# Effects of Sewage Sludge on the Parameters of the Crop Production and Influence on Some Phytopathogenic Soil Fungi

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**Abstract**

Many types of organic wastes, including sewage sludge are used for supplementing the soil with the organic matter. The effects of this kind of fertilize are often compared to those of manure. We observed that there was no harmful impact on the winter wheat health if sewage sludge were added to the soil. In the laboratory tests we showed that water extract of sewage sludge as well as humic substances isolated from them were not sources of nutritive substances for tested fungi. Although some changes made in sewage sludge after freezing or autoclaving made them friendly for fungi. *Pseudocercosporella* isolates were more sensitive for tested substances than *Fusarium culmorum*. We observed an inhibition of fungal growth but it was mostly due to bacteria.

**Keywords**: fungi; *Pseudocercosporella*; *Fusarium culmorum*; humic substances; sewage sludge; crop production

## INTRODUCTION

Reduction of humic substances, frequently observed in studies of soils, constitutes a significant indicator of soil degradation. Supplementing the soil with organic matter is therefore necessary. One of type of organic matter is sewage sludge, which is considered to use as a fertilizer and often compared to effects of manure. Simultaneously, the quality of plant and crop production often depends on the soil features. One of the problem is the plant health condition under the influence of sewage sludge, which can also influence on the phytopathogenic soil fungi as well as others microorganisms. In the soil treated with sewage sludge WIELGOSZ (1996) observed the increase of bacteria and actinomycetes number, simultaneous total fungi number did not changed. Similar observations had CHEN *et al.* (1994), SZWED *et al.* (1999) observed stimulation of plant growth parameters under the influence of organic matter originating from compost. Since now not many research was made on the influence of organic matter originated from sewage on fungi, although TUITERT *et al.* (1998) showed that composed household waste can suppress the *Rhizoctonia solani* development.

The aim of this work was to determine in the field, if supplementing of the soil with sewage sludge can influence on the plant health condition comparing to the cattle manure. We also studied the influence of sewage sludge and humic substances, isolated from the sewage sludge, on the three soil phytopathogenic fungi.

## MATERIALS AND METHODS

The field experiment was performed on the arable lands (Cambisoils developed from silts clay with low content of total organic carbon – 0.98%) in the Experimental Station in Glubczyce, Poland. Sewage sludge was added to soil 30, 50 or 80 mg/ha, comparing to not treated control and to soil with 30 mg/ha of cattle manure. Fields were sown with winter wheat and intensively cultivated. Test was made in four replications by the random plots method. Winter wheat

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resistance in the field was determined in the 9 degree scale (9 means healthy plants).

The sewage sludge used in the experiments (from The Sewage Treatment Plant of Opole) was stored for 12 years. The following chemical and physicochemical parameters were determined in sewage sludge samples: pH – 6.8, macroelements in mg/l: N-$NO_3$ – 412, P – 26, K – 130, Ca – 8 150, Mg – 182 and microelements in mg/l: Zn – 44, Cu – 2.95, Mn – 6.2, Fe – 75, B – 0.96, salinity – 1.35 g/l. Elemental composition of humic acids in sewage sludge, extracted by the SCHNITZER (1987) method characterized and atomic ratios calculated. The content of organic carbon and fractional composition of humus was also determined by Ponomariewa-Płotnikowa (DZIADOWIEC & GONET 1999).

In the laboratory tests three isolates of fungi were used: *Fusarium culmorum*, *Pseudocercosporella herpotrichoides* type R (*P. acuformis; Tapesia acuformis)* and *P. herpotrichoides* type W *(Ramulispora herpotrichoides; T. yallundae)*. Inocula (0.6 cm diam.) were prepared of three week old cultures. Water extracts were prepared as follows: 250 g dry weight of sewage sludge were shaken for 24 hours in 1 000 $cm^3$ of distilled water. Water solution consisting of (in mg/l): NO-$NO_3$ – 219, P – 62, K – 140, Ca – 1 360, Mg – 215, Na – 130, Zn – 0.1, Cu – 0.1, Mn – 0.3, Fe – 0.2. The total organic carbon was determined by Orlov methods (DZIADOWIEC & GONET 1999) and was 80 mg/l. Media: PDA and water agar (WA), were prepared as in MOLISZEWSKA and PISAREK (1996) with 20% or 50% of humic acid (HA) or water extract of sewage sludge (SS). Also autoclaved (AS) or frozen in –18°C (FS) for 24 hours samples of sewage sludge water extracts were used. Test was made in four replications. The fungi growth inhibition or stimulation was determined in the relation to the control according to the Abbott's formula modified by subtraction from each colony an inoculum diameter (KOWALIK & KRECHNIAK 1961). In the end of the test all inocula from dishes, were no growth was observed or growth was very minute, were transferred to PDA media to check its vitality, for *F. culmorum* also three 0.6 cm discs adjacent to each inoculum were taken and for others fungi a piece of medium from under the inoculum.

The model soil was prepared in tubes with clean and sterilized sand enriched by 2 $cm^3$ of 20%, 50% or 100% solution of HA or SS, in control tubes liquid Czapek Dox medium was added. All tubes were prepared in three replications. After three weeks, the fungi development was checked as in MOLISZEWSKA and PISAREK (1996) using the scale: 0 – no growth; 1 – a minute growth to 10% of a dish area; 2 – more than 10% to 50% overgrown area; 3 – more than 50% to 70% overgrown area; 4 – to 90% overgrown area; 5 – 100% overgrown area. For *F. culmorum* the measurement was made after three days and for other fungi after a week.

Statistical calculation was made by the variance method, significant differences between averages were measured according to Duncan's multiple range test ($P$ = 0.05).

## RESULTS

Fractional composition of sewage sludge humus showed that fulvic acids predominate and the $C_{HA}$:$C_{FA}$ value was 0.85. The value of the colour index of humic acids was 8.4, which, according to SCHNITZER (1987), indicates low content of carbon in condensed aromatic parts of humic substances (Table 1). Elemental composition of humic acids in atomic % showed content of C – 25.69, H – 39.98, N – 0.95, S – 0.19, O – 14.63. This means that the characteristic process of humification of analyzed fertilizer was oxidation. The calculated H:C index equal 1.56 indicates a high content of aliphatic side chains.

Field test showed that winter wheat yield was from 825 to 850 g/$m^2$. The soil amendment with sewage sludge has no influence on the yield and the results were not statistically different. Also the health condition of wheat leaves, ears and straws base were determined in scale as "9", in all cases plants were healthy.

Tests carried out in our laboratory showed that an addition of humic acid or sewage sludge extract to the medium can inhibit the growth of all the tested fungi, but on PDA medium amended with SS many

Table 1. Fractional composition of humic substances of sewage sludge (in % of C – total) by Ponomariewa-Płotnikowa

| $C_{total}$ | $C_{bitumin}$ | Fractions of HA | | | | Fractions of FA | | | | | HA/FA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Σ | 1a | 1 | 2 | 3 | Σ | |
| 14.09 | 16.05 | 4.68 | 16.19 | 7.45 | 28.32 | 4.26 | 7.45 | 12.76 | 8.94 | 33.41 | 0.85 |

bacteria colonies were observed (10–20 per 1 $cm^2$) and on dishes with HA 20%: 2–5 colonies of *Penicillium* sp. were denoted per dish; with HA 50%: 7–15 – *Penicillium* sp. on dish and to 20 bacterial colonies per 1 $cm^2$. On WA medium bacteria were not visible, but during checking the fungal vitality we observed bacteria development around pieces taken from test WA media and placed on PDA. Test showed that the inhibitory properties of HA or SS were mostly dependent on bacteria, although both substrates were not attractive as a carbon and nutritive source, that was clearly visible on the part of the test with WA medium (Table 2). We observed that both *Pseudocercosporella* were more sensitive for HA or SS than *F. culmorum* (Table 2). The addition of FS to PDA confirmed the great bacterial activity, because in PDA + 20% FS were more contaminated by them than PDA + 50% FS medium, where fungi growth stimulation was observed (Table 2). Also a part of the test with AS confirmed that inhibitory properties are due to bacteria (Table 2). The bacteria determination showed that they were: *Flaviomonas oryzihabitans* (from HA), *Bacillus amyloliquefaciens* (from SS). Checking the fungi vitality showed that pieces taken from WA + HA media always were strongly surrounded by bacteria and mycelium of non fungus was not visible, in other cases (PDA + HA or PDA + SS) fungi growth was observed.

Table 2. Influence of sewage sludge or humic acids on the growth of tested fungi in different media; a sign "minus" means the growth stimulation comparing to the control

| Medium | Growth inhibition or stimulation (%) | | |
|---|---|---|---|
| | *F. culmorum* | *P. herpotrichoides* type R | *P. herpotrichoides* type W |
| PDA + 20% HA | 48.90 c * | 21.96 c | 13.93 d |
| PDA + 50% HA | 88.86 d | 94.96 e | 100.00 g |
| PDA + 20% SS | 81.79 d | 100.00 e | 100.00 g |
| PDA + 50% SS | 87.09 d | 100.00 e | 93.50 f |
| PDA + 20% FS | 11.08 b | 74.45 d | 83.76 e |
| PDA + 50% FS | –33.62 a | –1.91 b | –10.43 c |
| PDA + 20% AS | –36.49 a | –34 20 a | –26.67 b |
| PDA + 50% AS | –40.09 a | 10.03 bc | –32.63 a |
| WA + 20% HA | 74.10 a | 100.00 a | 96.19 a |
| WA + 50% HA | 94.82 c | 100.00 a | 97.14 b |
| WA + 20% S | 86.19 b | 100.00 a | 100.00 c |
| WA + 50% S | 84.67 b | 100.00 a | 100.00 c |

*data indexed by the same letter are statistically not significantly different ($P = 0.05$) in the same column and for the same medium (PDA or WA)

Table 3. Influence of sewage sludge or humic acids on the fungi growth in the sand medium

| Addition of | The Petri dish overgrown area (averages according the scale) | | |
|---|---|---|---|
| | *F. culmorum* | *P. herpotrichoides* type R | *P. herpotrichoides* type W |
| Control | 4.7 c* | 3.4 c | 0.6 a |
| 20% HA | 3.3 b + ** | 1.2 a ** | 0.8 a** |
| 50% HA | 1.9 a + ** | 1.0 a ** | 1.0 ab ** |
| 100% HA | 2.4 ab + ** | 1.0 a ** | 1.0 ab ** |
| 20% S | 3.6 b | 1.9 b ** | 1.7 c ** |
| 50% S | 3.1 b | 2.0 b ** | 1.7 c ** |
| 100% S | 2.4 ab | 2.0 b ** | 1.4 bc ** |

*data indexed by the same letter are statistically not significantly different ($P = 0.05$) in the same column

**only bacteria or *Penicillium* spp. were observed

In the artificially composed model soil also strong bacterial activity from HA or SS was observed for both *Pseudocercosporella* spp. as well as in a case of *F. culmorum* treated by HA (Table 3). This test also confirmed that especially *Pseudocercosporella* isolates were sensitive for HA or SS, although their fungistatic activity was mostly due to bacteria.

## DISCUSSION

Data obtained in these research showed that raw sewage sludge extract as well as humic substances extracted from the soil treated by sewage sludge can not be used by fungi as a source of nutritive substance even in they are the only source of the carbon in the medium. The mechanism of their action is not clear, but now we can say that bacteria play an important role in the inhibition of tested phytopathogenic fungi. KUTER *et al.* (1988) found that mixtures of composted sewage sludge and peat turned suppressive when they had been stored three to four weeks before use, so this observation is similar to ours. The increase of bacteria and actinomycetes content in the soil amended with sewage sludge or compost observed CHEN *et al.* (1994), WIELGOSZ (1996) and SZWED *et al.* (1999). A possible inhibitory role of compost for fungi showed TUITERT *et al.* (1998). An enigmatic role played FS or AS, because they could not inhibit fungal development on PDA medium and mostly in the case where they were added the stimulation of fungi was observed. We think that probably some chemical changes made them more friendly for fungi, especially that we observed precipitates after freezing or autoclaving. This problem requires an additional research. In our research we shoved that tested fungi can not use HA or SS as a source of nutritive substances and they even can be inhibited by these substances, we also showed that an addition to the soil of sewage sludge did not influenced on the wheat health condition, so taking the biological activity of sewage sludge into consideration, we can say that they were not worse than manure in the field and that they can be used as a potential source of organic matter for the soil with its low content. Comparable research done by PISAREK (2001) showed that humification processes at composed sewage sludge and cattle manure were similar. This observations are confirmed by researches of others authors, they did not observed any negative influence of sewage sludge on plant and they even showed that humic substances can stimulate them (CHEN *et al.* 1994; PISAREK 1999).

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