ПРИКЛАДНАЯ БИОХИМИЯ И МИКРОБИОЛОГИЯ, 2012, том 48, № 4, с. 411–416

UDC 631.46

MICROBIAL ACTIVITY IN THE LANDFILL SOIL

© 2012 M. Swiontek Brzezinska, A. Burkowska, M. Walczak

Department of Environmental Microbiology and Biotechnology, Institute of Ecology and Environmental Protection, Nicolaus Copernicus University, Toruń, Poland

e-mail: swiontek@umk.pl

Received November 30, 2011

The research objective was to determine the activity of microorganisms in the soil exposed to direct influence of a landfill, as well as in the soil beyond its influence. Fluorescein diacetate (**FDA**) hydrolytic activity and respiration in the soil were determined. The highest number of cultivated bacteria was recorded at the site located within the zone of direct influence exerted by the landfill, whereas the least amount was found at a distance of 1000 metres from the landfill. In contrast, the largest numbers of molds were observed in the soil at a distance of 1000 m from the headquarters of the landfill. The highest FDA hydrolytic activity and biological oxygen demand (BOD_5) were recorded in the soil by the headquarters of the landfill, and the least parameters were revealed at a distance of 1000 m from the landfill. It was found a high correlation between the number of bacteria and FDA hydrolytic activity of soil and BOD_5 in the north-eastern of the landfill. However, in the same place, there is a low correlation between the number of molds, and FDA hydrolytic activity of soil and BOD_5.

A landfill is a construction object located and arranged in accordance with the regulations, and allotted for organized deposition of wastes with identified properties. There are different types of landfills: landfills for hazardous, inert, as well as other than hazardous and inert (municipal) wastes [1]. In Poland, municipal wastes are deposited in unorganized, semi-organized and organized facilities. Unorganized landfills are located in natural depressions and do not require special arrangements. Consequently, among others, uncontrolled emission of gases into the atmosphere takes place in a landfill of this type, as well as contamination of surface waters, surrounding areas with particulates (dust) and wastes of light ends through dispersion. Semi-organized landfills have a geomembrane, which isolates deposited wastes from a substratum. In this case, also emission of liquid and gaseous substances occurs. Organized landfills have a special location in accordance with hydrogeological and geotechnical criteria, as well as meets valid technical requirements. The advantage of landfilling as a method of waste disposal is the simplicity of the process, as well as high, short-term economic efficiency with low unit costs [2]. Waste deposition on landfills contributes to the development of substances, which are troublesome, hazardous and constituting a threat to the natural environment. The last might be manifested through: air contamination (physical, chemical and microbiological contamination, fetor, contamination with biogas), soil contamination, noise pollution and inconvenience related to the presence of birds, rodents, insects [3]. Soil contaminants are among the most difficult to remove. The contamination ensues through penetration of harmful and hazardous substances into the soil. It leads to contraindications for cultivation of deciduous plants within contaminated roadside zones, such as lettuce, beetroots or cabbage, due to accumulation of heavy metals in their leaves. Soil microorganisms are important as they may contribute to purification of the soil environment. The biological stability of solid waste is one of the main issues related to the evaluation of the long-term emission potential and the environmental impact of landfills [4]. The biological activity of soil in the landfill is an important element of landfill waste. Soil microorganisms play a fundamental role in eliminating pollution. Monitoring of their activity is the definition of indicator of the pace of the process of biodegradation of landfill pollution.

The aim of the study is to determine the activity of microorganisms in the soil to reveal direct influence of a landfill, as well as in the soil beyond its influence.

MATERIALS AND METHODS

Landfill sampling site. The research was carried out within the area of the city landfill in Torun, central Poland. In geomorphological respect, it is a fragment of a high terrace in the ice-marginal valley of the Vistula River. The relief of the area is basically flat and slightly inclined in the south-western direction. In the geological structure, one can distinguish quaternary Holocene and Pleistocene sediments. The Holocene is represented by a thin layer of soil and deposited materials of 0.3–0.8 m in thickness. Depositions consist of sands mixed with humus, debris, slag, garbage. Beneath the soil and depositions, there are sands of fluvial and glacial accumulation. In the vicinity of the

composting plant, they reach down to the depth of 9–10 m. Below that, down to the depth of 13 m, fluvial-glacial gravel is deposited, and beneath them – lacustrine and glacial clay. The landfill is classified as a disposal ground for wastes other than hazardous and inert, with a cell sectioned off for landfilling of hazardous wastes.

Sampling. The surface soil (10–15 cm) was collected from the north-eastern and south-western parts of the landfill site, at three locations (sites): 1 – within the direct impact, 2 – 500 m from the landfill and 3 – beyond the impact (1000 m from the headquarters of the landfill). Soil samples were collected between May and November 2008.

Soil microorganisms. The number of microorganisms in the soil was determined with Koch's plate technique by the surface inoculation on appropriate culture media [5]. The number of heterotrophic bacteria was defined using a plate count agar medium (Merck, Germany). Nystatin (0.1 g l^{-1}) was added to the medium in order to inhibit the growth of fungi. The plates were incubated for 7 days at 22°C. Molds were determined using the Rose Bengal medium (Merck, Germany). The plates were incubated for 14 days at 25°C.

Soil hydrolytic activity. Soil hydrolytic activity expressed as fluorescien diacetate (**FDA**) hydrolysis was determined following the method of Adam and Duncan [6]. It is known that FDA is a general substrate for several hydrolytic enzymes including esterases, lipases and certain proteases [7]. FDA hydrolytic activity was detected with spectrofluorimeter HITACHI F-2500 (Japan) measuring the product of hydrolysis (fluorescein). 100 μl of FDA (1 mg/ml) was added to the assay suspension of 1.0 g soil in 20 ml of phosphate buffer (pH 6.0). The assay mixture was placed on a rotary shaker at 100 rpm and incubated at temperature *in situ* for 60 min. The assay was terminated by with adding chloroform/methanol (2 : 1 v/v), followed by centrifugation at 10000 × g for 10 min at 4°C. In supernatant the released fluorescein was measured (excitation and emission wavelength 480 nm and 505 nm, respectively). All samples were analyzed in three replicates.

Soil respiration measurement. Biological oxygen demand (BOD) is a measure of the amount of oxygen used by microorganisms as they feed upon organic matter. BOD of soil was measured using the OxiTop® Control system [8]. It acts as a system of bottles hermetically closed by a manometric head which allows you to monitor the pressure inside the bottle. If microorganisms consume polymer, they use oxygen available in test bottles and release carbon dioxide, which is immediately trapped by the sodium hydroxide solution. Consequently, we record a pressure decrease in a test bottle. The BOD value was calculated automatically by the OxiTop® instrument. BOD measurements in the soil were carried out as described in the WTW instructions [9] with modifications. A 100 g of the soil were weighed out and placed in a BOD OxiTop® MG 1.0 bottle. The carriers with absorber CO_2 (0.4 g NaOH) were placed in a bottle. The measured values were recorded in the OC 110 control system, in "Pressure p" mode. Samples were incubated for 5 days at temperature *in situ* to get BOD_5 – the amount of dissolved oxygen consumed in 5 days by microorganisms. All samples were analyzed in three replicates.

Statistical analysis. Results were analyzed in STATISTICA 6.0, StatSoft, USA. Analysis of variance (ANOVA) facilitated comparison of site and part of landfill on abundances microorganisms, FAD hydrolysis and BOD_5. In addition, we determined correlation between abundance of microorganisms and hydrolytic activity and BOD_5.

RESULTS AND DISCUSSION

Soil microorganisms. The number of microorganisms in the soil of the landfill is presented in Table 1. It is obvious that the number of cultivated bacteria decreased together with the increase of the distance from the landfill. The highest number of these microorganisms was recorded at the site situated within the zone of direct influence of the landfill and the lowest number of bacteria was revealed at a distance of 1000 m from the landfill. It was found that the number of bacteria was much higher in the south-western part. On the other hand, the highest number of molds in the soil was recorded at a distance of 1000 m from the headquarters of the landfill and they were revealed in the south-western part, similarly to bacteria.

In the study of the influence of a municipal landfill on the soil microflora Nowak et al. [10] found that heterotrophic bacteria occurred in the largest numbers, actinomycetes were slightly less, and molds were the least numerous. The analysis of variance performed by the authors revealed that the number of bacteria and actinomycetes significantly depended on the sample collection site. The largest numbers of these microorganisms were recorded by the authors in the vicinity of the waste disposal ground. Their number decreased as the distance from the landfill increased. In contrast, the number of molds was not influenced by the soil sample collection site. The number and the activity of microorganisms are conditioned by different factors. The main factor is the amount of available organic matter, which comes mainly from plant residues, root secretions and partially from the biomass of microflora and microfauna.

Microbial activity in the soil. Microbial activity in the soil was expressed as fluorescein diacetate (FDA) hydrolysis and BOD_5. Values of the FDA hydrolysis revealed in the soil at landfill are presented in Fig. 1. The highest FDA hydrolytic activity was recorded in the soil at the headquarters of the landfill, and the least one was found in the soil at a distance of 1000 m from the landfill. In the north-eastern part of the landfill,

Table 1. The number of microorganisms in the soil at the landfill. The results are presented in CFU 10^3/g of dry weight (± – standard deviation, $n = 3$)

Date of sampling in 2008	Site*	The north-eastern part		The south-western part	
		cultivated bacteria	molds	cultivated bacteria	molds
15.05	1	4212 ± 1.1	12 ± 1.1	8512 ± 1.1	24 ± 2.2
	2	3523 ± 1.2	35 ± 2.2	5401 ± 1.5	65 ± 1.3
	3	2011 ± 1.1	45 ± 1.3	3200 ± 2.2	75 ± 1.1
17.06	1	5017 ± 1.3	4 ± 1.1	8121 ± 3.3	32 ± 1.3
	2	3211 ± 1.1	60 ± 1.2	6421 ± 1.5	70 ± 1.5
	3	2401 ± 1.2	35 ± 2.2	3511 ± 2.2	94 ± 3.3
10.07	1	6267 ± 2.2	19 ± 2.2	9312 ± 1.1	31 ± 2.3
	2	4121 ± 1.3	18 ± 1.1	6602 ± 1.3	81 ± 2.2
	3	3445 ± 1.2	19 ± 1.3	4311 ± 3.3	86 ± 1.1
27.08	1	6324 ± 1.2	9 ± 1.1	9814 ± 1.3	23 ± 1.2
	2	4621 ± 2.2	12 ± 2.2	7802 ± 2.2	33 ± 1.5
	3	3910 ± 1.1	27 ± 1.2	5232 ± 2.3	45 ± 2.2
17.09	1	5422 ± 1.3	14 ± 1.1	7511 ± 1.1	25 ± 3.3
	2	3021 ± 1.1	79 ± 1.1	4201 ± 3.3	41 ± 1.3
	3	2214 ± 1.2	12 ± 2.2	3325 ± 2.2	65 ± 1.1
16.10	1	3212 ± 2.2	5 ± 1.1	6311 ± 1.3	42 ± 1.3
	2	2714 ± 1.1	14 ± 1.3	3401 ± 1.5	35 ± 3.3
	3	1702 ± 1.2	17 ± 1.2	2547 ± 3.3	45 ± 1.5
27.11	1	2311 ± 2.2	3 ± 1.2	4311 ± 1.1	21 ± 2.2
	2	1201 ± 1.3	2 ± 2.2	2878 ± 2.3	12 ± 1.3
	3	931 ± 1.2	4 ± 1.1	1633 ± 2.2	31 ± 1.1

* 1 – soil taken directly from at the headquarters of the landfill, 2 – soil taken 500 m from the landfill, 3 – soil taken 1000 m from the landfill.

FDA hydrolytic activity ranged from 29.24 μg of fluorescein per g of dry weight on site 1 to 1.35 μg of fluorescein per g of dry weight on site III. However, in the south-western part of the landfill FDA hydrolytic activity changed from 39.85 μg of fluorescein per g of dry weight on site 1 to 2.61 μg of fluorescein per g of dry weight on site 3.

BOD_5 in the soil depends on the site of sampling and analyzed part of the landfill (Fig. 2). In the north-eastern part of the landfill BOD_5 ranged from 494 mg

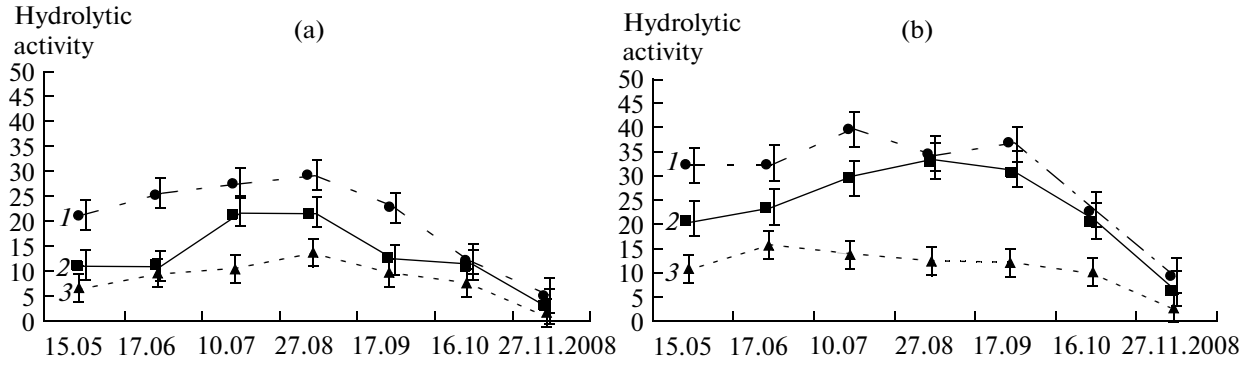

Fig. 1. FDA hydrolytic activity in the soil at a landfill in the north-eastern (a) and the south-western part (b).
1 – soil taken directly at the headquarters of the landfill, *2* – soil taken 500 m from the landfill, *3* – soil taken 1000 m from the landfill.

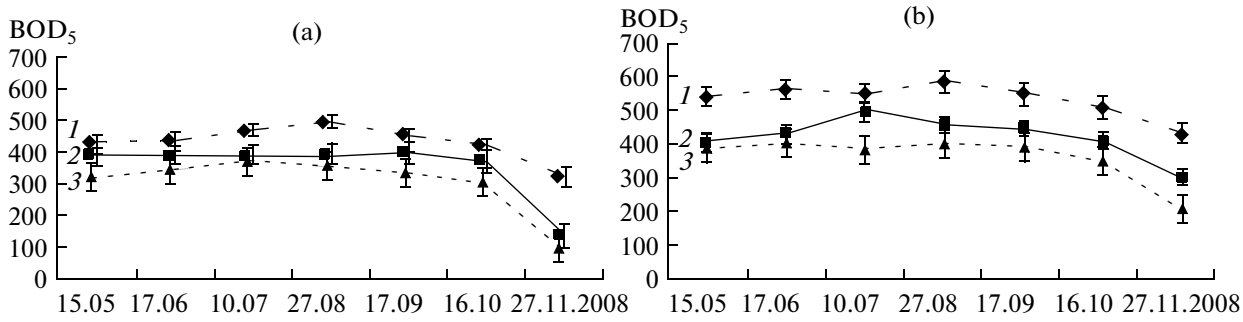

Fig. 2. BOD_5 in the soil at a landfill in the north-eastern (a) and the south-western part (b).
1 – soil taken directly at the headquarters of the landfill, *2* – soil taken 500 m from the landfill, *3* – soil taken 1000 m from the landfill.

of O_2 per kg of dry weight on site 1 to 102 mg of O_2 per kg of dry weight on site 3. However, in the south-western part of the landfill BOD_5 was higher, and changed from 587 mg of O_2 per kg of dry weight on site 1 to 207 mg O_2 per kg of dry weight on site 3.

Analysis of variance (Table 2) revealed statistically significant differences in the number of soil microorganisms and biological activity of the soil depending on the analyzed part of the landfill ($p < 0.001$). It was found a high correlation between the number of bacteria, FDA hydrolytic activity and BOD_5 in the north-eastern of the landfill (correlation coefficient of 0.87–0.92). However, there is a low correlation between the number of molds, FDA hydrolytic activity of soil and BOD_5 (Table 3).

Microbiological activity is a good measure of organic matter management in natural habitats, because 90% of the energy flows through microbiological decomposers [11]. Relevant techniques for measuring the total microbiological activity must be uncomplicated and sensitive, and the incubation period should be as short as possible. Spectrophotometric determination of hydrolysis using FDA turned out to be a simple, sensitive and quick method for determination of microbiological activity in the soil and garbage [12]. FDA is hydrolysed by many different enzymes, such as: proteases, lipases, esterases [7, 13] and fluorescein is the product of its decomposition, which is determined with the spectrofluorimetric method [12]. The results of our research revealed that hydrolytic activity in the soil was different at particular sites. Both in the north-eastern and south-western parts, the activity of hydrolases and the number of soil microorganisms, were much bigger than at the main cell of the landfill. Most probably, pollutants of the landfill significantly influenced the increase of this activity. The research by Schnürer and Roswall [12] indicates some differences in the hydrolytic activity between soil layers, which could reflect smaller amounts of soil organic matter in deeper layers. In the investigation of the activity of acid phosphatase and the content of available phosphorus, Garbolińska and Borkowski [14] observed a higher enzymatic activity in the surface soil than in a deeper layer, both in samples collected from a meadow and in beech-fir forest. In the case of an ecotone, and, thus, a transitional zone between different biocenoses, they did not record any significant differences in the enzymatic activity between soil layers. The authors suggest that this fact is probably caused by a higher activity of microorganisms in the surface soil layer and increased number of small plant roots. They are responsible for the production of enzymes in the soil. Enzyme activity is generally higher in rhizosphere than in bulk soil as a result of greater microbial activity, sustained by root exudates or due to the release of enzyme from roots [15–17]. Lalke-Porczyk et al. [18] recorded that the activity of hydrolytic enzymes in the soil samples connected with willow roots was two times higher as compared to the soil beyond the range of roots' influence. The overall enzymatic activity of soils consists of intra- and extracellular activity of microorganisms. It depends on several factors and according to Pancholy and Rice [19] the type of added

Table 2. Two – way ANOVA test comparing the influence of the site (distance from landfills – 1) and part of the landfill site (north-eastern or south-western parts of the landfill – 2) on the numbers of soil microorganisms, FDA hydrolytic activity and BOD_5

Source	Cultivated bacteria		Molds		FDA, hydrolytic activity		BOD_5	
	F-ratio	*P*	F-ratio	*P*	F-ratio	*P*	F-ratio	*P*
Site 1	19.26	**	7.39	*	19.8	**	79.5	**
Part 2	19.57	**	22.57	*	17.3	**	58.5	**

* Statistical differences at $P < 0.01$.

** Statistical differences at $P < 0.001$.

F-ratio: among-groups variance to the within – group variance;

site 1 – the statistical difference in the number of microorganisms and activity between the site 1, 2 and 3 (Table 1);

part 2 – the statistical difference in the number of microorganisms and activity between north-eastern and south-western parts of the landfill.

organic matter has the strongest influence. In the soil fertilized by liquid manure Kucharski and Wł adowska [20] found the highest activity of dehydrogenases in places fertilized with a lower dose of liquid manure rather than with a higher one, which is explained by the decreased fertility of soil fertilized with too high doses. The activity of hydrolytic enzymes is affected also by changes in the humidity and oxygenation of the soil.

The biological activity of microorganisms, apart from enzymatic activity, is a significant indicator that defines the intensity of organic matter transformations in water bodies. In our research, the OxiTop respirometric measuring system was applied, which permits to record the results at large time intervals. It is important when using sparingly decomposable compounds, which may occur in the landfill. A new generation of the OxiTop device for determination of the BOD enables to remember the measuring values from successive days, due to the electronic pressure-measuring system and memory. Vähäoja et al. [21] were looking for a detailed method for determining the biodegradation of various oils in subterranean waters. The method applied by the authors to determine the oxygen consumption with the use of OxiTop provided more accurate and more precise results as compared to traditional methods. Hufschmid et al. [22] had similar observations when investigating the contamination in liquid industrial wastes. The researches on the respiratory activity in the soil within the area of the landfill indicated that the activity was the highest within the zone of its direct influence. The BOD_5 decreased together with the increased distance from the headquarters of the landfill. Obviously, contaminants of the landfill had the influence on the increase in the respiratory activity of the soil. In the study of BOD_5 in the soil with the presence of shrimp wastes Swiontek Brzezinska et al. [23] found that BOD depended on temperature and incubation time, soil reaction, as well as shrimp wastes. The authors recorded the highest values of BOD_5 in the summer, when the presence of cephalic sections of shrimps was high and the main constituent of them was protein. In contrast, shells of shrimps were the least used by soil microorganisms, probably because they contained considerable amounts of a sparingly decomposable substance – chitin. When studying the soil respiration after addition of the sewage sludge, Quemada and Menacho [24] stated that the temperature and the water content influenced the soil respiration. Whereas according to Nadelhoffer et al. [25] soil respiration increased exponentially or linearly together with the temperature increase. Thus, the aforementioned environmental factors influenced the biological activity of the soil and diffusion of carbon dioxide [26]. The substances present in the soil are chemically instable and can be quickly oxygenated by soil microorganisms. Therefore, they affect the rate of microbiological soil respiration [27]. Soil respiration plays a critical role in determining a wide range of ecological phenomena, from the performance of individual plant to global atmospheric CO_2 concentrations [28].

Table 3. Correlation between the number of microorganisms, FDA hydrolytic activity of soil and BOD_5

Factor	Site	The north-eastern part		The south-western part	
		cultivated bacteria	molds	cultivated bacteria	molds
BOD_5	1	0.82	0.83	0.76	0.62
	2	0.87	0.57	0.73	0.62
	3	0.91	0.20	0.83	0.63
FDA hydrolytic activity	1	0.85	0.91	0.66	0.80
	2	0.82	0.32	0.72	0.70
	3	0.92	0.12	0.61	0.84

REFERENCES

1. Rosik-Dulewska, Cz., *Fundamentals of Waste Management,* Warsaw: Polish Scientific Publisher, 2002.
2. Bilitewski, B., Hördtle, G., Marek, K., *Waste Management Handbook*: *Theory and Practice*, Warsaw: Publishing House Seidel-Przywecki, 2006.
3. Leboda, R., and Oleszczuk, P., *Municipal Wastes and their Management*, Lublin: Publisher by the University of Maria Curie-Sklodowska University, 2002.
4. Cossu, R. and Raga, R., *Waste Management*, 2008, vol. 28, no 2, pp. 381–388.
5. Paul, E.A., Clark, F.E. *Soil Microbiology and Biochemistry*, Lublin: Publisher by the University of Maria Curie-Sklodowska University, 2000.
6. Adam, G. and Dunkan H., *Soil Biol. Biochem.*, 2001, vol. 33, no. 7, pp. 943–951.
7. Dick R.P., *Biological Indicators of Soil Health*. Oxford, UK: CAB International, 1997, pp. 121–156.
8. *WTW Applikationsbericht BSB 997 230; Respirometrishe BSB_5– Bestimmung von Häuslichem Abwasser mit dem OxiTop® Control–Order OxiTop®–Mass System,* Weilheim 1998.
9. Platen, H. and Wirtz, A., *WTW/OxiTop®/* Appliance, 1999.
10. Nowak A., Przybulewska K., Litwińczuk M. // *Ecological Aspects of Soil Microbiology.* Poznan: Publisher University of Agriculture, 1998, pp. 233–241.
11. Heal, O.W. and MacLean, S.F., *Unifying Concepts in Ecology*, Eds. Van Dobben W.H. and Lowe-McCannel, 1975, pp. 89–108.
12. Schnürer, J. and Rosswall, T., *App. Environ. Microbiol.*, 1982, vol. 43, no. 6, 1256–1261.
13. Rotman, B. and Papermaster, B.W., *Proc. Natl. Acad. Sci. USA*, 1966, vol. 55, no. 1, pp. 134–141.
14. Garbolińska, M. and Borkowski, A., *Acta Agr. Silv.*, 2006, vol. 49, pp. 203–211.
15. George, T.S., Richardson, A.E., and Simpson,R.J., *Soil Biol. Biochem.*, 2005, vol. 37, no. 5, pp. 977–988.

16. Marschner, P., Grierson, P.F., and Rengel, Z., *App. Soil Ecol.*, 2005, vol. 28, no. 3, pp. 191–201.
17. Grierson, P.F. and Adams, M.A., *Soil Biol. Biochem.*, 2000, vol. 32**,** no. 13, pp. 1817–1827.
18. Lalke-Porczyk, E., Swiontek Brzezinska, M., Donderski, W., and Walczak, M., *Ecology and Technology*, 2008, vol. 16**,** no. 5, pp. 86–89.
19. Pancholy, S.K., Rice, E.L., *Soil Sci. Soci. Am. Proc.*, 1973, vol. 14, pp. 47–50.
20. Kucharski, J. and Władowska, E., *Acta Agr. Silv.*, 2004, vol. 42**,** pp. 265–270.
21. Vähäoja, P., Kuokkanen, T., Välimäki, I., Vuoti, S., and Perämäki, P., *Anal. Bioanal. Chem.*, 2005, vol. 381, pp. 445–450.
22. Hufschmid, A., Becker – Van Slooten, K., Strawczynski, A., Vioget, P., Parra, S., Péringer P., and Pulgarin, C., *Chemosphere*, 2003, vol. 50, no. 1, pp. 171–176.
23. Swiontek Brzezinska, M., Lalke-Porczyk, E., and Donderski, W., *Pol. J. Environ. Stud.*, 2010, vol. 19, no. 3, pp. 627–633.
24. Quemada, M. and Menacho, E., *Biol. Fertil. Soils*, 2001, vol. 33, pp. 344–346.
25. Nadelhoffer, K.J., Giblin, A.E., Shaver, G.R., and Linkins, A.E., *In: Arctic Ecosystems in a Changing Climate. An Ecophysiological Perspective*. Chapin III F.S., Jeffries R.L., Reynolds J.F., Shaver G.R., Svoboda J. (Eds.), San Diego: Academic Press, 1992, pp. 281–300.
26. Kelting, D.L., Burgerm, J.A., and Edward, G.S., *Soil Biol. Biochem*. 1998, vol. 30, no. 7, pp. 961–968.
27. Silora, L.J. and McCoy, J.L., *Biol. Fertil. Soils*, 1990, vol. 9**,** pp. 19–24.
28. Liu H.S., Li L.H., Han X.G, Huang J.H., Sun J.X., and Wang H.Y., *App. Soil Ecol.* 2006, vol, 32, pp. 284–292.