THE SOIL MICROBIAL COMMUNITY RESPONSE TO ADDITION OF FRESH SLUDGE ARISING FROM WASTEWATER TREATMENT

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Abstract. *Numerous studies concerning the use of sludge were done on the nutritional properties of their in order to increasing production, only some studies have been done in our country in terms of ecological impact on soil microbial composition, richness or diversity. The main objective of our research was to assessment the impact of activated sludge on soil microflora and monitoring the presence of pathogenic bacteria with a high risk of contamination for human and animals in case of two cultures of forage crops Dactylis glomerata, and Medicago sativa. For this reason we initiated a field experience in spring of 2012 on Chernozem soil type from SDE Timisoara. The field experience ordered in Latin rectangle model assessed the impact of sludge applied in three doses respectively 15, 20 si 40 tones•ha-1 on. The microbial assays involved estimation of bacteria number, actinomycetes and fungi, and in the second weeks were analyzed the richness of pathogenic bacteria like total number of enterococcus bacteria, coliform bacteria and Escherichia coli. The results show that there are different answers regarding the number of edaphic microorganisms for the treatment administered. So, the larger difference compared with the control was recorded for bacteria number from soil under D. glomerata grass culture for dose of 40 t / ha sludge. In case of fungi the largest number were determined for 15 t ha sludge on soil under D. glomerata grass, and M. sativa culture also. But it was noted that increasing the quantities of sludge applied both to D. glomerata and M. sativa, determine decreases of the number of fungi in soil. After 2 weeks from sludge application did not find any presence of biological contaminants as fecal pollution indicators like Escherichia coli. A higher persistence showed enterococcus bacteria. In conclusion application of wastewater sludge causes temporary changes in the structure and composition of the soil microbial community for a period of time determined by the crop plant and weather. It seems that a drought period installed after applying the sludge has determined a fastest elimination of the pathogenic bacteria.*

Keywords: *edaphic bacteria, actinomycetes, Escherichia, enterobacteria*

INTRODUCTION

It is know that implementation of the Council Directive on Urban Waste –Water Treatment from 1991has lead to increasing of sewage sludge production in the European Union countries (EC, 1991). In our country the use of sewage sludge is regulated by Directive Order 344/2004 (MO 959, 2004) from their technical norms for use in agriculture in order to environmental and soil protection. The sewage sludge from waste water treatment have more than 95% of moisture so in order to reduce its mass and volume can be subjected to different processes. The most frequently treatments are drying in this way resulting biosolids with 18-35% of dry matter content and organic compounds stabilized. From biological point of view aerobic stabilization is the best practice to reduce the human hazardous microorganisms and ensuring the sludge sanitation. Furthermore introducing of additional post-treatments for sludge as thermal drying or composting for the improvement of biosolids properties is often necessary (SHANAHAN ET AL, 2010; ROIG, et al, 2012).

It is largely know that sludge can be use in agriculture and may improve soil physicochemical properties, soil porosity, bulk density, aggregate stability, water holding capacity and

increasing of soil organic matter (TEJADA M.2009) . Also, the sludge can affect soil biological properties by increasing the soil microbial biomass and its mineralization potential (CLARKE AND SMITH, 2011). On the other hand, the accumulation of heavy metals contained in the sludge is an important factor to consider within sludge managements as these compounds can have a notable affection on the soil functioning and its biodiversity (TAS, 2010), (ANDRES, et al, 2011).

In the present paper the main objective was the assessment of activated sewage sludge impact on soil microflora and monitoring the presence of pathogenic bacteria with a high risk of contamination for human and animals.

MATERIAL AND METHODS

In spring of 2012 were initiated two fields experiences in SDE Timisoara area on Chernozem soil type. The fields of forage crops *Dactylis glomerata*, and *Medicago sativa* were ordered in Latin rectangle model, see Table 1, for assess the impact of sludge applied in three doses respectively 15, 20, respectively 40 tones•ha^{-1}.

Table 1.

Graphical representation of the field experiment with variants and repetitions ordered in Latin rectangle model for each forage crop

R_{II} V_3	V_1	V_2	5 m 3 m V_0	V_3	V_1	V_2	V_0
R_{II} V_2	V_3	V_0	V_1	V_2	V_3	V_0	V_1
R_I V_0 control	V_1 15 t·ha^{-1}	V_2 20 t·ha^{-1}	V_3 40 t·ha^{-1}	V_0	V_1	V_2	V_3

The fresh sludge was obtained from wastewater treated with activated sludge by aerobic digestion coming from the new wastewater treatment plant from Timisoara. The new station has the cumulative volume of sewage basins of 106,000 cubic meters and one third part of each receives a reduced air supply to favor denitrification process. From aeration basin the activated sludge is discharged into storage tanks. Phosphate binders chemical treatments as $Fe_2(SO_4)_3$ were applied in three-point first in distribution room of biological basins, second at the end of denitrification process and the last in room distribution at the exit of the aeration basin. From the storage tanks, sludge is sent to press dehydration. On the feed line press of sludge was administered cationic polyelectrolyte that promotes dehydration. The raw sludge used has 19 to 22% dry matter content. The levels of metal and organic contaminants in the final sludge product were measured in accord to SR ISO and are in accord with limits considered by Ord 344/2004 concerning application of technical regulations for environmental and especially soil protection when sewage sludge is used in agriculture (Table 2.). From the analysis of sewage sludge arising from SC Aquatim S.A. Timisoara is noted that it has a neutral pH (7.37), 83.42% water content, and the total organic carbon content is about 64%.

Table 2.

Parameters of the soil and sewage sludge content used and the maximum allowable concentration of heavy metals in sludge for agricultural use (according to Ord 344/2004).

Parameters/ elements (units)	Chernozem soil (on dry content)	Sewage sludge (on fresh content)	Sewage sludge (on dry content)	Maximum allowable concentration of heavy metals in sludge for agricultural use (on dry content) (Ord 344/2004)
pH	7.46	7.37	-	-
Total nitrogen (%)	0.31	1.89	11.39	-
PAL	14.19		-	-
KAl	246.0		-	-
Total Phosphor,(%)	0.534	1.03	6.21	-
Total Kalium (%)	1.23	0,23	1.38	-
Water content (%)		83,42	0	-
TOC (%)		64,00	386	-
Copper (ppm)	2.6	31,45	189.68	500
Manganese (ppm)	386.0	460,2	2775	-
Lead (ppm)	12.4	7,65	46.1	300
Zinc (ppm)	73.6	109,8	662.2	2.000
Calcium (%)	0.86	0,36	2.17	-
Magnezium (%)	0.41	0,13	0.78	-
Chromium (ppm)	16.12	10,07	60.73	500
Nickel (ppm)	26.80	4,97	29.97	100
Cadmium (ppm)	0.28	0,23	1.38	10

To accomplish the main objective were conducted (1) the estimation of total number of mesophilic microorganisms on the group, respectively bacteria, actinomycetes and fungi after 10 days from sewage sludge application, and (2) detection of fecal pollution indicator bacteria like total coliform bacteria (TCB), *Escherichia coli*, and total enterococcus bacteria (TEB) in soil variants treated with sewage sludge after 2 and 4 weeks from application. The soil microbial determination were made on specific culture media, respectively for eubacterias Nutrient Agar (meat extract 1 $g \cdot L^{-1}$, yeast extract 2 $g \cdot L^{-1}$, peptone 5 $g \cdot L^{-1}$, NaCl- 5 $g \cdot L^{-1}$, agar-15 $g \cdot L^{-1}$), for actinomycetes Gause synthetic medium (soluble starch 20 $g \cdot L^{-1}$, KNO_3-1 $g \cdot L^{-1}$, $MgSO_4$-0.5 $g \cdot L^{-1}$, NaCl-0.5 $g \cdot L^{-1}$, K_2HPO_4-0.5 $g \cdot L^{-1}$,$FeSO_4$ -10mgL^{-1},agar15 $g \cdot L^{-1}$, distilled water), and for fungi Martin medium (glucose 10 $g \cdot L^{-1}$, peptone- 5 $g \cdot L^{-1}$, K_2HPO_4-0.5 $g \cdot L^{-1}$, KH_2PO_4-0.4 $g \cdot L^{-1}$, MgSO4-0.5 $g \cdot L^{-1}$, rose Bengal- 30mg, agar-12 $g \cdot L^{-1}$, yeast extract 0.5 $g \cdot L^{-1}$, streptomycin sulfate -30mg, distilled water).

Detection of fecal pollution indicator bacteria were made in accord to SR ISO ISO 9308-1/2004 AC 2009 for TCB detection and *Escherichia coli* using TSA medium (pancreatic digest of casein -15 $g \cdot L^{-1}$, soy peptone -5 $g \cdot L^{-1}$, NaCl- 5 $g \cdot L^{-1}$, agar -15 $g \cdot L^{-1}$, distilled water) and TBA medium (casein enzymatic hydrolysate -20 $g \cdot L^{-1}$, bile salts mixture -1.5 $g \cdot L^{-11}$, agar - 15 $g \cdot L^{-1}$, distilled water). For TEB detection was used Slanetz-Bartley medium (tryptose -20 $g \cdot L^{-1}$, yeast extract - 5$g \cdot L^{-1}$, K_2HPO_4 - 4 $g \cdot L^{-1}$, glucose -2 $g \cdot L^{-1}$, sodium azide 0.4 $g \cdot L^{-1}$, TTC - $g \cdot L^{-1}$, bacteriological agar 10 $g \cdot L^{-1}$) were made in accord to SR EN ISO 7899-2/ 2002.

RESULTS AND DISCUSSIONS

After 10 days from sewage sludge application on forage fields of *D. glomerata* and *M. sativa* the richness of soil bacteria is variable depending by sewage sludge quantity that was administered, see Figure 1A. Thus, in the variant V40G, with 40 t·ha^{-1} of fresh sludge it was recorded the biggest number of soil bacteria, the difference compared to control is statistically assured as very significant. At the same time, from the soil fields cultivated with *M. sativa* the richness of soil bacteria were registered decreases, mostly in V20L and V40L on addition of fresh sludge, the differences are statistically assured in the both cases. Concerning the composition of bacteria from soil microflora it was determined a high frequency of occurrence on plates for genus *Arthrobacter*. These bacterial species were appeared with frequency of 33% higher for V40G variant compared to bacteria isolated from control soil.

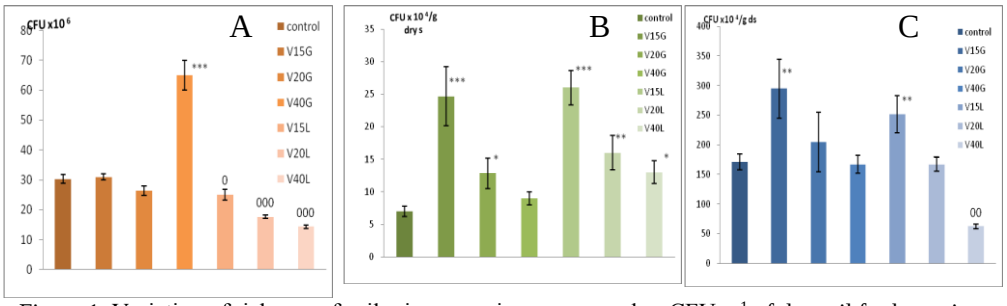

Figure 1. Variation of richness of soil microorganism expressed as CFU·g^{-1} of dry soil for bacteria (A), fungi (B), and actinomycetes (C)

Concerning the effect of fresh sewage sludge on richness of fungi from the figure 1 B we can see that incorporation of 15 t / ha sludge stimulates and supports the growth of fungi in the soil, in both fields grasses *D. glomerata* and *M. sativa*. The addition of greater amounts of sewage sludge to 20 and 40 t/ha has led a decreasing of the number of fungi from soil perhaps for cumulative effects of chemical compounds that generate ecotoxicological effects.

As shown in Figure 1C actinomycetes responded positively to the administration of 15 t / ha sewage sludge while increasing the dose determines an inhibiting effect, the difference from the control being evaluated as significant. Among actinomycetes *Streptomyces* sp was predominant followed by *Nocardia* sp.

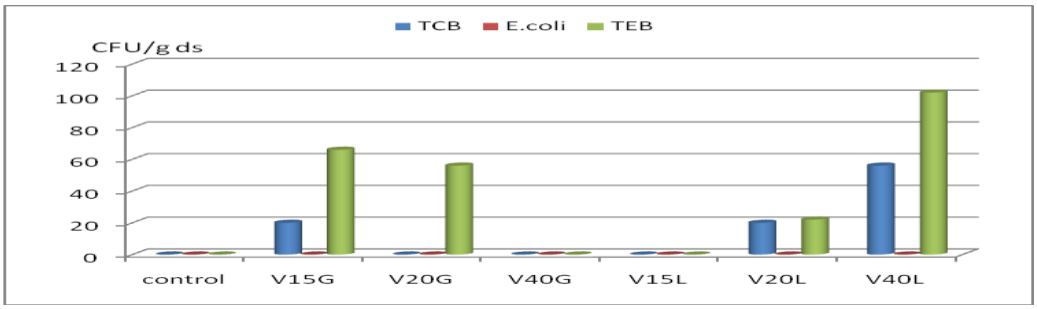

Figure 2. Detection of TCB (total coliforms bacteria), E. coli and TEB (total enterococcus bacteria) after 2 weeks from application of raw sludge sewage in *D.glomerata* (15G, 20G, 40G) and *M. sativa* (15L, 20L, 40L) fields.

The initial charge of raw sewage sludge with potentially pathogenic bacteria for humans was high. The highest number was detected in case of TCB about $115,04x10^4$ CFU/gr fresh sludge, follow by TEB, $88,4x\ 10^4$ CFU/gr fresh sludge and *E. coli* $50,1x\ 10^4$ CFU /gr fresh sludge. After 2 weeks *E coli* was totally inactivated, number of these bacteria it was null in every samples of variants. However, it seems that the enterococcus bacteria have a better resistance in edaphic environment since TEB were recorded a bigger number after 2 weeks in V40L and smaller in V15G, V20G and V20L. The coliformbacteria were still there in soil after 2 weeks from application, TCB was detected in V15G, V20L and V40L.

CONCLUSIONS

Our results clearly show that a amount of equivalent corresponding dose of 15 t / ha fresh sewage sludge has a positive influence on soil indigenous fungi and actinomycetes. Also, how much the amount of fresh sludge is higher the number of TEB is larger after two weeks of time. *E coli* disappear from the soil due to the phenomenon of „self-purification". On the other hand a drought period installed after applying the sludge has determined a fastest elimination of human pathogenic bacteria.

Despite the potential of sewage sludge to have harmful effects on the soil microorganisms, it is important to note that the amount of metal elements and the pH of soil would limit using the raw sewage. Thus it is necessary to promote a sustainable use of sewage sludge in agriculture in order to promote soil fertility and to avoid secondary effects concerning ecotoxicological aspects on soil microflora.

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