



Article Biomonitoring and Assessment of Dumpsites Soil Using Phospholipid Fatty Acid Analysis (PLFA) Method—Evaluation of Possibilities and Limitations

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Abstract: Dumped waste is not only a problem from an aesthetic point of view, but also has an environmental polluting effect, or can even pose a direct danger if the waste is dumped in illegal landfills in an uncontrolled manner with unknown composition. In the case of soil pollution, the assessment of the changing microbial state can be used as an indicator of initial changes, since waste as a pollutant impacts the diversity of the landfill's microbial community. The degree of change depends on the qualitative and quantitative composition of the pollutants, which can be measured through the microbial phospholipid fatty acid (PLFA) pattern. The aim was a comprehensive assessment of the soil microbiological and toxicological hazards of various illegal landfill. Cluster-analysis of the average principal component revealed significant differences between the experimental sites. In comparison with the control site, the percentage of fatty acid biomarkers of Gram-positive bacteria was significantly higher in the contaminated areas, as well as the ratio of trans/cis isomerization in the case of 16:1w7 and 18:1w7 fatty acids. The inverse tendency was observed in the relative quantities of fatty acid biomarkers of Gram-negative bacteria compared to Actinomycetes, and in the fungal-bacterial ratio.

Keywords: PLFA; illegal landfill; pollution; soil biology; community structure

1. Introduction

Through human activities, processing and consuming 'unnatural' materials lead to the production of wastes, which often get to the ecosystem in increasing quantities. In some cases, these wastes are not able to take part in the natural cycles, or if they get in, cause such a disturbance that the biological processes cannot counteract, and consequently, they damage the elements of the natural environment and endanger humans as well [1]. Illegal landfills are most often created near municipal solid waste treatment plants, along roads, on side roads, in unfenced private plots (especially on the edge of populated areas), and next to various natural resources (surface waters, fields, forests) [2]. Their further importance is that they can pose a risk not only to the soil but also to human or animal health indirectly through the plants grown on the soil [3].

Certain microorganism genera, such as *Azoarcus*, *Phaeodactylibacter* or *Illumatobacter* are especially responsive to soil environmental effects [4]. These responses are reflected by the changes in biomass, community structure, different enzymatic activities, and biomarkers [5]. Contaminants generally have continuous effects on in situ microbiota, and after a considerable time, the induced changes integrate into the structure and activity of the communities [6,7]. As a result of contamination, microorganisms disappear or



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). appear, and often their metabolic activity is altered, changing based on the composition of the contaminants [8].

Phospholipid Fatty Acids (PLFA) have several properties that make them useful as indicators of environmental stress [9]. They are key components of the microbial membrane and as such can respond to stress from both the intracellular and extracellular environment [10]. Several researchers used PLFA to evaluate the soil biomass and determine the structure of microbiota for examining the effects of agricultural fertility [11], soil pollution [12], for ecotoxicological testing [13], or inspecting the incorporation of marked substrates [14]. Several microbial taxa can be analyzed by their whole biomarker patterns [10,11]. PLFA pattern measurement is usually used in soil studies to measure differences between bacterial and fungal biomass. By dividing the amount of the mole percentage values of the fungal fatty acid markers by the sum of the mole percentage values of the bacterial fatty acid markers, a fungal to bacterial (F:B) ratio can be determined [15]. In other cases, the ratio of Gram-positive to Gram-negative bacterial lipids (GP:GN) indicates the relative dominance of these bacterial groups in the soil [16].

In the case of Rajapaksha's 60-day-long laboratory experiments, heavy metal pollution revealed that bacteria are more sensitive to metals than fungi thorough better able to exploit organic matter to which metals are bound [17,18].

In the case of recultivation, the modified microflora of waste-contaminated areas can influence the soil status. Microbial diversity, as well as the proper accomplishment of the task of a particular species in the system of matter circulation, are essential factors of soil health, since fundamental soil functions, such as carbon and nitrogen cycle or organic matter transformation, are based on bacterial activities [19]. Furthermore, changes in the fatty acid profile of the soil microbial community can be indicators of environmental loads on soils such as heavy metal pollution, waste deposition, organic pollution, etc. [20]. To be able to calculate actual biomass values from the soil's bacterial/fungal ratios one needs reliable extraction methods. Such extraction data will be different in the case of the PLFA technique. Since there are currently no reliable conversion factors yet to compare the results of different techniques, it is meaningless to evaluate the actual values of the bacterial/fungal ratios estimated in this way [21]. However, changes in the ratios due to environmental factors, such as dumps, provide reliable results, irrespective of the type of tpollution [22,23].

Based on the above, environmental or human impacts on the soil, as well as changes resulting from soil use, have a significant impact on its microbial community, resulting in measurable changes in it. Therefore, this knowledge provides important information for predicting the effects of changes in land use. The main objective of our research was to find out what changes in soil microbial communities are caused by improperly deposited municipal waste as a potential source of pollution. Furthermore, we searched for the answer to how the quantity and ratio of phospholipid fatty acid-methyl ester biomarkers changed in light of the polluting effects of wastes of different compositions.

2. Materials and Methods

2.1. Sampling Sites

The sampling sites were abandoned public areas near settlements that are used for illegal landfill in Szabolcs-Szatmár-Bereg County, Hungary. Samples were collected at three sampling sites: in the immediate vicinity of Gelénes (48°19′ N, 22°44′ E), Beregdaróc (48°21′ N, 22°50′ E) and Beregsurány (48°14′ N, 22°58′ E) settlements near the Ukraine border (Figure 1). The illegal landfill of Beregsurány (**Bs**) is situated southeast of the settlement. Along the way to Gelénes-Barabás, the illegal landfill of Gelénes (**G**) functions as an adequately built regional dumpsite. The control area, Beregdaróc forest (**Co**), is the only part of the oak-hornbeam forest (Querco robori-Carpinetum), that more or less remained in its initial stage. We designated this area southeast of Beregdaróc, in the immediate vicinity of the Beregdaróc landfill (**Bd**). It had been a clay pit, which has been filled with communal wastes. Presently, this site is in an advanced stage of succession. The distance between the



two areas is approximately 800 m. All sampling sites are located within a 2 km radius circle and can describe the same botanical and pedological characteristics.

Figure 1. (**A**): Regional orientation map for the study area. (**B**): The Hungarian-Ukraine border location. The studied area is shown by the grey box. (**C**): Location of sampling sites along the studied area. The location of the map is shown in (**B**).

2.2. Sampling

Sampling points were marked out randomly on each plot site. 250 g core samples were taken from the depth of 0-20 cm without the municipal waste cover on the soil. Each soil sample was composed of five soil cores taken within 10 m^2 .

Soil Chemical Analysis

Soil pH was measured potentiometrically in 0.1 KCl, and cation exchange capacity (CEC) was determined in the 0.1 HCl extract. The total nitrogen (Nt) was determined by Kjeldahl's method [24]. Soil organic carbon (SOC) was measured using the wet oxidation method [25]. Potentially Toxic Elements (PTE) levels in soil samples were determined by analysis of Cd, Cu, Fe, Pb, and Zn after acid digestion, using inductively coupled plasma mass spectrometry (ICP-AES). The analysis was done in three replicates.

2.3. Phospholipid Fatty Acid Analysis (PLFA)

The composition of microbial communities in the soil samples was determined by the fatty acid methyl ester content. Lipid extraction and separation of the fatty acid methyl esters were performed according to Sasser (1990) [26] and Ibekve and Kennedy (1998) [27]. Samples were examined with HP 7890A (Agilent Technologies) gas chromatograph, with FID detector (300 °C). Type of the Colonna was HP Ultra 2 (5% diphenyl-95% dimethylpolysiloxane, 25 m × 0.2). Identified peaks with bacterial methyl ester standard (FAME 37, 24 bacterial fatty acid, Supelco). Fatty acid peak areas were converted to nmol g^{-1} using internal standards.

Certain groups of microorganisms were identified on the basis of the following fatty acids: i-15:0, a-15:0, 15:0, i-16:0, i-17:0, a-17:0, i-16:1, $16:1\omega7t$, $16:1\omega7c$, 17:0, cy17:0, $18:1\omega7c$, $18:1\omega7t$, cy19:0 general bacterial biomarkers [28,29]. Fatty acid $18:2\omega6$ is the biomarker of fungi [23,29]. Bacterial/fungal proportion was counted as a quotient of the quantity of $18:2\omega6$ fatty acid and the amount of bacterial fatty acids. The $16:1\omega7t$, $16:1\omega7c$, cy17:0, $18:1\omega7c$, $18:1\omega7c$, $18:1\omega7c$, $18:1\omega7t$, $16:1\omega7t$, $16:1\omega7t$, $16:1\omega7c$, cy17:0, $18:2\omega6$ fatty acid and the amount of bacterial fatty acids. The $16:1\omega7t$, $16:1\omega7c$, cy17:0, $18:1\omega7c$, $18:1\omega7t$ and cy19:0 fatty acids are biomarkers of Gram-negative bacteria [30–32] The i-15:0, a-15:0, 15:0, i-16:0, i-16:1, i-17:0, a-17:0, 17:0 fatty acids are biomarkers of Grampositive bacteria [32]. The following fatty acids are biomarkers of Actinomycetes 10Me16:0, 10Me17:0, 10Me18:0 [33]. Total microbial biomass was estimated by summing all identified lipid concentrations [34,35].

2.4. Statistical Analysis

For each parameter, submitted data were analyzed by two-factor of variance (ANOVA). Changes in the PLFA pattern of microbial communities of the sampling sites were determined by principal component analysis. Calculations were carried out from the correlation matrix. We consider those principal components, the value of which was higher than 1. We applied varimax rotation. For Cluster Analysis of sampling sites, we used the average values of principal components. Groups were formed based on Euclidean distance. Created groups of fatty acids were developed with the Cluster Analysis of principal component weights. Statistical data analysis was performed using IBM SPSS Statistics 20.0 software.

3. Results

3.1. Main Parameters of the Soils Sampling Area

Waste can be described with diversified qualitative and quantitative compositions on the examined dumpsites. High organic matter content of wastes and potentially toxic elements (PTE) are the two primary sources of pollution; however, considering the proportions, differences can be observed among the sampling sites.

The total copper content of soils (Table 1) is significantly larger on dumpsites (G 47.9 mg kg⁻¹, Bs 43.2 mg kg⁻¹, Bd 34.8 mg kg⁻¹) than in the control area (19.5 mg kg⁻¹). However, it does not exceed the limit value of this contaminant (75 mg kg⁻¹). A similar tendency can be observed in the case of Pb. The total lead content of the dumpsite soils (G 67.18 mg kg⁻¹, Bs 46.1 mg kg⁻¹, Bd 43.7 mg kg⁻¹) is more significant than that of the control area (15.39 mg kg⁻¹). Higher values of Cd and Zn in dumpsite soils are not only significantly different from the control area but also exceed the limit values (Cd 1 mg kg⁻¹, Zn 200 mg kg⁻¹), which can be explained by the anthropogenic effects. The limit values were considered according to MSZ 21470-50: AAS, ICP regulation.

D (Unit	Sampling				
Parameter		G	Bs	Bd	Со	
pH (KCL)		4.33	4.81	4.52	5.22	
SOC	$(g kg^{-1})$	$25.8\pm0.3~b$	54.2 ± 0.4 a	$32.4\pm0.2b$	$19.6\pm0.4~\mathrm{c}$	
Total N	$(g kg^{-1})$	7.5 ± 0.3 a	9.2 ± 0.4 a	8.2 ± 0.2 a	$4.2\pm0.1b$	
WHC	%	$22\pm0.1~\mathrm{a}$	25 ± 0.4 a	24 ± 0.3 a	$24\pm0.2~\mathrm{a}$	
CEC	%	$27\pm0.2b$	$26\pm0.3b$	$27\pm0.2b$	31 ± 0.3 a	
CaCO ³	%	0.00 a	0.00 a	0.00 a	0.00 a	
Total Cu	$ m mgkg^{-1}$	$47.9\pm0.3~\mathrm{a}$	43.2 ± 0.3 a	$34.8\pm0.2~ab$	$19.5\pm0.3~\mathrm{b}$	
Total Zn	$mg kg^{-1}$	$234.8\pm0.3~\mathrm{a}$	$237.5\pm0.3~\mathrm{a}$	$228.2\pm0.2~\mathrm{a}$	$23.2\pm0.4b$	
Total Pb	$mg kg^{-1}$	$67.18\pm0.3~\mathrm{a}$	$46.1\pm0.2b$	$43.7\pm0.4~\mathrm{b}$	$15.39\pm0.5~\mathrm{c}$	
Total Cd	$mg kg^{-1}$	2.6 ± 0.2 a	3.2 ± 0.3 a	$1.8\pm0.2~\mathrm{ab}$	$0\pm0b$	

Table 1. Physico-chemical properties of the soils sampling sites.

ANOVA: Tukey's B-test (n = 6). Within a column, the values which are marked with the same characters (a, b, or c) do not significantly differ from each other. (p < 0.05).

3.2. PLFA Analysis

Table 2 shows a summary of PLFA derived from bacteria (Gram+, Gram-, Actinomycetes) and fungi extracted from plot soils. The relative amount of fatty acids was given in mol %. The microbial biomass was significantly higher in the contaminated soils (Bd 71.82 \pm 0.4 nmol g⁻¹, Bs 85.65 \pm 0.3 nmol g⁻¹, G 79.75 \pm 0.2 nmol g⁻¹, *p* < 0.05) than in the control (55.21 \pm 0.3 nmol g⁻¹, *p* < 0.05).

Table 2. Phospholipid fatty acids derived from bacteria (Gram+, Gram-, Actinomycetes) and fungi extracted from experimental plot soils.

Sampling	Gram+ Biomarker Fatty Acids (mol %)		Gram– Biomarker Fatty Acids (mol %)		Actinomycetes Biomarker Fatty Acids (mol %)		Fungi Biomarker Fatty Acid (mol %)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Co	24.7352 a	0.02	21.3526 c	0.02	9.1203 b	0.02	4.6113 b	0.01
Bd	53.3526 b	0.02	14.2145 b	0.02	4.2516 a	0.01	1.9933 a	0.03
Bs	79.3526 c	0.01	4.2516 a	0.00	2.0516 a	0.01	1.5481 a	0.01
G	70.1302 c	0.01	6.3627 a	0.02	3.2536 a	0.01	1.7017 a	0.02

ANOVA: Tukey's B-test (n = 6). Within a column, the values which are marked with the same characters (a, b, or c) do not significantly differ from each other. (p < 0.05).

The biomarker fatty acid ratio was significantly higher in the studied dump site area (Bs 79.35 nmol g^{-1} ; G 70.13 nmol g^{-1} ; Bd 53.35 nmol g^{-1}), indicate Gram-positive bacteria, compared to the control site (24.73 nmol g^{-1}). In the soils of the dump site Gram-positive organisms were predominant, and the genus Bacillus, with good survival, was dominant. An inverse trend was observed for Gram-negative bacteria. Significantly higher values were detected in the control site (21.35 nmol g^{-1}) compared to the dump site soils (Bs nmol g^{-1} ; Gelénes 6.36 nmol g^{-1}). The highest values were measured in the control area (21.35 nmol g^{-1}), which is significantly higher than the results from the landfill sites (Bs nmol g^{-1} ; Gelénes 6.36 nmol g^{-1}).

The results for Beregdaróc site (14.21 nmol g^{-1}) were significantly higher than the other two sites, but still not higher than the control site. The relative amounts of the biomarker fatty acids, which indicate the presence of the Actinomycetes genus, showed no significant difference (Bd 4.25 nmol g^{-1} ; Bs 2.05 nmol g^{-1} ; G 3.25 nmol g^{-1}), while the control site values were significantly higher (9.12 nmol g^{-1}). A similar trend was observed for fungi: their abundance was significantly lower in the landfill area (G 1.70 nmol g^{-1} ; Bs 1.54 nmol g^{-1} ; Bd 1.99 nmol g^{-1}) compared to the control (4.61 nmol g^{-1}).

The fungal/bacterial biomass ratio can be used more effectively than the relative population size for the characterization of the different pollution levels (Table 3). Fungal/bacterial ratio can be given through the fatty acid biomarkers. This ratio is significantly

higher in the control area (0.0699) than in the dump sites soils, among which are no significant differences (Bd 0.0270, Bs 0.0184, G 0.0209). The decreasing ratio in dump sites and the number of fungi biomarkers can be explained which negatively correlates with the disturbance intensity [36]. On these sites, vehicle traffic and soil disturbance harm the development of the fungal mycelia.

Table 3. The fungal/bacterial ratio and the ratio of trans/cis isomers of $16:1\omega7$, and $18:1\omega7$ extractions from soils.

Sampling	Fungal/Bacteria Ratio	Transz/Cisz Ratio 16:1ω7	Transz/Cisz Ratio 18:1ω7
Со	0.0699 b	1.6449 a	0.5886 a
Bd	0.0270 a	1.9235 a	1.1044 b
Bs	0.0184 a	2.4323 b	1.1114 b
G	0.0209 a	2.5601 b	1.1254 b

ANOVA: Tukey's B-test (n = 6). Within a column, the values which are marked with the same characters (a, b, or c) do not significantly differ from each other. (p < 0.05).

Increasing $16:1\omega7$ and $18:1\omega7$ fatty acid trans/cis isomer ratios in bacterial cell membranes are indicative of ongoing environmental stress effects [36,37]. Our results show higher $16:1\omega7$ and $18:1\omega7$ fatty acid trans/cis isomer ratios in the soil of the dump sites compared to the soil of the control area. For $16:1\omega7$ fatty acid, the trans/cis isomer ratio was significantly higher in the soils of the Gelénes (2.5601) and Beregsurány (2.4323) sample sites compared to the soils of Beregdaróc (1.9235) and the control site (1.6449). For $18:1\omega7$ fatty acid, the trans/cis isomer ratio was significantly higher in all three landfill samples (Bd 1.1044; Bs 1.1114; G 1.1254) compared to the control samples (0.5886).

3.3. Results of Principal Component Analysis (PCA)

The following 18 variable were used for the analysis: i-15:0, a-15:0, 15:0, i-16:0, i-17:0, a-17:0, i-16:1, 16:1 ω 7t, 16:1 ω 7c, 17:0, cy17:0, 18:1 ω 7c, 18:1 ω 7t, cy19:0, 18:2 ω 6, 10Me16, 10Me17, 10Me18. The total variance of the principal components, which were determined on the basis of eigenvalues, could be characterized by large total variances (PCA1 60,686%; PCA2 19,091%; PCA3 14,074). The cumulative variance value of the first three principal components is 93.851% (Table 4).

Table 4. Eigenvalues and total var	iances regarding the first three	principal components (>1.0)
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Principal Component	Eigenvalue	Total Variance (%)	Cumulativity (%)
1. (PCA1)	10.927	60.686	60.686
2. (PCA2)	3.436	19.091	79.777
3. (PCA3)	2.533	14.074	93.851

Considering fatty acids (Figure 2), i-15:0, i-16:1, $18:1\omega7c$, i-17:0, a-15:0, $16:1\omega7c$, 15:0, a-17:0, and $16:1\omega7t$ correlated with each other and connected to the first principal component, while cy17, 10Me18, $18:1\omega7t$, 10Me16, 10Me17 fatty acids mainly belonged to the second principal component and cy19, 17:0, i-16:0 fatty acids mainly belonged to the third principal component.



Figure 2. Matrix of principal components. The PCA1 parameters are marked in green, the PCA2 parameters in yellow, and the parameters belonging to PCA3 in gray.

3.4. Cluster Analysis

The four different sampling sites' results had been analyzed by cluster analysis to reveal the similarities. Based on the PLFA analysis, the subject of comparison was the microbial community structure. Based on the cluster analysis dendrogram, it can be concluded that there were significant similarities between the G and Bs sites, and that the soils in Bd site were different from these sampling sites. Furthermore, all three landfills showed significant differences compared to the control site (Figure 3).



Figure 3. Dendrogram showing the results of the cluster analysis grouping of sampling sites. *X*-axis distance is based on the Squared Euclidean Distance.

Cluster analysis of the principal component weights was also carried out to determine the fatty acids characteristic of the sampling sites. The soil of the control (Co) site was well characterized by two major fatty acid biomarkers of the Actinomycetes group (10Me17 and 10Me16), the fungal biomarker (18:2 ω 6) and the fatty acids of Gram-negative bacteria (cy17:0, cy19:0, C17:0, C18:1 ω 7c, C18:1 ω 7t). In addition to Gram-positive bacterial fatty acid biomarkers (i-15:0) and Gram-negative bacterial fatty acid biomarkers (16:1 ω 7t and 16:1 ω 7c), the Beregdaroc (Bd) samples were characterized by a third Actinomycetes group fatty acid biomarker (10Me18). Biomarker fatty acids of Gram-positive bacteria are distributed between the soil samples of the Gelénes and Beregsurány dumpsites. Fatty acids a-15:0, i-16:1 and i-17:0 are mainly characteristic of the soils of landfill Beregsurány, while fatty acids a-17:0, i-16:0 and C15:0 are more characteristic of dumpsites Gelénes (Figure 4).





4. Discussion

Our examinations indicate that the composition of soil microbial communities on the sampling sites is determined by both the vegetation cover and the microorganisms which can adapt to the waste deposition. The organic carbon and nitrogen content of all three dumps soils were significantly higher than that of the control site. The increased activity on Bd results from the abandoned status of the dumpsite. The area is covered by vegetation

and soil, and mineralization of the formerly deposited waste has begun. On the dumpsite of Bs, organic matters are in an advanced state of degradation. On the dumpsite of G, plastic wastes are dominant, and easily degradable organic materials can be found in small quantities. Considering the biomass value, the amount of control sites is lower than that of dumpsites, which can be explained by the absence of wastes and the composition of microbiome, which characterizes the intact forest soils and is mainly dominated by fungi instead of bacteria. According to Béni et al. [38], the composition of microbial communities in forest soils is characterized by the dominancy of fungi, which results from the natural processes of humic forest soils.

The composition of microbial communities changes in dump sites both qualitatively and quantitatively. This also concerns the natural diversity, which is related to soil depth and characterizes the unpolluted areas [39]. The surface layer of dump soils is damaged. Vehicle traffic is frequent in the area, which leads to the compression of the upper soil layer. The dominance of fungi characterizes microbial communities in undisturbed areas [40]. The disturbance is especially disadvantageous for fungi, and as several publication mentions, physical disruption leads to the dominance of bacteria in the soil [39–41].

The direct layering of wastes on the soil surface changes temperature, humidity, and oxygen supply. A large amount of easily utilizable organic matter from waste gets to the soil, which serves as a carbon source and another nutrient source for microorganisms. At the same time, the PTE of waste harm the life functions of soil microorganisms. The process of PTE adsorption on microorganisms takes place through the binding of the cell wall and/or the cytoplasmic membrane. The metal ions are connected with the negatively charged groups on the surface and penetrate into the cell. Cations that have a large relative atomic mass, such as Hg(II), Cd (II), and Ag (I), can interact with SH-groups of enzymes and inhibit their activity, while others can enter into interactions and then inhibit the physiological ions. Such ions are the Cd (II) instead of Zn (II) or Ca (II), Ni (II), and Co (II) instead of Fe (II), and Zn (II)instead of Mn (II) [42]. Consequently, microorganisms, Gramnegative bacteria, Actinomycetes, and fungi of the surface soil layer become repressed. This leads to the propagation of Gram-positive bacteria (especially Bacillus genus [43] on dumpsites), which have more effective survival ability due to spores and are able to adapt to disadvantageous conditions.

Fungi are sensitive to the PTE content of the soil. The heavy metal content of the examined sites was significantly higher than the control area, which negatively influenced the amount of fungal biomass in the soil of the sampling sites. Hinojosa et al. [44] found that in case of increasing pollution, the proportion of fungal biomarker fatty acid (18:2w6c) decreased in PTE polluted soils. Consequently, a significantly higher fungal and bacterial biomass could be calculated in unpolluted and remediated areas than in polluted ones. According to our results, relative amounts of biomarker fatty acid of Actinomycetes are not significantly different from each other. In contrast, their quantity is significantly higher in control samples. Based on the scientific literature, members of the Actinomycetes genus react differently to increasing heavy metal content of the soil [45,46]. Lechevalier [47] found that the quantity of methyl branched-chain fatty acids (which mainly characterize the family of Actinomycetes) decreased due to increasing Cd, Cu, and Zn pollution. Frostegård et al. [23], found that the quantity of 10Me16:0, 10Me17:0, and 10Me18:0 fatty acids increased in heavy metal-contaminated forest soils, while could observe the reverse process in the case of cultivated soils.

Microbial metabolism can be inhibited by the influence of environmental stress, to which the bacteria mainly react by the rigidification of the cytoplasmic membrane. Membrane fluidity modulators are different according to the Gram type. Gram-negative bacteria mainly alter the ratio of unsaturated fatty acid to saturated fatty acid while Gram-positive bacteria regulate the amounts of forming branched-chain fatty acids or the lengths of their fatty acid chains to control the membrane phase in response to negative environmental effects [48]. Consequently, a non-specific permeability can develop, inducing modified (increased or inhibited) activity of transport processes [49]. During PLFA Analysis, one of the

biomarkers which indicate environmental stress is the process when cis monounsaturated fatty acids transform into trans monounsaturated fatty acids through an isomerization process. In the experimental dump sites the proportion of trans-cis isomerization increased in the case of $16:1\omega7$ and $18:1\omega7c$ fatty acids compared to control samples. According to the scientific literature, the ratio of trans-cis isomers in heavy metal polluted areas (0.10) was lower than on the control site (0.16) [48]. In limed soils (0.07), the proportion of trans-cis isomers ($16:1\omega7$) was lower than in unlimited soils (0.12) [31]. Isomerization of monounsaturated fatty acids is modified by the following stress effects: high temperature [50], organic pollutants [51], the lack of nutrients [52], osmotic stress [53], extreme acidic pH [54] and PTE pollution [55].

5. Conclusions

In summary, different contaminants of waste deposition cause qualitative and quantitative changes in the composition of the soil microbial community. The quantity of biomarker fatty acids of Gram-positive bacteria is significantly larger on dumpsites than in uncontaminated areas. Quantity of Gram-negative bacteria, Actinomycetes, and fungi decreases on dumpsites. The fungal/bacterial ratio also decreases in the soil under the influence of waste deposition and waste management. In the case of $16:1\omega7$ and $18:1\omega7$ fatty acids, the proportion of trans-cis isomers increases in dump soils.

The fatty acid composition of bacteria is determined not only by the genetic endowment but also by the available easily absorbed nutrients and the pollution of toxic substances concentrations in landfills. The changed microflora of the polluted areas can affect the condition of the "soil health" that can have an effect after its recultivated. The diversity of microorganisms and the proper discovery of the role and task of each species in the material circulation system is an essential factor from the point of view of soil homeostasis. Based on our results the phospholipid fatty acids have a valuable indication value in the case of description and monitoring of microbial communities under stress conditions. Therefore, the change in the fatty acid composition of bacteria can be an indicator of the environmental burdens on the soil, such as heavy metal pollution, waste disposal, the introduction of organic pollutants, etc.

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