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Application of hydrogeological and biological research for the lysimeter experiment performance under simulated municipal landfill condition

Dominika Dąbrowska¹ · Marek Sołtysiak¹ · Paulina Binińska² · Justyna Michalska² · Daniel Wasilkowski² · Agnieszka Nowak² · Vahid Nourani^{3,4}

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Abstract

The size and chemical composition of leachates migrating into the aquifer are dependent on the parameters of the waste and the storage conditions. Lysimeter studies allow us to determine the size and chemical composition of leachates as well as the leachate water balance. Lysimeter studies were conducted on a 230-L municipal waste sample for 6 months. During the tests, the specific electrolyte conductivity, pH, Eh, and temperature, as well as the chemical composition, microbiological analysis, and profiling of physiological population level using EcoPlate™ microarrays were measured in collected leachate samples. During the entire experiment, the amounts of inflow and outflow from lysimeters were measured. To assess the existence of significant differences in the chemical component concentrations in leachates, use of Principal Component Analysis was taken into account. The maximum EC value from leachate from the lysimeter was 33 mS/cm. High concentrations of ammonium ion (up to approx. 1400 mg dm⁻³), chlorides (up to approx. 6800 mg dm⁻³), and iron (up to approx. 31 mg dm⁻³) were observed in the effluents. The number of enterococci in May reached 53,000 cells/100 ml. By contrast, the number of these microorganisms was about 15,000 and 16,000 CFU/100 ml in January and April, respectively. Community-level physiological profiling indicates that the activity and functional diversity of microorganisms were higher in the leachate samples obtained in winter compared to effluents collected from lysimeters in spring.

Keywords Lysimeter · Leachates · Microbiology · BIOLOG

Introduction

Many landfills do not have ground seals, so that pollutants leached from waste migrate to the ground and water environment. In most cases, the exploitation of unsealed landfills has ended; however, their impact on the groundwater environment [8] still occurs.

Determination of the amount and composition of leachates arising from landfill is necessary for designing the leachate drainage system and determining what volume of leachate may be used for recycling through the spraying of waste [13].

Due to the fact that the study of the groundwater hazard generated by leachate from existing landfill is quite expensive and the duration of such research can be relatively long, much cheaper, and shorter static or dynamic leaching tests are very often selected [40–42]. Thus, lysimeter studies seem to be a promising alternative. These tests are used both to assess the size of irrigation requirements for cereals [32, 39, 46] to demonstrate the effect of compost [10, 11, 33] and to simulate the conditions prevailing in landfill [3, 27, 35]. The lysimeter experiment allows us to determine the value of contamination loads and the dynamics of their elution [1, 35, 41].

According to the literature, microorganisms—including pathogens and potential pathogens that may be serious

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threats to human health and wellbeing—are often present in leachates [16, 24, 31, 49] which reported a high number of bacteria in leachates from 22 landfills located in Upper Silesia. Therefore, determination of the number of appropriate indicator organisms is necessary to assess the impact of landfills on the surrounding environment and water. It is considered that the potential presence of pathogens in leachates that enter the environment can be inferred via the monitoring of faecal coliforms, enterococci, *Pseudomonas aeruginosa*, and *Clostridium perfringens*. Moreover, the determination of *Escherichia coli* belonging to the coliforms is recommended as an indicator of the degree of recent faecal pollution [48].

Compared to statistical and dynamic leaching tests, lysimeter studies are carried out less frequently due to the higher cost of the experiment [19] and the size of the required sample of soil or waste. Due to the physiochemical properties or microbiological hazards of municipal waste, most lysimeter studies were conducted on metallurgical and coal waste [18, 41].

Hitherto lysimeter studies on municipal waste have been carried out in several research centres. These tests significantly differed in duration [2, 5, 7], size of the waste sample tested, purpose of the research, and scope of laboratory tests [8]. The size of the waste sample varied from 15 to 390 L [5, 6]. The lysimeter studies of Bilgili [5] and Cossu [7] focused only on the assessment of the impact of waste aeration on the composition of leachates from landfill bioreactors, whereas Hantsch [17] analysed the effect of waste aeration intensity on the value of chemical oxygen demand (COD) in leachate from a lysimeter filled with old landfill waste. Some lysimeter experiments focused only on one group of leached pollutants; for example, Kajiwara [21] examined the washability of brominated flame retardants from pilot-scale lysimeters in Japan, whereas Karnchanawong and Limieniaepkan [22] monitored the leachability of heavy metals contained in batteries in Thailand. In other studies, Sarto [39] assessed the changes in biochemical oxygen demand (BOD₅) and COD values, as well as solutes, in leachate from municipal solid waste landfill in Indonesia.

Determination of effective parameters that are crucial for prediction of leachate impact on the environment is very important. Rafizul and Alamgir [36] and Ahsan [1] analysed the content of landfill gases in three individual lysimeters in Bangladesh and, moreover, performed a statistical analysis (one-way ANOVA) for Ca, Na, K, Mg, Cu, Cr, Cd, Ni, Pb, Mn, Fe, and Zn ion concentrations.

A major environmental concern for modern civilization is the growing tendency to discharge wastes to the environment [38]. Hence, microbial communities are crucial indicators of environmental quality because of their critical role in biochemical cycles and the decomposition of organic matter [47]. The biochemical activities of microorganisms

are sensitive tools for assessing the ecological status of the environment, since they respond quickly to adverse changes [9, 38]. However, it is only in a few cases that leachates have been evaluated in terms of bacterial presence [30].

One of the methods that is easily applied and time efficient for monitoring microbial metabolic status is the BIOLOG[®] System (BIOLOG Inc., Hayward, USA). The usage of this method in environmental studies is well documented in the literature. For instance, it was used for studying changes of the metabolic activity of microbial communities in wetland mesocosms after exposure to acid mine drainage [46], in sewer sediment deposits at different temperatures [4], in industrial wastewater systems [29], during sewage purification [37], and in degraded soil after amendment [12]. The wide use of the BIOLOG[®]-System as an alternative to the conventional microbiological methods allows for quick assessment of metabolic activity and functional diversity of the microbial communities.

The aim of this article is to present the results of a 6-month pilot phase of lysimeter studies conducted on a sample of municipal waste. The tests included leaching balance analysis, determination of leachate chemical composition, and microbiological analysis. To assess the existence of significant differences in the chemical composition and the quality of the leachates, Principal Component Analysis (PCA) was used [25]. A novelty in lysimeter studies was the profiling of the physiological population level (CLPP) in the leachate. CLPP was performed at different seasonal times, using the BIOLOG[®] System and EcoPlates[™] to monitor the metabolic activity and functional diversity of microbial population in leaked wastewater.

Materials and methods

Construction of the lysimeter

The lysimeter was filled with municipal waste with 20 03 01 European Waste Code with a moisture value equals to 29.3% from single-family houses, collected from landfill in Tychy–Urbanowice. The average sift composition of the waste is shown in Fig. 1.

A stand-pipe field lysimeter test was conducted within the area of the MASTER Waste and Energy Company, owner of the municipal waste landfill in Tychy–Urbanowice. Based on the novel project (unpublished data), the lysimeter was made of PVC pipe with a diameter of 0.42 m and a height of 1.8 m. It was equipped with a drainage system composed of a drainage layer of gravel, bowls with a conical end connected to PVC ducts, which drains leachates to the receiver. From the surface, the waste layer was not tightly covered with a filtration layer of gravel. From the top, the lysimeter was isolated to prevent the infiltration of precipitation but not the complete isolation

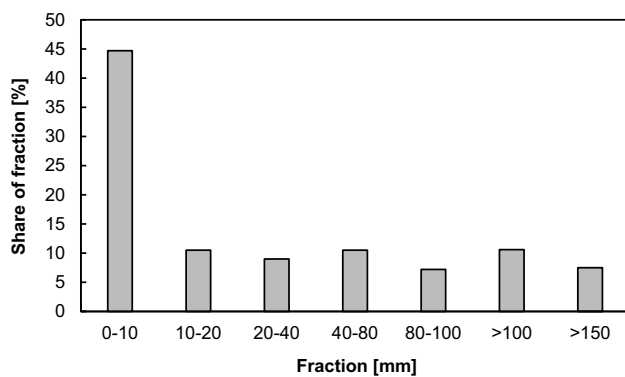


Fig. 1 The average share of fractions in a tested sample of municipal waste



Fig. 2 Lysimeter research stand

of oxygen (Fig. 2). Leachates samples were collected with the frequency of once a month, but the observation of the amount of leachate was carried out each day.

Lysimeter research conducting

The lysimeter experiment was carried out from November 2016 until May 2017. During the experiment, the lysimeter was precipitated with distilled water with a volume equal

to the average monthly rainfall calculated on the basis of rainfall volume measurements for the municipal waste landfill in Tychy–Urbanowice in the period 2004–2009 [20]. The flow rate constant using a rain machine and the lysimeter supply with water was impulsive. The temperature value in this period was also observed. The results of average monthly rainfall amounts and temperature are summarized in Table 1.

In each month of the experiment, the total volume of the obtained effluents was measured, and the values of specific electrolytic conductivity, temperature, pH, and Eh were determined. In addition, the characteristic indicators of groundwater pollution in the area of municipal waste landfills (Na, K, Ca, Mg, Fe, Al, Mn, Ni, Cu, Sr, S, Cl, SO₄, HCO₃, NO₃, NO₂, NH₄, PO₄, N Kjeldahl, and TOC) were determined at the accredited chemical laboratory every month, according to the requirements of the Regulation of the Minister of Environment of 30th April 2013 on landfills. These pollutants included inorganic salts, metals, and heavy metals which were selected to analysis as the main pollutants of groundwater near MSW landfills. These parameters may be used to a synthetic assessment of the impact of the pollution source on groundwater. There are a few indices which can be employed to do that, e.g., heavy metal pollution index, the metal pollution index, the Nemerow index comprehensive evaluation method, and the Backman's contamination index [26].

PCA analysis was also performed to reveal the characteristics of leachates composition. This analysis uses the orthogonal transformation to convert a set of results of possibly correlated variables into a set of values of linearly uncorrelated variables (principal components) which reflects both common and unique variance of the variables. The first principal component accounts for as much of the variability as possible, and each succeeding principal component accounts the remaining variability. It was performed in a few steps: creation of the observation sample matrix, determining the correlation matrix of variables, computing eigenvalues of the correlation matrix of variables [50], determining the principal component, and, finally, obtaining comprehensive results.

Due to the small supply of the lysimeter in April 2017, the results from chemical analyses of May and April 2017 were averaged. At the end of the experiment, the total load of pollutants leached from the waste was calculated.

Table 1 Average monthly amounts of precipitation in 2004–2009

Month	December	January	February	March	April + May
Average amounts of precipitation (mm/month)	39.30	56.15	48.15	70.00	22.80
Average temperature (°C)	2.0	−3.0	3.0	9.0	15.0

Microbiological analyses

In January, April and May 2017, collected leachate samples were subjected to microbial analyses, which included the determination of the number of total bacteria, *E. coli* and coliform bacteria, enterococci, *P. aeruginosa*, and *C. perfringens*. The number of total culturable heterotrophic bacteria (mesophilic and psychrophilic) was evaluated by serial tenfold dilutions of leachate samples (according to [52]). In turn, the number of coliforms was determined by fermentation on Eijkman Lactose Medium (according to [53]). The detection and quantification of *E. coli* and coliform bacteria, enterococci, *P. aeruginosa*, and *C. perfringens* was done by membrane filtration methods using filters with a 0.45 µm pore size on TTC Medium [54], Slanetz Bartley Medium [55], Pseudomonas Agar CN [55, 56], and m-CP Medium [51], respectively. All microbiological analyses were performed in triplicate.

After incubation, the number of colony-forming units (CFU) of the analysed microorganisms was given in 1 ml of leachate (in the case of mesophilic and psychrophilic bacteria) and in 100 ml of leachate (for the other species/groups of bacteria analysed).

Community-level physiological profiling

The physiological diversity of microorganisms from leakage waters of waste dumps in January and April was determined using the BIOLOG® System. For this purpose, the community-level physiological profiles (CLPPs) in leakage water samples were obtained. The rate of the carbon source utilization, as an indicator of active microorganisms, was assessed by the reduction of tetrazolium violet redox dye to purple formazan and colour development [44]. Therefore, EcoPlates™ contained 31 different carbon sources (three replicates) which were inoculated with 120 µl of 100-fold leakage water diluted with 0.9% NaCl. Next, the plates were incubated at 22 °C for 92 h and the colour development was followed by reading spectrophotometrically the absorbance at 590 nm using a MicroStation™ reader [14].

The microbial functional diversity indices were expressed as the average well-colour development (AWCD) and Shannon–Wiener index (H'). Furthermore, values of substrate richness (R) were determined as the number of utilized substrates which were divided into six groups presented in Table 2 [14, 44].

Furthermore, to evaluate utilization of low-molecular-weight, dissolved organic nitrogen and phosphorus compounds nitrogen use index (N-index) and phosphorus use index (P-index) were calculated, according to [37]. N-index and P-index were expressed as a proportion of total substrate utilization due to substrates that contained nitrogen (amines, amino acids, D-glucosaminic acid, and N-acetyl-D-glucosamine) and phosphorus (DL-α-glycerol phosphate and glucose-1-phosphate), respectively.

Data analyses

All of the absorbance values on the EcoPlates™ were used to calculate the following:

$$\text{AWCD} = \sum \text{OD}_i / 31.$$

OD_i is the optical density of each well and 31 is the sum of substrates on EcoPlates™ for one replicate:

$$H' = - \sum_{i=1}^n p_i \log p_i,$$

where p_i is the proportional colour development of the well divided by the total colour development of all wells on the plate.

The data were represented as the mean ± the standard deviation (SD) of triplicate. All of the analyses were performed using the Office Package (Microsoft, Inc., USA) of the Statistica® 13.1 PL (StatSoft® Inc., USA) software package.

Table 2 Groups of substrated on EcoPlate™ ([14], modified)

Compound group	Carbon source
Carbohydrates (Carb)	Glucose-1-phosphate, D,L-α-glycerol phosphate, β-methyl-D-glucoside, N-acetyl-D-glucosamine, α-D-lactose, D-cellobiose, D-mannitol, D-xylose, i-erythritol
Polymers (Poly)	glycogen, α-cyclodextrin
Carboxylic acids (CA)	α-Ketobutyric acid, D-glucosaminic acid, D-galactonic acid γ-lactone, D-galacturonic acid, D-malic acid, pyruvic acid methyl ester, itaconic acid, γ-hydroxybutyric acid, 2-hydroxybenzoic acid, 4-hydroxybenzoic acid
Amino acids (AA)	Glycyl-L-glutamic acid, L-arginine, L-asparagine, L-phenylalanine, L-threonine, L-serine)
Amines (A)	Phenylethylamine, putrescine
Surfactants (Surf)	Tween 40, Tween 80

Table 3 The amount of supply and the volume of leachate during the lysimeter experiment

Month	December	January	February	March	April + May
Water supply (cm ³)	5191	7412	6356	9240	11,740
Volume of leachates (cm ³)	3930	5450	3225	4030	11,530

Table 4 Parameters measured in the field

Month	December	January	February	March	April + May
EC (mS/cm)	17.17	30.06	33.50	30.80	28.00
pH	6.88	6.63	7.00	7.18	6.98
Eh (mV)	-268	-225	-260	-180	-330

Results

The lysimeter experiment was conducted from December 2016 to May 2017. During the experiment, the leachate water balance was determined. Potential evapotranspiration calculated based on Thornthwaite method was equal to 181 mm. The amount of lysimeter supply and the volume of leachates obtained in the following months are presented in Table 3. Effective infiltration coefficient for this lysimeter was equal to 69.5%.

The amount of water supply during the whole experiment was greater than the obtained leachate. The largest differences between the size of the supply and the volume of leachate were found in February and March, which may be related to atmospheric conditions. Negative temperatures contributed to the freezing of waste and retention of leachate inside the lysimeter. By contrast, the increase in temperature may be the reason that the volume of leachate obtained in April and May was almost equal to the volume of water delivered to the lysimeter during these months.

The values of physiochemical parameters measured in leachate from the lysimeter in the field indicate a large convergence of the groundwater parameters obtained from a piezometer located on the top of an inactive municipal waste landfill in Tychy–Urbanowice [8]. Simultaneously, electrolytic conductivity was significantly higher than the values of this parameter in leachate captured by the drainage system of the new municipal waste landfill in Tychy–Urbanowice. Table 4 presents values of electrolytic conductivity, pH, and Eh measured in the field.

The statistical values of chemical components of leachates are presented in Table 5.

The chemical composition of the effluent was determined primarily by the high concentrations of bicarbonates, chlorides, and sulfates found in the effluent analysed. Among dominant chemical components in the effluent, the concentration of bicarbonates varied the most, whereas the content of ammonium ions did not change

Table 5 Statistical values of leachate chemical parameters (mg dm⁻³)

	Mean value	Minimum	Maximum	St. dev.
Al	1.75	0.24	4.82	1.85
B	3.40	1.08	7.12	2.68
Ba	0.28	0.02	0.56	0.20
Cr	0.08	0.01	0.20	0.07
Cu	0.50	0.07	1.41	0.55
Fe	12.61	3.87	31.10	11.08
Mn	2.89	0.34	5.16	2.26
Ni	0.45	0.14	1.29	0.48
Sr	1.72	0.25	4.68	1.84
Zn	1.98	0.34	4.10	1.58
V	0.06	0.03	0.12	0.04
Cd	0.00	0.00	0.01	0.00
Co	0.04	0.00	0.13	0.05
Pb	0.06	0.06	0.17	0.07
Ca	1002.60	650.00	1590.00	353.78
Mg	468.00	241.00	630.00	143.99
Na	3179.60	1638.00	4280.00	1007.67
K	2784.80	1483.00	3590.00	793.92
NH ₄	1181.60	647.00	1411.00	305.92
Cl	5597.80	3725.00	6820.00	1292.42
SO ₄	4218.60	738.00	6610.00	2538.61
NO ₂	4.32	1.60	6.70	2.23
HCO ₃	6056.38	591.99	14,331.00	5726.51
PO ₄	7.47	0.80	13.00	4.64
N	2364.40	1499.00	3989.00	995.30
TOC	5849.20	3388.00	7856.00	2030.94

significantly during the months which the experiment was conducted. The presence of large concentrations of ammonium ion in the effluents (Fig. 3) may be the reason for low values of redox potential that indicated strong reduction conditions in the lysimeter what can be associated with activity of sulfate-reducing bacteria (SRB) that use sulphur-containing substances, e.g., sulfate as electron acceptors, and use the organic matter for sulfate reduction. Sulfate reduction undergoes under anaerobic conditions which is supported by data about Eh.

Nevertheless, the content of most of the individual components in the effluent was reduced, which indicates their gradual elution. During the experiment, the most significant decrease in content occurred in the case of sulfates (Fig. 3). The highest concentrations for Mg, NH₄, K, Na, SO₄, and Cl were found in the effluent from February.

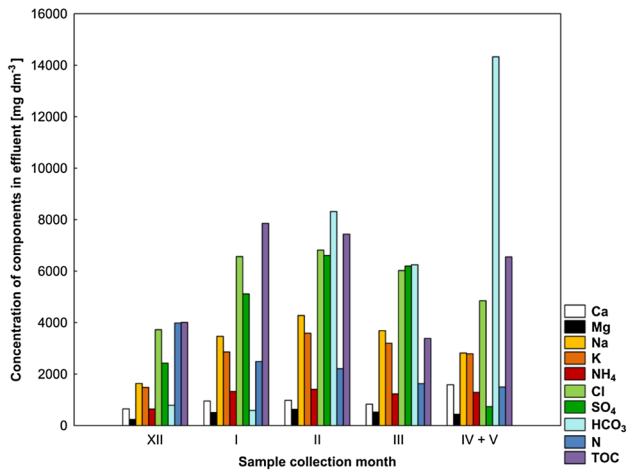


Fig. 3 Changes of the concentration of dominant chemical components in the effluent from the lysimeter

In the following months, the content of ions in the effluent ranged from 0 to 35 mg dm^{-3} . The concentration of iron varied the most among all measured ions (Fig. 4). The final concentration values of iron suggest that it may have leached further out of the waste. Leaching activation of boron, zinc, and manganese from the lysimeter occurred in March. During the experiment, the almost complete elution of phosphates, copper, and nickel was observed. Boron deserves attention in the toxicity study of leachate from landfills. It is a very mobile compound, with a slow dissolution, and its high concentration in landfill leachates is associated with the presence of plastics, detergents, and medicines in waste. Its concentration in the lysimeter is comparable with concentrations in groundwater in the region of the landfill in Tychy [8].

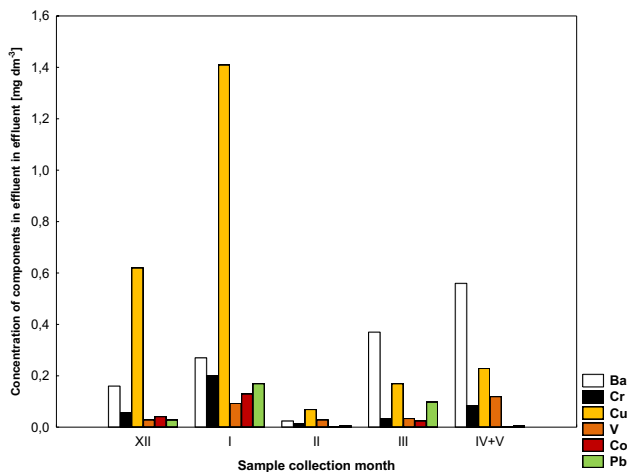


Fig. 4 Changes of the concentration of selected chemical components in the effluent from the lysimeter

Among the chemical components of leachates, the concentrations of which did not exceed 1 mg dm^{-3} in effluents, concentration of barium ions varied the most. Furthermore, concentration of this chemical steadily increased in the subsequent months of lysimeter test conducting. The concentration of cadmium, cobalt, and lead decreased to almost zero during the experiment (Fig. 5). Heavy metals are a threat, due to their adverse effect on the natural environment. The toxicity of leachates may result from cadmium and lead contamination. Their concentrations in the effluent from the lysimeter are even 300 times higher (concentration of Cd) in relation to contaminated waters from the P18 piezometer located on the top of an old municipal waste landfill in Tychy [8].

Figure 6a–c shows the total load of pollutants eluted from the lysimeter during the experiment. Among the calculated values, attention should be paid to the load of washed chlorides, sodium, ammonium, iron, and boron.

As part of the PCA analysis, the relationships between chemical parameters from leachates were determined. The correlation coefficient matrices for the most important parameters are shown in Table 6. The default p value for highlighting is .05; significant t tests with p less than or equal to this value are shown in red colour.

The eigenvalues, contribution rates, and cumulative contribution rates are shown in Table 7.

As can be seen from Table 7, owing to the contribution rate of the fourth principal component is less than 10%, the first three components would be taken as final principal components. These three values can explain the 92.98% leachate composition information of the experiment.

The results of the analysis indicate that the first principal component is related to Na, Cl, NH_4 , SO_4 , and HCO_3 . These are typical indicators of groundwater pollution in the area

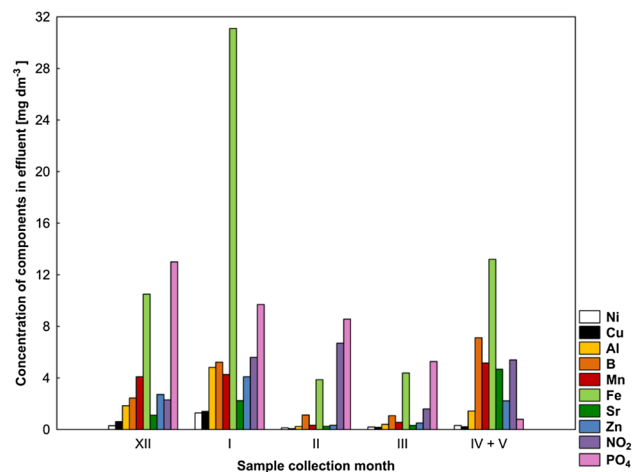
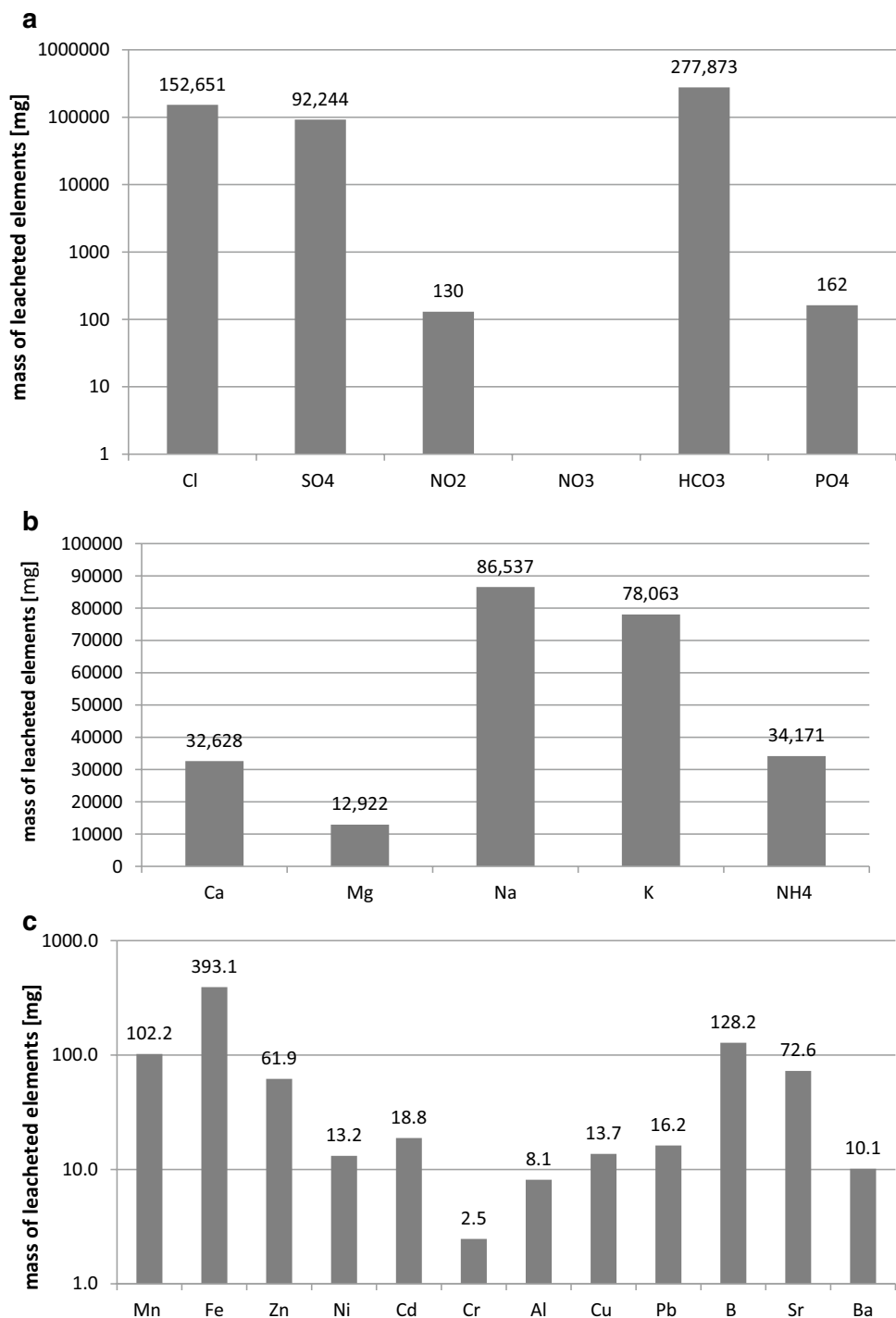


Fig. 5 Changes in subordinate ion concentration in the effluent from the lysimeter

Fig. 6 **a** Concentration of main anions eluted from lysimeter. **b** Concentration of main cation eluted from lysimeter. **c** Concentration of other ions eluted from lysimeter



of municipal landfills. The second principal component is associated with N and PO₄. These are typical agricultural contaminants (fertilizers used in the region). The third component is associated with manganese contamination.

Concentrations of various bacterial groups or species determined in leachates are presented in Table 8.

The microbiological analyses showed that the number of heterotrophic bacteria varied significantly in leachate

samples collected in the following months. Total number of psychrophilic bacteria in the leachate remained constant during the experiment, which may indicate that the effluent provided easily digestible organic matter for these bacteria [45]. On the contrary, the number of mesophilic bacteria was significantly higher in leachates collected in May as compared to effluents collected in January and April. Yet, by contrast, it was reported a dominance of psychrophilic bacteria rather

Table 6 Matrix of the correlation coefficients

	Al	B	Cr	Cu	Fe	Ni	Sr	Zn	V	Co	Mg	Na	K	NH ₄	Cl
Al	1.00	0.55	0.98	0.98	0.99	0.97	0.35	0.94	0.52	0.93	-0.21	-0.20	-0.29	-0.02	0.06
B	0.55	1.00	0.66	0.39	0.65	0.49	0.97	0.67	0.97	0.25	-0.21	-0.28	-0.16	0.17	-0.22
Cr	0.98	0.66	1.00	0.93	1.00	0.97	0.47	0.90	0.66	0.88	-0.10	-0.11	-0.15	0.14	0.14
Cu	0.98	0.39	0.93	1.00	0.94	0.95	0.17	0.91	0.35	0.98	-0.24	-0.21	-0.33	-0.11	0.07
Fe	0.99	0.65	1.00	0.94	1.00	0.98	0.45	0.92	0.64	0.89	-0.12	-0.12	-0.18	0.11	0.13
Ni	0.97	0.49	0.97	0.95	0.98	1.00	0.27	0.84	0.51	0.95	0.01	0.03	-0.06	0.18	0.29
Sr	0.35	0.97	0.47	0.17	0.45	0.27	1.00	0.51	0.94	0.02	-0.23	-0.31	-0.16	0.14	-0.31
Zn	0.94	0.67	0.90	0.91	0.92	0.84	0.51	1.00	0.56	0.80	-0.49	-0.50	-0.55	-0.26	-0.26
V	0.52	0.97	0.66	0.35	0.64	0.51	0.94	0.56	1.00	0.25	0.01	-0.06	0.06	0.38	-0.01
Co	0.93	0.25	0.88	0.98	0.89	0.95	0.02	0.80	0.25	1.00	-0.11	-0.06	-0.20	-0.03	0.21
Mg	-0.21	-0.21	-0.10	-0.24	-0.12	0.01	-0.23	-0.49	0.01	-0.11	1.00	0.99	0.99	0.93	0.94
Na	-0.20	-0.28	-0.11	-0.21	-0.12	0.03	-0.31	-0.50	-0.06	-0.06	0.99	1.00	0.98	0.90	0.96
K	-0.29	-0.16	-0.15	-0.33	-0.18	-0.06	-0.16	-0.55	0.06	-0.20	0.99	0.98	1.00	0.94	0.89
NH ₄	-0.02	0.17	0.14	-0.11	0.11	0.18	0.14	-0.26	0.38	-0.03	0.93	0.90	0.94	1.00	0.86
Cl	0.06	-0.22	0.14	0.07	0.13	0.29	-0.31	-0.26	-0.01	0.21	0.94	0.96	0.89	0.86	1.00

Table 7 Eigenvalues, contribution rates, and cumulative contribution rates

Principal component	Eigenvalue	Contribution rate (%)	Cumulative contribution rate (%)
1	10.22	39.32	39.32
2	7.65	29.44	68.76
3	6.30	24.23	92.98
4	1.82	7.02	100.00

than mesophilic microorganisms in leachates from 22 landfills located at Upper Silesia. Mesophilic bacteria occurring naturally in the human body and along with faecal matter enter the environment, thus generating a serious contamination due to the presence of pathogenic bacteria among them [28, 45]. It has been proved that pathogenic microorganisms migrate from leachates into soil and surface and

groundwater. Bacteria including coliforms, *C. perfringens*, *L. monocytogenes*, and psychrophiles were detected in water collected at various distances from the landfills [23]. *E. coli* was determined in the leachate collected in May, while coliform as well as enterococci were constantly present in effluent samples collected during the experiment. Interestingly, *P. aeruginosa* was not detected in the leachates, whereas *C. perfringens* was detected only in effluents collected in May.

The highest number amongst other microbial groups investigated was found in May, when the number of enterococci reached 53,000 cells/100 ml. By contrast, the number of these microorganisms was about 15,000 and 16,000 CFU/100 ml in January and April, respectively.

In general, the microbial population in leakage waters of waste dumps in January had a higher metabolic activity (AWCD) and was more diverse (H') than in April. Microorganisms present in the leachate samples collected in January were able to use more sources of carbon on EcoPlate™ (R),

Table 8 The number of tested bacterial group/species in leachates

Sampling date	The number of tested bacterial group/species in leachates							
	Total number of bacteria (CFU/ml)		<i>E. coli</i> (CFU/100 ml)	<i>Enterobacter</i> sp. <i>Klebsiella</i> sp. (CFU/100 ml)	<i>Salmonella</i> sp. <i>Shigella</i> sp. <i>Proteus</i> sp. <i>Pseudomonas</i> sp. (CFU/100 ml)	<i>Enterococci</i> (CFU/100 ml)	<i>P. aeruginosa</i> (CFU/100 ml)	<i>C. perfringens</i> (CFU/100 ml)
	Mesophilic bacteria	Psychrophilic bacteria						
January 2017	1.7×10^7	5.9×10^7	–	1.6×10^2	6.0×10^2	1.6×10^3	–	–
April 2017	1.8×10^7	4.5×10^7	–	1.9×10^2	2.0×10^2	1.6×10^3	–	–
May 2017	8.6×10^7	4.0×10^7	8.4×10^2	2.0×10^4	7.1×10^3	5.3×10^4	–	6.2×10^3

CFU colony-forming unit

Table 9 Mean values of the average well-colour development (AWCD), Shannon–Wiener (H'), substrate richness values (R), and N-use and P-use indices for leaked wastewater in January and April sampling

Index	January	April
AWCD	0.387 ± 0.042	0.255 ± 0.033
H'	3.460 ± 0.410	2.450 ± 0.340
R	25 ± 2	13 ± 2
N-use, %	26.83 ± 2.57	30.64 ± 7.19
P-use, %	4.13 ± 0.23	2.33 ± 0.74

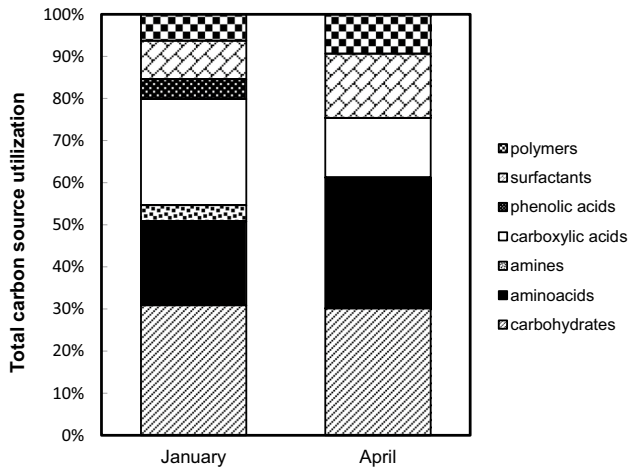


Fig. 7 The pattern of carbon source utilization for microbial population in leaked wastewater in January and April

compared to their ability to utilize substrates in April. The P-use index was higher in winter, whereas the N-use index was similar for both samples, independent of the season. On the basis of changes of ion composition in the effluent (Figs. 3, 5), it could be supposed that the nitrogen-containing compounds were not depleted during the experiment, while phosphorus-containing substrates could be a limiting factor,

especially, that samples collected in April/May contained very low concentration of orthophosphates (Fig. 5). All indices are shown in Table 9.

The season sampling affected the carbon source utilization pattern. It was found that the intensity of usage the carboxylic acids and amines decreases in leaked wastewater in spring, while amino acids, surfactants, and polymers increased (Fig. 7). The main shift in particular carbon source usage was connected to the more intense utilization of β -methyl-D-glucoside and D-malic acid in winter, and pyruvic acid methyl ester, Tween 40, L-serine, N-acetyl-D-glucosamine, glycogen, and glycy-L-glutamic acid in spring (Fig. 8).

The evaluation of the potential for substrate utilization by microbial assemblages in relation to nutrient availability is crucial for understanding microbial processes in wastes. In this study, the functional diversity of the microbial community in the simulated municipal landfill conditions was assessed. Due to the fact that the diversity of microbial communities is closely related to the composition of waste water, all microbial and biochemical analyses were conducted in leakage waters of waste dumps. Surprisingly, it was indicated that the activity of microorganisms and values of diversity indices were higher in winter compared to spring. This can be explained by the substrate quality and availability of nutrients in wastes during winter and spring, which are major factors controlling microbial decomposition processes. Initially, wastes were potentially rich in organic matter and could be the source of nutrients for microorganisms. However, carbon sources and nutrients may be depleted over time and that may lead to a reduction of microbial functional diversity. It could be confirmed by the gradual decrease of TOC concentration from January to May with simultaneous HCO_3^- concentration increase as an inorganic carbon source (Fig. 3). Depletion of nutrients in the lysimeter may be also indicated by a decrease in the P-use index value during the experiment as a result of a reduction in orthophosphates concentration (Table 2, Fig. 5).

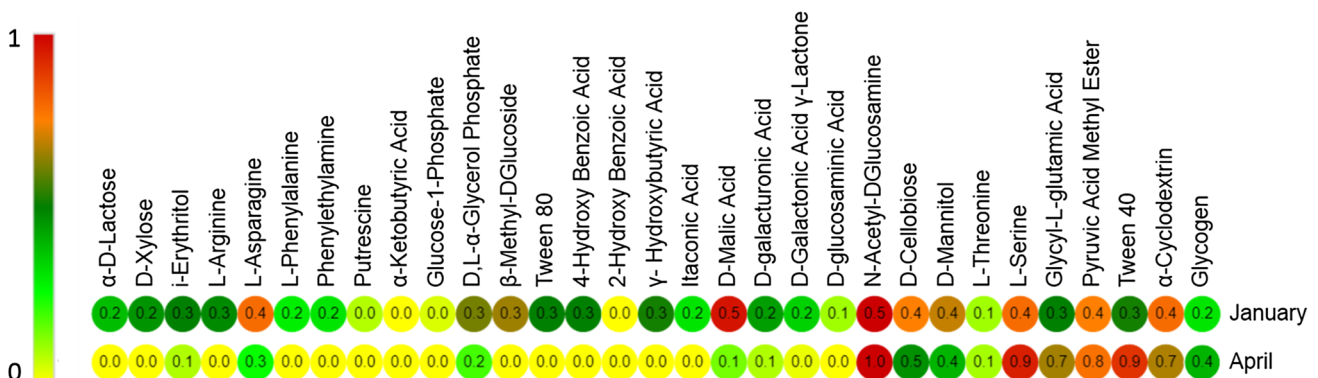


Fig. 8 The intensity of particular carbon source utilization for microbial population in leaked wastewater in January and April

Summary and conclusions

The lysimeter experiment is a promising alternative to the classic leaching tests. Lysimeter studies are conducted on a larger sample of land or waste and for a longer period of time. The results obtained during the lysimeter tests better reflect the processes occurring in the landfill. Knowledge of the quantitative balance of leachates and their chemical composition allows us to design a system of sealing landfills from the ground, but also can indicate the environmental hazard that may be generated by old and disused landfills.

The results of the pilot phase of the lysimeter experiment at the municipal landfill in Tychy–Urbanowice indicate that leaching of individual pollutants from municipal waste occurs at various rates. Some of the indicators of groundwater pollution in the area of communal waste landfills, such as boron or iron ions, are washed out by leaps and bounds.

The value of electrolytic conductivity measured during the experiment is comparable to the values obtained from sampling of the piezometer located on the top of an inactive municipal waste landfill in Tychy–Urbanowice. The microbiological analysis showed that the leachates were contaminated with bacteria of sanitary importance including coliforms and enterococci, from groups including *Salmonella* sp., *Shigella* sp., *Pseudomonas* sp., *Proteus* sp., and *C. perfringens*. It can be concluded that the leachates from landfills may be an epidemiological hazard to the natural environment. For this reason, monitoring bacterial indicators of leachate quality is an important issue. In addition, municipal landfills are dynamic systems, resulting in differences in the number and diversity of microorganisms over time, due to atmospheric conditions, the degree of decomposition of waste, the content of chemical compounds, and the age of the landfill itself [15, 43].

The volume of waste used in the experiment and the duration of the experiment indicate that it is sufficient to conduct research on the leachability of contaminants due to the fact that changes in the number of bacteria as well as concentrations of individual physicochemical parameters are noticeable and the leaching rate of these components can be determined. In future research, it is planned to modify the experiment by adding sensors that enable constant control of the washing rate of the waste solid and conductivity changes using neural networks for further prediction [34].

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