Scientific Papers. Series A. Agronomy, Vol. LVII, 2014
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF ORGANIC AMENDMENTS EFFECTS ON SOIL MICROORGANISMS AND ENZYMATIC ACTIVITIES OF DEGRADED CHERNOZEM

Irina SENICOVSCAIA

"Nicolae Dimo" Institute of Pedology, Agrochemistry and Soil Protection, Chisinau, 100 Ialoveni str., MD 2070, Chisinau, Republic of Moldova

Corresponding author email: irina_sen@mail.ru

Abstract

Microbiological and enzymatic properties of the long term stored municipal sewage sludge from 11 cities of the Republic of Moldova were investigated in connection with the problem of its utilization. The microbial biomass content in the sewage sludge fluctuated from 0.32 to 6.72 mg C g^{-1} waste, ratio between microbial and organic carbon content – from 0.70% to 5.09%, coli-titer index from >0.1 to 0.0001 respectively. As a result of keeping wastes and mineralization processes intensification, organic carbon, microbial biomass and enzymatic activity reduced. Microbiological and biochemical parameters depended on storage duration of wastes and organic matter content. The correlation coefficient between organic carbon content and microbial biomass constituted 0.74, catalase activity – 0.68. Using of the municipal sewage sludge as a fertilizer for reducing of the soil degradation is possible only after the preliminary composting in the combination with sugar industry waste materials (sugar lime) and manure. As a result of composting wastes sanitary indicators were improved. Organic amendments ramped up microbiological and enzymatic properties of degraded chernozem. Wastes amended soil showed a significant increase in the microbial biomass, enzyme activities and the humus content in 12 years after the application of composts.

***Key words**: coli-titer, compost, degraded chernozem, enzymatic activities, microbial biomass, sewage sludge.*

INTRODUCTION

Recycling of the municipal sewage sludge is the actual problem in the Republic of Moldova. The amount of the sewage sludge produced annually in the country is about 35.5 thousand tons of the dry weight or 71.0 thousand tons with 50% of humidity (Rusu et al., 2012). The organic matter content in the waste with the natural moisture constitutes in average of 43.5% and the content of heavy metals does not exceed the maximum permissible concentration after the purification (Rusu et al., 2012).

The motivation for using sewage sludge on agricultural land lies in its value as a nutrient-rich fertilizer and source of organic material. For several decades, controlled application of sewage sludge to agricultural land has been a major route for disposing of sludge (Tsurkan et al., 1989; Banaru et al., 2003; Arhip, 2011; Rusu et al., 2012).

The sewage sludge is the valuable fertilizer, but may represent an environmental hazard from the standpoint of the fecal pollution and the possible threat for worsening epidemiological situation. It is known that sewage sludge and municipal solid waste samples can contain hazardous substances and pathogenic organisms and are liable to undergo biological action (Gerba, 1987). Untreated sewage sludge contains a wide variety of pathogens, including bacteria, viruses, fungi, cysts and eggs of parasites. These pathogens can potentially pose a risk to the environment, public health and food safety when sludge is applied to agricultural land (Santamaría and Toranzos, 2003). The permanent microbiological monitoring of the long stored precipitations and soils after their application in the republic is not carried out, although during the long period the wastes management had been searching the ways in order to obtain economic benefits and reduce the negative effect on the environment.

Standards for the treatment of sewage sludge are proposed in numerous regulations and guidance on best practice for its beneficial use. They are also been described in many codes of practice covering the use of sludge in agriculture of different countries (Board on Environmental Studies and Toxicology, 2002;

Standing Committee of Analysts, 2003; Sanitary regulations and standards, 2010). A variety of treatment methods, such as composting, aerobic and anaerobic digestion, alkaline stabilization, conditioning, dewatering and heat drying, is used in wastewater-treatment plants to reduce pollutants and to destroy pathogens (European Commission, 2001).

Using of the municipal sewage sludge as a fertilizer for reducing the soil degradation is possible only after the preliminary composting. Composting of sewage sludge has been practiced long ago and is a satisfactory method for handling these wastes (Tsurkan et al., 1989). The problems with composting wastewater sludge are the high moisture content, the density of the sludge particles, pathogenic microorganisms and a potential high oxygen demand. Usually sewage sludge is combined with other wastes. In this procedure the sludge is first mixed with bulking products such as straw. The promising way of the sludge' utilization is their mixing with the sugar industry waste material, an alkaline reaction, for the purpose of the decontamination from pathogens.

The purpose of the research was to evaluate the microbiological status of sewage deposits in cities of the country and to determine the effect of composts with the sewage sludge on microbiological and enzymatic properties of the leached chernozem, degraded as the result of the long-term agricultural utilization.

MATERIALS AND METHODS

Sewage sludge samples from 11 cities of the Republic of Moldova were investigated in connection with the problem of its utilization. The storage duration of wastes ranged from 1 to 20 years. Composts with the sewage sludge and straw of 2% were examined in the dynamics in 1 and 3 months and in 1 and 1.5 years.

Experimental site. The site was located in the center of the country, in the Ivancha village, Orhei region. The long-term arable soil without fertilizers (control), sewage sludge and composts with sewage sludge treatments were tested in 12 years after the application of fertilizers. Investigations were carried out on plots with the sunflower, soybean and the winter wheat. In 1986 sewage sludge was introduced in the dose of 40 t ha^{-1}. The other plot was amended with the compost from the 40 t ha^{-1} of sewage sludge, 40 t ha^{-1} of defecate and 40 t ha^{-1} of manure. Organic fertilizers were introduced under the first crop rotation links culture (Serjentu et al., 1991). The soil at the site is the leached chernozem with humus content of 4.22%, total nitrogen – 0.22%, phosphorus – 0.105%, potassium – 1.90%; hydrolytic acidity was 2.5 me 100 g^{-1} and pH = 6.6-7.2 in the topsoil (Serjentu et al., 1991).

Soil samples were collected from the 0-30 cm layer of the experimental plots in the spring.

Microbiological properties. The microbial biomass carbon was measured spectrophotometrically by the rehydratation method based on the difference between carbon extracted with 0.5 M K_2SO_4 from fresh samples and from samples dried at 65-70°C for 24h, with K_c coefficient of 0.25 (Blagodatsky et al., 1987). Counts of microorganisms were obtained on agar plates (Zvyagintsev, 1991). The number of lactose-positive E. coli (coli-titer) was determined by the fermentation method with the subsequent seeding at the *Endo* medium. The CO_2-producing capacity of composts was measured by the titration method (Zvyagintsev, 1991).

Enzymatic activity. The potential enzymatic activity was determined in samples of the air-dry soil and waste. The catalase activity was determined by the volumetric method by the rate of hydrogen peroxide's decomposition during its interaction with the soil and by the volume of released oxygen (Haziev, 2005). The urease activity was measured by estimating the ammonium released on incubation of soil with buffered urea solution by colorimetrical procedures (Haziev, 2005). The dehydrogenase activity was determined colorimetrically by the presence of triphenylformazan (TPF) from 2, 3, 5-triphenyltetrazolium chloride added to air-dry soil (Haziev, 2005). The polyphenoloxidase and peroxidase activities were determined colorimetrically using hydroquinone as a substrate (Karyagina and Mikhailovskaya, 1986).

Soil chemical properties. Organic carbon was analyzed by the dichromate oxidation method.

Microbiological, enzymatic and chemical

indices were evaluated by the analysis of variance and correlation analysis.

RESULTS AND DISCUSSIONS

Microbiological characteristics of municipal sewage sludge. Sewage sludge is the material with a slightly alkaline reaction (pH = 7.5) and a considerable humidity (47.8%); it has a high content of organic matter, in average of 43.5% in terms of the natural moisture content (Rusu et. al., 2012). As a result, the raw waste has a high number of bacteria, CO_2-producing capacity and dehydrogenase activity (Table 1). It has been determined that sewage sludge is a biologically active material, in which bacteria are predominant among the microbial community. The number of fungi is insignificant. Sewage sludge contains methane bacteria in the amount of 350 $\cdot 10^3$ CFU g^{-1}. The traditional indicator, such as the contents of *E.coli* demonstrated a high degree of the fecal contamination of the waste, coli-titer was 0.0000001. This value significantly exceeds the sanitary standard for the sewage sludge which has been developed in different countries (Standing Committee of Analysts, 2003; Sanitary regulations and standards, 2010).

The long term stored municipal sewage sludge demonstrates the substrate which is enriched with microorganisms (Table 2).

Table 1. Microbiological and biochemical characteristics of the fresh sewage sludge (Kishinev city)

Heterotrophic bacteria	***Nocardia***	**Actinomycetes**	**Methane bacteria**	**Fungi**	**CO_2**	**Dehydrogenase activity**
CFU* $g^{-1}\cdot 10^6$			CFU* $g^{-1}\cdot 10^3$		mg 100g^{-1} 24h^{-1}	mg TPF 10g^{-1} 24h^{-1}
1286.8	249.3	22.8	350	5.4	1.91	11.8

Table 2. Microbiological and enzymatic indices, the carbon content in the municipal sewage sludge in the Republic of Moldova

City	**Storage period, years**	**C_{org}, %**	**Microbial biomass C, mg C g^{-1} waste**	**C_{MB}/C_{org}, %**	**Catalase, cm^3 O_2 g^{-1} waste min^{-1}**
Balti	2-3	11.42	5.81	5.09	11.3
	10	2.63	0.36	1.36	0.9
Floreshti	1	13.26	4.40	3.32	5.4
	10	5.93	1.40	2.36	2.9
Edinets	3-4	21.06	6.52	3.10	5.4
	4-6	4.40	0.32	0.72	0
Faleshti	2-3	19.10	5.77	3.02	10.2
	10	9.18	0.83	0.91	3.7
Ungheni	7-8	17.98	3.49	1.94	2.4
	8-10	18.10	3.84	2.12	0.8
Kishinev	1	11.36	2.54	2.24	6.8
	2-3	15.65	1.53	0.98	3.0
	5-6	13.26	2.38	1.79	6.1
Tighina	1	28.46	6.72	2.36	39.1
	2	11.73	3.15	2.68	10.3
	5	7.52	0.87	1.16	0.4
Orhei	3	12.42	2.03	1.64	2.8
	4-5	4.59	0.64	1.39	0.8
Hyncheshty	1	17.29	3.09	1.79	10.1
	5	6.06	1.81	2.99	3.4
	7-8	10.25	1.10	1.07	2.0
Chimishliya	8-10	13.17	2.01	1.53	1.2
	15-20	13.23	2.31	1.75	3.7
Kahul	1	23.34	1.62	0.70	19.0
	4-5	13.94	1.27	0.91	0.7
	7-8	11.76	2.25	1.91	0.4

The quantity of the microbial biomass in the waste with the 1-4 years of storage is 1.53-6.72 mg C g^{-1} dry waste that in 4-18 times greater than this value in soils.

One ton of the waste contains 3.0-13.4 kg of dry microbial biomass.

The share of the microbial carbon in the total content of the organic carbon in the sewage sludge constitutes of 0.70-5.09%.

The catalase activity can reach values 39.1 cm^3 O_2 g^{-1} waste min^{-1}.

E. coli content in investigated samples is from 0.0001 to 0.1 and above 46% of the samples are characterized by a titer of 0.001; this indicator is 0.01 in 27% of the samples and 0.1->0.1 in 18% of sewage sludge samples. Coli titer is at the level 0.0001 only in 9% of the samples.

All sewage sludge samples were divided into 3 groups according to the duration of the retention period (Table 3).

As a result of the keeping wastes and mineralization processes intensification, the organic carbon content, the total biomass of microorganisms, the ratio between the microbial and total carbon and the catalase activity in the sludge had been reduced, sometimes to the level of the average values of this index in the zonal soils.

Sewage sludge keeping at landfills leads to the marked reduction in the number of *E. coli*. The changes in the index of coli-titer mean that contamination of wastes reduces and their sanitary state improves through the storage.

Microbiological and biochemical parameters depended on storage duration of wastes and organic matter content.

The correlation coefficient between organic carbon content and microbial biomass constituted 0.74, catalase activity 0.68 accordingly.

The dynamics of microbial processes during composting of municipal sewage sludge. The maximum number of heterotrophic microorganisms and actinomycetes was determined in the initial period of composting of the sewage sludge with a straw (Table 4).

Their abundance was reduced in 3.1-11.0 and 1.9-24.5 times respectively as a result of mineralization processes in the compost.

The greatest number of nitrifying bacteria and CO_2 emissions was detected after 3 months of composting. The polyphenoloxidase and the peroxidase activities ranged from 4.0 to 8.0 and from 8.0 to 25.0 mg 1.4-p-benzoquinone $10g^{-1}$compost 30 min^{-1} accordingly. The highest activity of these enzymes was registered after 3 months of composting. Probably the synthesis of humic substances in the compost, in which these enzymes participate, is the most intensive in this period. Composting of the sewage sludge leads to the significant reduction in the number of *E. coli*. Composted waste had a standard titer *E. coli* only after 1.5 years.

Table 3. Influence of the storage duration on the carbon content and microbiological indicators in the sewage sludge (mean values)

Storage period, years	C_{org}, %	Microbial biomass C, mg C g^{-1} waste	C_{MB}/C_{org}, %	Catalase, cm^3O_2 g^{-1} waste min^{-1}	Coli-titer
1-5 (n=11-12)	16.93 ± 3.43	3.92±1.21	2.42 ± 0.74	11.2±6.9	0.0001-0.001
5-10 (n=11)	10.56 ± 2.95	1.68±0.63	1.56 ± 0.42	1.9±1.3	0.0001-0.01
>10 (n=3-4)	5.91	0.86	1.54	2.1	0.001->0.1

Table 4. Dynamics of microbiological and enzymatic indicators in the compost from the sewage sludge and straw (n=3 for each indicator)

Index	1 month	3 month	1 year	1.5 year
Heterotrophic bacteria, CFU g^{-1} compost • 10^6	352.8	114.8	32.0	50.3
Nitrifying bacteria, CFU g^{-1} compost • 10^3	8.0	151.8	17.9	3.5
Actinomycetes, CFU g^{-1} compost • 10^6	412.0	216.3	143.2	16.8
CO_2 emission, mg 100 g^{-1} compost 24 h^{-1}	223.1	339.8	104.3	120.1
Coli-titer	0.0000001	0.000001	0.0001	0.01
Polyphenoloxidase, mg 1,4-p-benzoquinone 10 g^{-1} compost 30 min^{-1}	4.0	8.0	4.8	5.8
Peroxidase, mg 1,4-p-benzoquinone 10 g^{-1} compost 30 min^{-1}	8.0	25.0	15.0	14.0

Effects of organic amendments on soil microorganisms and enzymes.

The use of sewage sludge with the sugar industry waste material and manure aiming to the reduction of degradation processes in the leached chernozem has prolonged after-effects. The use of the compost consisting of sludge (40t ha^{-1}) and sugar lime (40t ha^{-1}) as well as sludge in the dose of 40t ha^{-1} with sugar lime (40t ha^{-1}) and manure (40t ha^{-1}) contributed to the increase of the microbial biomass content from 307.6-380.2 to 363.2-441.6 μg C g^{-1} soil in 12 year after the application into the leached chernozem (Table 5).

Table 5. Microbiological indices and the carbon content in the leached chernozem (12 years after the application of wastes)

Variant	Humus, %	C_{org}, %	Microbial biomass (MB), µg C g^{-1} soil	C_{MB}/C_{org}, %
Sunflower				
Control	3.72	2.16	308.3	1.43
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1}	4.04	2.34	428.2	1.83
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1} + manure, 40 t ha^{-1}	3.98	2.31	441.6	1.91
Soybean				
Control	3.57	2.07	307.6	1.49
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1}	3.57	2.07	375.9	1.82
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1} + manure, 40 t ha^{-1}	3.91	2.27	363.2	1.60
Winter wheat				
Control	3.79	2.20	380.2	1.73
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1}	3.98	2.31	415.8	1.80
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1} + manure, 40 t ha^{-1}	3.92	2.27	428.5	1.89

Growth of the share of microbial carbon in the organic carbon content was observed. Significant changes were determined on the plot with sunflower where the increase of the proportion of microbial and organic carbon was on the 28.0-33.6%. The humus content at the fertilized plots was also higher than the control plot, on average by 0.17-0.25%. *E. coli* has been not detected.

Soil enzymes under organic system with wastes were active even in 12 years after the administration of composts use. Urease activity in the chernozem increased on average of 1.3-2.2 times under the influence of composts and dehydrogenase activity of 1.5-1.9 times respectively (Figures 1 and 2).

A long-term soil management practice with wastes application led to increases in the polyphenoloxidase activity by 2.0-4.3 times and the peroxidase activity by 34.6-62.4% compared to the unfertilized control plot (Table 6). The correlation coefficient between humus content and microbial biomass constituted 0.77, dehydrogenase activity 0.72. Thus, the stabilization of indicators of the microbial community and the humus content at the higher level, compared with the initial, indicates the prolonged action of composts with sewage sludge. As a result of the interaction of wastes with soil microorganisms, soil obtains the other quality.

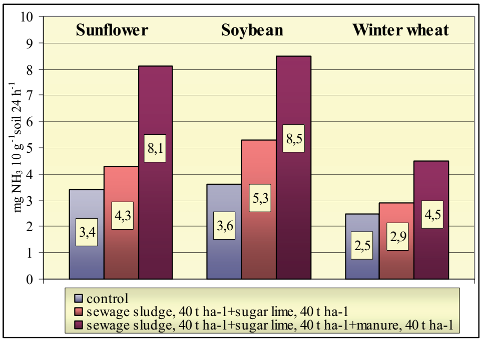

Figure 1. Urease activity of the leached chernozem in conditions of the application of composts with the sewage sludge ($LSD_{0.5}$=1.9)

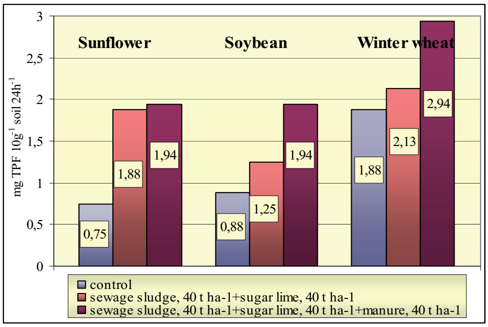

Figure 2. Dehydrogenase activity of the leached chernozem in conditions of the application of composts with the sewage sludge ($LSD_{0.5}$=0.56)

Table 6. Polyphenoloxidase and peroxidase indices of the leached chernozem (12 years after the wastes application)

Variant	Polyphenoloxidase	Peroxidase
	mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	
Sunflower		
Control	2.5	19.5
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1}	3.5	23.5
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1} + manure, 40 t ha^{-1}	4.0	32.8
LSD 5%	0.8	6.5
Soybean		
Control	2.0	19.5
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1}	7.5	33.5
Sewage sludge, 40 t ha^{-1}+ sugar lime, 40 t ha^{-1} + manure, 40 t ha^{-1}	16.3	31.0
LSD 5%	6.8	7.2
Winter wheat		
Control	2.8	22.5
Sewage sludge, 40 t ha^{-1} + sugar lime, 40 t ha^{-1}	3.3	25.8
Sewage sludge, 40 t ha^{-1} + sugar lime, 40 t ha^{-1} + manure, 40 t ha^{-1}	10.8	36.0
LSD 5%	4.3	6.9

CONCLUSIONS

Sewage sludge is a biologically active material but dangerous for the environment with regard to its fecal contamination. As a result of keeping wastes at landfills and mineralization processes intensification, organic carbon, microbial biomass, the number of *E. coli* and enzymatic activity reduced. Microbiological and biochemical parameters depend on storage duration of wastes and organic matter content. Composting of sewage sludge with straw leads to the significant reduction of *E. coli* number. The microbiological testing of sewage sludge should be carried out in each concrete case. The soil management practice with the sewage sludge application and sugar industry waste material and manure contributes to increase the level of microbiological and enzymatic indices in chernozems, which were degraded as a result of the long-term utilization. Composts with sewage sludge have the prolonged action on the microbial community and humus content. The regular microbiological monitoring of the sewage sludge and fertilized lands is mandatory for the environmental safety of soils and crop products.

REFERENCES

Arhip O., 2011. Possibility for the capitalization of sewage sludge. In: Materials of the In. Sc. Sym. "Codrii", Shtiintsa, Kishinev, p. 26-29.

Banaru A., Arhip O., Senicovscaia I., 2003. The use of sludges from the municipal wastewater treatment for the fertilization of soils. In: Abs. of the Sc. Conf. "Balance of the scientific activity of MSU in 2000-2002", Kishinev, p. 341-342.

Blagodatsky S.A., Blagodatskaya E.V., Gorbenko A. J., Panikov N. S., 1987. Rehydration method for the determining of the microbial biomass in the soil. In: Pochvovedenie, Moscow, 4, p. 64–71.

Board on Environmental Studies and Toxicology (BEST), 2002. Biosolids applied to land: Advancing standards and practices. The National Academic Press, Washington, DC.

European Commission, 2001. Biological treatment of biowaste. Working Document, 2nd draft. Directorate-General Environment. Brussels.

Gerba C.P., 1996. Microbial pathogens in municipal solid waste. In: Palmisano A.C., Barlaz M. (Eds). Microbiology of solid waste. CRC Press, New York, p. 155-173.

Haziev F.H., 2005. Methods of soil enzymology. Russian Academy of Sciences, Ufa.

Karyagina L.A., Mikhailovskaya N.A.., 1986, Determination of polyphenoloxidase and peroxidase activities in the soil. In: Journal of the Academy of Sciences of BSSR, Minsk, 2, p. 40-41.

Rusu A. et al., 2012. Guide of utilization of organic fertilizers. Pontos, Kishinev, p. 19-24.

Sanitary regulations and standards of SANPIN 2.1.7, 2010. "Hygienic requirements of the use of urban wastewater and sludge for the irrigation and fertilization", Moscow.

Santamaría J., Toranzos G.A., 2003. Enteric pathogens and soil: a short review. In: Int. Microbiol., 6(1), p. 5-9.

Sergentu E.P., Tsurkan K.P., Baraga V.M., Rozmirets A.E., 1991. Efficiency of the application of different organic fertilizers in crop rotation on the leached chernozem. In: Application of organic fertilizers in Moldova. Kishinev, p. 14-21.

Standing Committee of Analysts (SCA), 2003. The Microbiology of Sewage Sludge. Part 1. An overview of the treatment and use in agriculture of sewage sludge in relation to its impact on the environment and public health, Nottingham, UK.

Tsurkan M.A., Arhip O.D., Rusu A.P., 1989. Municipal wastes and methods for their utilization. Shtiintsa, Kishinev.

Zvyagintsev D.G. (Ed.), 1991. Methods of soil microbiology and biochemistry. MSU, Moscow.