chernozem soil of Szeged for 50 days

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