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Chemical Composition and Toxicological Evaluation of Landfill Leachate from Białystok, Poland

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Abstract: Leachates from landfills constitute a very complex environmental matrix with potentially toxic properties for both the environment and humans. Due to this fact, toxicological tests were carried out on landfill leachate (LL) obtained from the local landfill in Hryniewicze (Poland). The analyses included physicochemical studies of leachates and their impact on soil and plants, and studies conducted in bacterial models and human cell models. The results obtained indicate high contents of metals and organic matter, especially dangerous PAHs, in the tested leachate. This results in the influence of LL on changes in the content of assimilation pigments and oxidative stress observed in plants grown in soil fed with leachates. The effect of leachate on the growth of *Sporosarcina pasteurii*, *Staphylococcus aureus*, *Lactobacillus rhamnosus*, *Saccharomyces boulardii* and *Candida albicans* varied depending on the strain and LL dose. A particularly significant increase in proliferation after exposure to LL was noted for *S. aureus*. In studies conducted on human cancer cell lines representing three types of glioblastomas and one type of colorectal adenocarcinoma, a particularly significant increase in the viability of cells treated with LL was noted for the DLD-1 cell line. The results obtained, especially the stimulation of the growth of cancer cells and an increase in the number of pathogenic bacteria, indicate the potential toxic properties of the tested leachates. This is confirmed by the high level of oxidative stress in plants. The results indicate the need for continuous monitoring of waste landfills and leachates generated there.

Keywords: landfill leachate; oxidative stress; cytotoxicity; soil; plant; cancer; bacteria

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1. Introduction

Waste landfills are facilities built for depositing mainly municipal waste. They must meet certain conditions, such as proper location, and technical and operational parameters. In Poland, despite attempts to reduce the number of landfills, they are still one of the most common methods of waste disposal [1,2]. Some of the waste is recycled, some is subject to thermal transformation and composting, but nearly 40% of waste is still stored in a traditional way. This method poses the greatest threat to the natural environment and humans [3].

A landfill is a kind of biochemical reactor in which continuous chemical reactions, and biochemical and microbiological transformations take place. The changes taking place are influenced by atmospheric factors and the presence of microorganisms [4,5]. A landfill is a highly heterogeneous environment, which results from the fact that different transformations occur simultaneously in different areas and different stages of decomposition processes coexist [5,6].

According to EU Council Directive 1999/31/EC, landfill leachate (LL) is described as "any liquid percolating through landfilled waste and emitted from/or contained in a landfill". Leachates are generated by direct infiltration of rainwater through the landfill body, but also as a result of changes taking place in the landfill itself [7]. The composition of leachates depends on many factors, including the composition of the stored waste, the method of its storage, the density of the stored waste, the age of the landfill and the rate of formation of new layers. The technology of landfill construction and the method of its operation are also important. The composition of leachates includes organic components (organic nitrogen, volatile fatty acids, alkanes, alcohols, aldehydes, terpenes, and aromatic hydrocarbons), inorganic components (ammonium nitrogen, nitrates III and V, sulphates, bicarbonates, chlorides, sodium, and potassium) and heavy metals (arsenic, mercury, iron, cadmium, copper, nickel, and lead). Key indicators in monitoring leachate from landfills are pH, specific electrolytic conductivity, total organic carbon, the sum of polycyclic aromatic hydrocarbons and the content of individual heavy metals, such as copper, zinc, lead, cadmium, chromium (VI) and mercury [8,9].

When considering the potential toxicity of landfill leachates, it should be taken into account that they are mixtures of many substances, which may act synergistically or even additively with each other. However, the complexity of these mixtures makes it impossible to determine which substance is toxic in the leachate. It should also not be forgotten that, in addition to penetrating into soils and groundwater, leachates may be emitted in the form of bioaerosols and biogas. Bioaerosols contain microbiological factors that are potentially dangerous and infectious to humans, while biogas is produced as a result of uncontrolled biochemical transformations deposited in a landfill [10,11].

Research on the impact of waste landfills on human health is often associated with significant methodological limitations. One such limitation is the difficulty in estimating the level of population exposure to chemicals found in leachates. In our previous experiment, we also examined the potential toxicity of leachate from a landfill near Bialystok, but we focused on different research models and conducted the experiment in a different way. In the above-mentioned manuscript, we demonstrated stimulation of the growth of bacterial cells exposed to a wide range of LL concentrations, while in the case of human cells we obtained a toxic effect for healthy cells and a stimulating effect for the growth of cancer cells [12].

The aim of this work is to examine the impact of leachate from a local municipal waste landfill on various elements of the ecosystem, including humans. For this purpose, the chemical composition of leachates, their physicochemical properties, their influence on soil properties and plant metabolism, and the effect of leachates on selected bacterial strains and selected cell lines were analyzed. The novelty of this research study is the application of a wide range of bioassays, including plant metabolism analysis and microbial bioassays, and in vitro human cell culture, as a biological model for toxicological studies. This approach will allow for the complex estimation of LL influence on different ecosystem elements, including soil, plant, human and environmental microbiomes and the human organism.

2. Materials and Methods

2.1. LL Sample Collecting

Samples of landfill leachate were collected in December 2022 from a solid waste landfill located in the northeastern part of Poland (Hryniewicze) and prepared according to our previous studies [12]. The main physicochemical properties of the LL are summarized in the Table 1.

2.2. Pot Experiment Design and Plant Sampling

In order to determine the effect of LL on plants, a pot experiment was prepared according to the method described by Wydro et al. [13]. The properties of the soil on which the experiment was carried out are summarized in Table 1. Nitrogen doses were calculated according to the following data: a single dose of 85 kg/ha means 50% of the N dose, 50 LL, while 170 kg/ha means 100% of the N dose, 100 LL. Seeds of a lawn grass mixture containing *Lollium perenne*, *Festuca rubra* and *Poa pratensis* were sown before LL application.

The prepared pots with plants were watered with distilled water until the humidity reached 60–70% of the maximum water-holding capacity. The pot experiment was conducted for 6 weeks, after which plant samples were collected to determine the physiological parameters. In addition, soil filtrates were prepared according to the procedure described by Ming et al. and Wydro et al. [13,14].

Table 1. Physicochemical parameters of landfill leachate (LL) and soil before starting the experiment.

Parameter	Matrice		Parameter	Matrice	
	LL	Soil		LL	Soil
pH	7.85	8.70	BOD [1] (mg/L)	61	-
TOC [3] (mg/L)	-	1.23	COD [2] (mg/L)	1880	-
EC [4] (mS/cm)	9.87	0.275	N [6]	329.2 mg/L	0.138%
P [5] (mg/kg)	-	317	BOD/COD	0.032	
Metals	mg/L	mg/kg	Metals	mg/L	mg/kg
Cr	0.33	9.8	Ni	0.08	7.6
Mn	0.004	294.6	Cu	0.04	14.27
Fe	1.19	5232.5	Zn	0.08	63.43
Cd	0.01	0.40	Pb	0.03	27.41
Ions	mg/L		Ions	mg/L	
PO_4^{3-}	19.5	-	Cl^-	2568.2	-
SO_4^{2-}	104.7	-	NO_3^-	1523.6	-
N-NH_4	205.7				
PAHs	ng/L	µg/kg	PAHs	ng/L	µg/kg
Naphthalene	180.84	0.0309	Benzo[a]anthracen	4.80	1.3132
Acenaphthylene	10.90	0.0002	Chrysene	14.30	1.0465
Acenaphthene	40.95	0.0095	Benzo[b]fluoranthene	11.40	1.1196
Fluorene	136.84	0.0115	Benzo[k]fluoranthene	10.24	0.5245
Phenanthrene	475.90	0.0438	Benzo[a]pyrene	3.09	0.2603
Anthracene	39.29	0.0456	Indeno[1,2,3-cd]pyrene	8.15	0.4532
Fluoranthene	60.78	0.8361	Dibenzo[a,h]anthracene	2.56	0.0227
Pyrene	57.10	0.7296	Benzo[ghi]perylene	-	0.2723

[1] BOD—biochemical oxygen demand; [2] COD—chemical oxygen demand; [3] TOC—total organic carbon; [4] EC—electrical conductivity; [5] P—phosphorus; [6] N—nitrogen.

2.3. Soil Filtrates Physicochemical Characteristics

Physicochemical properties of soil filtrates, such as pH, total organic carbon (TOC), heavy metals (Ni, Cr, Mn, Fe, Cd, Cu, Zn and Pb) content, electrical conductivity (EC), inorganic ions (PO_4^{3-}, SO_4^{2-}, Cl^-, NO_3^-) content and 16 PAHs content were analyzed according to [12].

2.4. Assimilation Dyes Content in Plants

1 g of fresh plant sample was ground in a mortar with the addition of calcium carbonate and 20 mL of 90% methanol. The obtained suspension was filtered through filter paper. The absorbance in the plant extract was determined at the following wavelengths: 470 nm, 665 nm, 652 nm to determine the content of chlorophyll a (Chl*a*), chlorophyll b (Chl*b*) and carotenoids (carot). After adding 50% HCl to the extract, the absorbance was determined at a wavelength of 654 nm to determine the content of total pheophytins (Phe(*a* + *b*)). The dye contents were determined from Equations (1)–(4) and expressed as mg/g of fresh weight (mg/g fw). Moreover, the ratio of Chla/Chlb was calculated.

$$\mathrm{Chl}a = ((16.82 \times A_{665} - 9.28 \times A_{652}) \times V)/(m \times 1000) \quad (1)$$

$$\mathrm{Chl}b = (36.82 \times A_{652} - 16.54 \times A_{665}) \times V)/(m \times 1000) \quad (2)$$

$$\mathrm{Carot} = ((1000 \times A_{470} - 1.91 \times \mathrm{Chl}a - 95.15 \times \mathrm{Chl}b) \times V)/(m \times 1000) \quad (3)$$

$$\mathrm{Phe}(a + b) = (23.1 \times A_{654} \times V)/(m \times 1000) \quad (4)$$

where:

A_{665}, A_{652}, A_{470}, A_{654}—absorbance at wavelengths 665 nm, 652 nm, 470 nm, 654 nm, respectively;
Chl*a*—chlorophyll a; Chl*b*—chlorophyll b; carot—carotenoids; Phe(*a* + *b*)—total pheophytins;
M—sample weight (g);
V—volume of extract (mL).

2.5. Analysis of Oxidative Stress Parameters in Plants

In the plant samples, the activity of catalase (CAT), NADH-dependent substances, thiobarbituric acid reactive substances (TBARS), and superoxide dismutase (SOD), and the content of protein thiol groups (–SH), were analyzed according to Łozowicka et al. 2021 [15]. Each sample was analyzed three times.

2.6. Microbial Cell Viability and Cytotoxicity in Human Cells

The following strains were used to test the viability of microorganisms after treatment with LL: *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231), *Sporosarcina pasteurii* (ATCC 6453), *Lactobacillus rhamnosus* (ATCC 53103), and *Saccharomyces boulardii* (ATCC MYA-796). The tested microorganisms were cultured at 37 °C in Mueller Hinton II Broth medium. After 24 h, the cultures were diluted in fresh MH II Broth to obtain 10^6 CFU/mL inoculum. The details have been described previously in [12]. Antimicrobial activity after LL application was analyzed according to the methodology described previously by Liang et al. and Jahn et al. [16,17]. Each sample was analyzed three times.

The cytotoxic effect of leachate was examined in three different glioblastoma cell lines (LN-229, U-118MG, A-172) and one colorectal adenocarcinoma cell line (DLD-1), which were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in DMEM (Gibco, San Diego, CA, USA) supplemented with 10% FBS (Gibco, San Diego, CA, USA), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37 °C in a humidified atmosphere of 5% CO_2 in air. Leachate cytotoxicity was measured according to Carmichael et al. using an MTT cytotoxicity assay test [18].

2.7. Estimation of Chronic Daily Intake (CDI) and Cancer Risk (CR)

Chronic daily intake (CDI) and cancer risk (CR) were calculated for estimating the carcinogenic risk for selected LL components. CDI and CR factors were calculated according to [19–22].

2.8. Data Analysis

The effects of leachate and soil filtrate application on the studied parameters were determined using analysis of variance (ANOVA). When significant differences were detected depending on the level of the experimental factor (LL dose), the differences between the means were evaluated by Tukey's test at $p < 0.05$. For cytotoxicity and viability tests, the differences between the control and treated cells were established using Dunnett's test at $p < 0.05$, $p < 0.01$ and 0.001. Relations between soil filtrate properties and plant physiological characteristics were identified by Pearson correlation analysis, and correlations were significant at $p < 0.05$.

3. Results

3.1. Physicochemical Properties of Soil Filtrates

Prepared soil filtrates from various experimental variants contain various components (ions, heavy metals, PAHs, etc.) in forms that can potentially be taken up by plants together with soil water (Table 2). The sum of PAHs in the filtrate in the control soil was 1006.78 ng/L, while in the filtrate where the soil was treated with N-50% and N-100% it was 1350.53 ng/L and 1688.29 ng/L, respectively, which was approximately 34% and 67% more compared to the control. Among all analyzed PAHs where N-100% was applied, the highest amounts were observed for benzo[a]anthracene, chrysene, benzo[b]fluoranthene, and fluoranthene, at 356.67 ng/L, 284.82 ng/L, 239.51 ng/L and 209.12 ng/L, respectively. In the filtrate from the control soil, the highest amount of benzo[k]fluoranthene was observed—225.6 ng/L—while the highest amount of phenanthrene—286.75 ng/L—was observed in the filtrate treated with N-50%. Among all the analyzed metals in the filtrate, the highest values were observed for Fe, around 2.3 µg/L. However, among the tested ions in the filtrate, the highest values were obtained for Cl^- and NO_3^- treated with N-50% and N-100% leachate.

Table 2. Physicochemical parameters of soil filtrates.

Parameter	Dose			Parameter	Dose		
	Control	N-50%	N-100%		Control	N-50%	N-100%
pH	8.7	8.23	7.8	TOC (mg/L)	119.40	570.70	293.90
Metals		µg/L		Metals		µg /L	
Ni	0.004	0.004	0.004	Cd	0.001	0.002	0.003
Cr	0.008	0.008	0.005	Cu	0.033	0.030	0.038
Mn	0.026	0.023	0.026	Zn	0.043	0.036	0.055
Fe	2.245	2.360	2.337	Pb	0.005	0.006	0.007
Ions		mg/L		Ions		mg/L	
PO_4^{3-}	3.72	3.56	4.82	Cl^-	1.32	51.54	153.15
SO_4^{2-}	2.76	5.47	13.47	NO_3^-	0.75	29.6	63.4
PAHs		ng/L		PAHs		ng/L	
Naphthalene	23.48	115.63	75.96	Benzo[a]anthracen	61.30	179.64	356.67
Acenaphthylene	0.16	4.27	2.34	Chrysene	77.96	134.41	284.82
Acenaphthene	7.23	14.92	8.83	Benzo[b]fluoranthene	81.75	120.92	239.51
Fluorene	9.41	43.83	19.78	Benzo[k]fluoranthene	225.66	101.26	141.00
Phenanthrene	28.57	286.75	115.01	Benzo[a]pyrene	67.43	15.71	30.85
Anthracene	18.91	11.12	2.67	Indeno[1,2,3-cd]pyrene	45.42	15.08	36.86
Fluoranthene	159.15	102.03	209.12	Dibenzo[a,h]anthracene	19.83	3.40	3.29
Pyrene	139.93	186.24	159.14	Benzo[ghi]perylene	40.58	15.30	2.46

3.2. Assimilation Dyes Content in Plants

The doses of leachate used in the tests influenced the content of chlorophyll a and b and their proportion in grass leaves. The amount of chlorophyll a was at a similar level and amounted to approximately 0.16 mg/g FM (Figure 1), while the content of chlorophyll b in the aboveground parts of grass was statistically significantly higher where N-100% was used. The total chlorophyll content in grasses was from 0.273 mg/g FM (N-50%) to 0.365 mg/g FM (N-100%). The calculated chlorophyll a/b ratio indicates the stress that the plants may have experienced after the leachate was applied. The application of N-100% significantly reduced the a/b ratio and its value was 0.72 mg/g FM. In turn, after application of N-50%, the a/b ratio increased to 1.6 (Figure 1). Analysis of the carotene content in grass leaves indicates an increase in their content with the leachate dose applied. The highest amounts of carotenes were observed where N-100% was used. The content of pheophytin a + b was the highest in the control and the lowest—0.15 mg/g FM—where the leachate dose of N-50% was used.

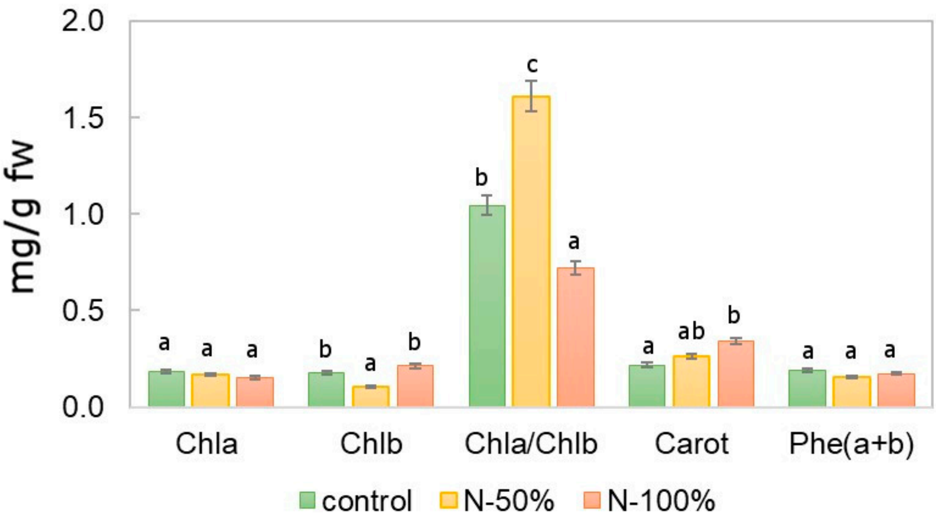

Figure 1. Content of Chl*a*, Ch*b*, carot, Phe(*a* + *b*) and Chl*a*/Chl*b* in plants after application of LL in doses of C (control), N-50% and N-100%. The same letters above bars mean nonsignificant differences between treatments assessed by the Tukey test at $p < 0.05$.

3.3. Oxidative Stress Parameters in Plants

Figure 2 shows the parameters of oxidative stress in plants after the application of leachate (N-50% and N-100%). Catalase activity ranged from 0.74 U/mg protein to 1.39 U/mg protein. Treatment with leachate at a dose of N-100% increased the activity of this enzyme by approximately 87% compared to control samples. The activity of NADH-dependent peroxidase in control samples and samples treated with leachate at a dose of N-50% were at a similar level of 0.12 U/mg protein and 0.15 U/mg protein, respectively. However, in plants treated with the highest dose of leachate, a significant statistical increase in NADH-dependent peroxidase by approximately 133% was observed compared to the control. The content of –SH groups ranged from approximately 68 nM/mg protein to approximately 78 nM/mg protein (Figure 2). A slight decrease in the number of -SH groups was observed after the application of N-50% and N-100% leachate, approximately 8% and 16%, respectively, compared to the control. The TBARS content in plants increased with the increase in the leachate dose and at the highest dose it was at the level of 1.27 nM/mg protein (Figure 2). Compared to control samples, an increase in TBARS content was observed by approximately 29% for N-50% and 84% for N-100%. SOD activity in the tested samples ranged from 18.6 U/mg protein to 40.7 U/mg protein. After applying the leachate to the soil, a significant increase in SOD activity was observed compared to control samples by approximately 32% for N-50% and 118% for N-100%.

Table 3 shows the Pearson correlation coefficients for the physiological parameters of plants and the properties of soil filtrates. The obtained results indicate a positive significant correlation between Cd and parameters such as TBARS, SOD and -SH groups (0.79; 0.87 and 0.76, respectively), and a negative one for Chla (−0.89). Moreover, a positive significant correlation was detected between Pb and -SH groups (0.68), and a negative one between Pb and Chla (−0.91). In the case of TBARS and SOD, a positive significant correlation with Cl^- concentration was observed (0.89 and 0.98, respectively). The content of 16 tested PAHs correlated positively with CAT (0.89) and SH groups (0.65) and negatively with Chla (−0.85).

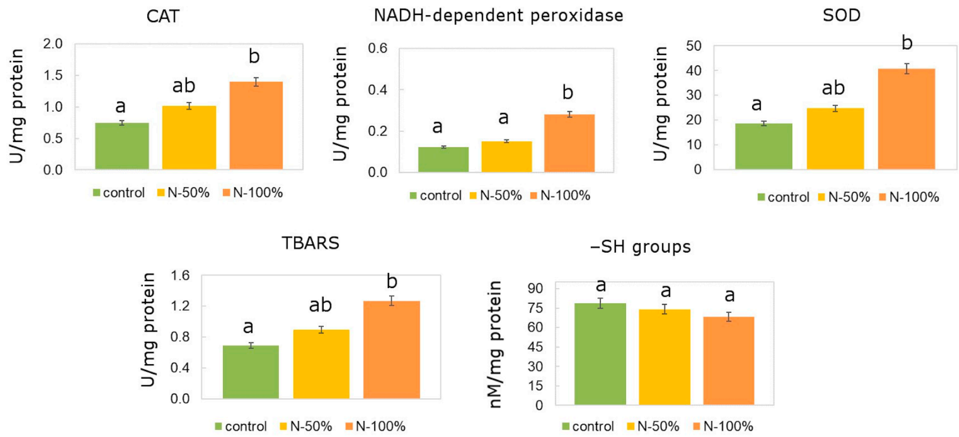

Figure 2. The main parameters of oxidative stress (CAT, NADPH-dependent peroxidase, SOD, TBARS and -SH groups) in plants after application of LL in doses of C (control), N-50% and N-100%. The same letters above bars mean nonsignificant differences between treatments assessed by the Tukey test at $p < 0.05$.

Table 3. Correlation coefficients (r) between the physicochemical properties of soil filtrates and the physiological parameters of plants.

Soil Filtrates Characteristics	CAT	NADH	TBARS	SOD	-SH Groups	Chl*a*	Chl*b*	Chl*a*/Chl*b*	Carot	Phe(*a* + *b*)
pH	−0.67	−0.93	−0.98	−0.96	0.33	**0.83** *	−0.30	0.34	−0.98	0.42
TOC	0.29	0.04	0.23	0.14	−0.33	−0.39	−0.75	0.72	0.23	−0.20
Cd	0.52	0.94	**0.79**	**0.87**	**0.76**	**−0.89**	0.33	−0.36	0.79	−0.40
Cr	−0.41	−0.39	−0.94	−0.96	0.89	0.06	−0.76	0.78	−0.93	−0.12
Mn	0.10	0.35	0.16	0.25	−0.05	0.01	0.94	−0.93	0.16	0.62
Fe	0.69	0.48	0.64	0.57	−0.72	−0.76	−0.37	0.34	0.64	−0.70
Cu	0.69	0.85	0.74	0.80	−0.66	−0.61	0.94	−0.96	0.73	0.48
Zn	0.70	0.86	0.74	0.80	−0.67	−0.62	0.94	−0.95	0.74	0.47
Pb	0.80	0.94	0.39	0.97	**0.68**	**−0.91**	0.33	−0.36	0.39	−0.40
PO_4^{3-}	0.86	0.96	0.89	0.93	−0.83	−0.80	0.83	−0.85	0.49	0.23
SO_4^{2-}	0.74	0.03	0.69	0,87	−0.98	−0.66	0.57	−0.60	0.29	−0.14
Cl^-	0.54	0.89	**0.89**	**0.98**	−0.49	−0.58	0.50	−0.53	0.51	−0.21
NO_3^-	0.35	0.65	0.39	**0.98**	−0.25	−0.25	0.37	−0.40	0.29	−0.35
16PAHs	**0.89**	0.74	0.29	0.97	**0.65**	**−0.85**	0.32	−0.36	0.42	−0.40

* Bolded values mean significant correlation coefficient at $p < 0.05$.

3.4. Microbial Cell Viability

Figure 3 shows the effect of leachate on the growth of *S. pasteurii*, *Staphylococcus aureus*, *Lactobacillus rhamnosus*, *Saccharomyces boulardii* and *Candida albicans*. Their viability was measured using the colorimetric method with an MTT reagent. The results obtained for *C. albicans* show that the stimulating effect after using the leachate was observed at concentrations ranging from 0.8% to 12.5%. However, a statistically significant inhibitory effect on the growth of *C. albicans* of approximately 22% was observed only at the highest concentration of 50% as compared to the control. Regarding the effect of leachate on the viability of *S. pasteurii*, it was observed that treatment with leachate at concentrations of 0.8%, 1.6%, 3.10%, 6.30%, 12.5%, 25% and 50% showed a slight decrease in the growth of *S. pasteurii* of 2% to 7% compared to the control. In turn, *S. aureus* leachate treatment resulted in an increase in cell viability of 2% to 5% at concentrations of 0.8%, 1.6%, 3.10%, and 6.30% compared to the control, while a decrease in relative cell viability was observed at the highest leachate concentrations used (from 12.5% to 50%). The highest inhibitory effect on S. aureus was observed after using the leachate at a concentration of 50% and was approximately 51%.

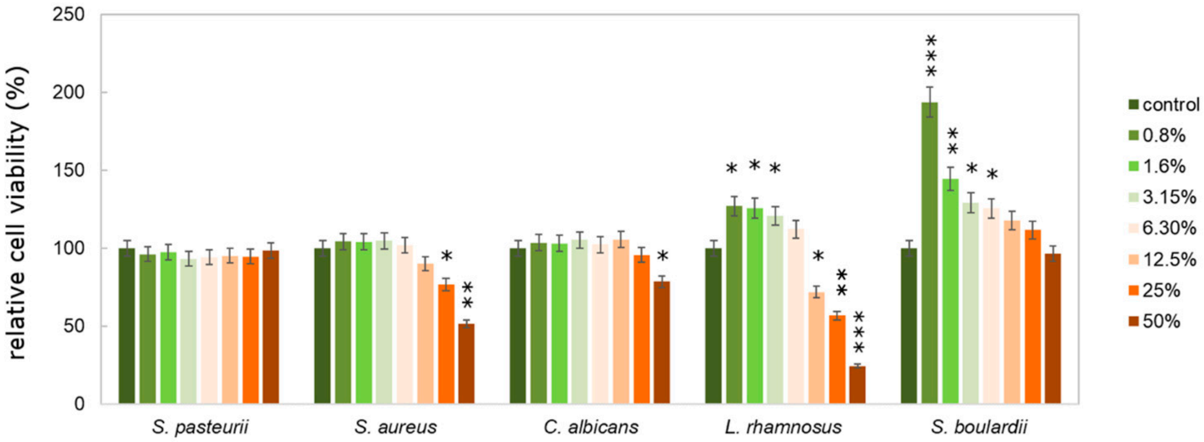

Figure 3. Relative viability of microbial cells (*S. pasteurii*, *S. aureus*, *C. albicans*, *L. rhamnosus* and *S. boulardii*) treated with LL in different concentrations. Each value presented as a column with bars represents the mean with the standard deviation (±SD). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ describe significant effects between treatments and control assessed by Dunnett's test.

In turn, treatment with the leachate of probiotic bacteria *L. rhamnosus* and *S. boulardii* had a stimulating effect on the relative viability in the lowest concentrations of 0.8%, 1.6%, 3.10%, and 6.30%. The highest relative cell viability was observed in *S. boulardii* at a concentration of 0.8% and amounted to 193%. However, in *L. rhamnosus*, the highest inhibitory effect on relative viability was observed after applying the highest concentration of 50% and amounted to 76% compared to untreated cells. However, in the case of *S. boulardii*, treatment with leachate at a concentration of 50% resulted in a slight growth decrease of approximately 4% compared to control cells.

3.5. Human Cell Viability

In the case of human cells, the vast majority of them observed an increase in cell proliferation under the influence of the analyzed leachates (Figure 4). All cell lines used as models in this experiment were cancer lines. Both in the case of the DLD-1 colorectal adenocarcinoma line and in the case of all glioblastoma lines, increases in proliferation were observed, with the exception of the U118-MG line, which was the only one that responded by inhibiting cell proliferation as a result of exposure to leachates. However, it should be noted that even in this U118-MG cell line, an increase in the level of cell proliferation above the control level was noted under the influence of the two lowest analyzed leachate concentrations—0.1% and 0.5%. Statistical analysis indicates particularly high increases in proliferation under the influence of LL in the DLD-1 cell line. Virtually no concentration tested dropped below the control level, but particularly high levels of proliferation were observed in the concentration range of 0.1% to 2%. This seems obvious considering that it is a gastrointestinal cancer, which is the most exposed to any xenobiotics/contaminants entering the body through food.

3.6. Chronic Daily Intake (CDI) and Cancer Risk (CR) for Selected LL Components

LL contains many potentially dangerous substances such as heavy metals and PAHs. CR was estimated for selected particularly hazardous metals and PAHs (Table 4). It was found that the tested leachates have carcinogenic properties and pose a danger to human health. Among the tested compounds, Cd and Cr appear to exceed values indicating high toxicity. The remaining analyzed compounds do not exceed the standards adopted for toxicological safety.

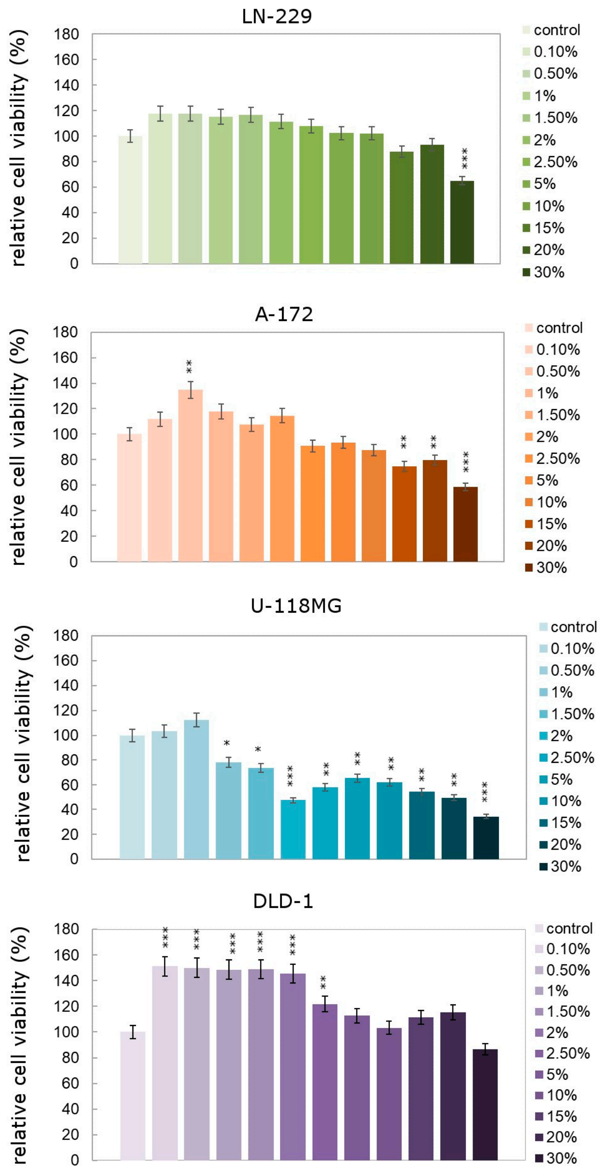

Figure 4. Relative viability of cells exposed to grading concentrations of landfill leachate. Each value presented as a column with bars represents the mean with the standard deviation (±SD). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ describe significant effects between treatments and control assessed by Dunnett's test.

Table 4. Chronic daily intake (CDI (mg kg^{-1} day^{-1})) and cancer risk (CR) for selected LL hazardous components.

Parameter	Conc (mg/L)	CDI (mg/kg/day)	CSFing	CR
Cr	0.33	9.0×10^{-3}	0.5	4.7×10^{-3}
Cd	0.01	3.0×10^{-4}	15.0	4.3×10^{-3}
Pb	0.03	9.0×10^{-4}	8.5×10^{-3}	7.3×10^{-6}
Benzo[a]anthracen	4.8×10^{-6}	1.4×10^{-7}	0.7	1.0×10^{-7}
Chrysene	1.4×10^{-5}	4.1×10^{-7}	7.30×10^{-3}	3.0×10^{-9}
Benzo[b]fluoranthene	1.1×10^{-5}	3.3×10^{-7}	0.7	2.9×10^{-7}
Benzo[k]fluoranthene	1.0×10^{-5}	2.9×10^{-7}	0.07	2.1×10^{-8}
Benzo[a]pyrene	3.1×10^{-6}	8.8×10^{-8}	7.3	6.4×10^{-7}
Indeno[1,2,3-cd]pyrene	8.2×10^{-6}	2.3×10^{-7}	0.7	1.7×10^{-7}
Dibenzo[a,h]anthracene	2.6×10^{-6}	7.3×10^{-8}	7.3	5.3×10^{-7}

4. Discussion

Leachates from municipal waste landfills are characterized by diverse chemical composition, which is one of the factors that significantly complicate their chemical analysis and testing of their toxic properties. The composition of leachates changes over time and depends on the type of waste stored and the method of landfill operation. Data from the literature indicate that in most cases leachates contain high concentrations of nitrogen, the content of which, like other organic compounds, changes during the operation of the landfill [23,24].

Due to the complexity of the environmental matrix constituted by leachates, the methodology for examining them should include various scopes of research, both physicochemical and biological. The results obtained during physicochemical analyses indicate a relatively high content of PAHs, especially fluorene and phenanthrene, as well as BOD and COD. The high content of organic matter, total nitrogen and nitrates in the leachate indicates a high potential danger to the natural environment as a result of possible penetration of the leachate into, for example, groundwater. These results are consistent with data from the literature and show some analogies to our earlier work [12]. In a study by Jabłońska-Trypuć et al., the authors also analyzed leachate from the landfill in Hryniewicze, but both experiments differ in the dates of sampling and the biological models used. In our previous experiment we focused mainly on different bacterial strains and human cell lines representing healthy cells and cancerous cells. For the first time in the LL study, here we used a comprehensive approach covering a wide range of biological tests. We started analyzing the impact of LLs on the environment by examining their impact on plants. The key aspects turned out to be the content of assimilation dyes and oxidative stress parameters.

In plants, chlorophyll a and chlorophyll b are the main photosynthetic pigments that are an important element of proper functioning. In our studies, the addition of leachate to the soil had a significant impact on the content of chlorophyll a and b, carotenoids and pheophytin in the aboveground parts of the grass mixture. In most cases, the chlorophyll a to b ratio was relatively low (less than 1) (Figure 1). The reason for this may be the addition of leachate, as well as the properties of the urban soil on which the lawn grass grew, which could have been an additional stress factor. This may be explained by the particularly similar chlorophyll a to b content in plants collected from control pots. As noted by Hänsch and Mendel [25], the proper functioning of photosynthetic pigments may be disturbed by heavy metals contained in urban soil, which contribute to physiological and anatomical changes in plants. In our own research, an imbalanced ratio of chlorophyll a to b and an increase in carotene content could be observed at the highest dose of leachate used. Prasad observed a similar trend in his research [26], where he indicated the negative impact of metals on the inhibition of chlorophyll production and thus on the biochemical processes occurring in plants, i.e., respiration, photosynthesis, and transpiration.

Pheophytin is a functional component of the reaction center of photosystem II, where it participates in electron transfer from P-680. The increase in the pheophytin content in plants may be an expression of chlorophyll destruction. Among the assimilation pigments, chlorophyll a is more susceptible to pheophytinization than chlorophyll b [27]. Based on the results obtained, it can be seen that treating plants with doses of N-50% and N-100% of the leachate does not affect the increase in the sum of pheophytins a and b in the leaves, and even causes their value to decrease by approximately 11% compared to the control. Moreover, a positive correlation between the sum of the pheophytins and the content of chlorophyll a was observed, which may indicate the mutual relationship between these pigments during environmental stress.

Reactive oxygen species (ROS) in low concentrations are needed by organisms because they function as signaling molecules, participate in programmed cell death, participate in tissue development, and regulate gene expression. Antioxidant systems protect the body against excessive accumulation of ROS, including the enzymatic and nonenzymatic systems [28]. The literature sources show that the enzymatic activity of peroxidase is considered a very important indicator of oxidative stress in plants. In response to stress factors in the plant's environment, there is usually an increase in the activity of this enzyme in its tissues [29], which was also observed in our own research. As Huang et al. pointed out [30], peroxidase is one of the enzymes that participate in maintaining the balance between ROS production and their removal. However, catalase activity is related to the removal of hydrogen peroxide, which is a reactive oxygen species [28]. In our own research, the formation of H_2O_2 in plant tissues may be a defensive reaction to the dose of leachate that was applied in the experiment. According to Møller et al. [31], H_2O_2 transported by aquaporins in the cell membrane will not only cause oxidative damage but will also participate in the regulation of cell signaling. However, as shown by Kärkönen and Kuchitsu [32], an increase in the H_2O_2 content in cells may be the result of cell differentiation and the formation of a cell wall. Moreover, it is closely related to and interacts with hormones that regulate development processes in plants and the aging of plant cells. SOD is the main intracellular antioxidant that is involved in protecting the cell against ROS [33]. When it comes to plants, we can distinguish three isoforms of SOD, such as the Mn-SOD isoform found in the mitochondria; the chloroplastic Fe-SOD isoform; and the chloroplastic and cytosolic Cu/Zn-SOD isoform [34]. According to the literature, nutrient deficiencies and the presence of metals in the soil may increase SOD activity and also cause the appearance of new isoforms [33,35]. In our own research, an increase in SOD activity was observed with the applied dose of leachate, which suggests that the metals (Pb and Cd) contained in these leachates could induce the activity of this enzyme and counteract stress in plants. Moreover, a positive correlation between SO_4^{2-} and Cl^- ions is also observed. Oxidation of the -SH groups of proteins indicates that the plant is under oxidative stress because this metabolite can act as a detoxifier and antioxidant [36]. In our study, a decrease in -SH groups was observed, which may indicate that the plants had started to activate their defense system. Zagorchev et al. [37] report that plant thiols are involved in the response of plants to many stress factors, and their quantities in plants, redox status, and regulation are important criteria for understanding the interconnections between various biochemical pathways and plant tolerance to stress.

The formation of TBARS in plants indicates the generation of free radicals, and this indicator can be used as a marker in biological systems [38]. TBARS analysis in plants showed that after the use of leachate, the amount of this indicator in plant leaves increased statistically significantly, which indicates a high level of damage to cell membranes, especially in plants where the highest dose of leachate was applied. According to Turan [39], changes occurring in the properties of cell membranes, such as an increase in their permeability to H+ ions and other elements, indicate a significant level of membrane damage and an increase in lipid peroxidation.

Among the bioassays selected by researchers to analyze LL toxicity are often microbial bioassays. Their most important advantages are speed of execution, repeatability of results and good quality-to-cost ratio. The most common tests are the test using *Vibrio fisherii*, based on the bioluminescent properties of this bacterium, and the test using the *Salmonella* strain. In our research, we chose a wide spectrum of bacterial strains and one species of fungus. There were significant differences between the tested strains in the intensity of the proliferative response to the tested leachates. This is consistent with data from the literature indicating differences in sensitivity to chemical substances contained in leachates and other environmental pollutants [40–42]. In our work, we selected so many different strains that the results of toxicological analysis based on microbial bioassays were as reliable as possible. Different strains of bacteria and species of fungi represent taxonomically, phylogenetically, and structurally a wide range of microorganism diversity in the environment and, at the same time, their different sensitivities to xenobiotics [43,44]. Similarly to Zawierucha et al., we noticed an evidently large amount of metal ions, especially such as lead [45]. Heavy metal ions are highly toxic to living organisms, and even trace amounts may cause an effect due to their accumulation in living cells. Bacterial cells are more resistant to the presence of many pollutants, including heavy metals. Therefore, we observed a smaller effect of the leachate in them than in the case of human cells. The results obtained are similar to those that we presented previously [12]. TU was calculated according to Kalka J., and it amounted to 6.19 for *Lactobacillus rhamnosus* and 17.85 for *Saccharomyces boulardii*, which indicates that the studied LL is toxic for *L. rhamnosus* because the TU value is below 10 [46].

In the literature on toxicological analyses of landfill leachates and other xenobiotics, some in vitro biological models based on selected human cell lines have been described. These lines are primarily HepG2, MCF-7, lymphocytes, CHO, MVLN, and NIH/3T3 [47]. Typically, cell lines for toxicity testing are selected due to the route of exposure to the tested substances. Toxic compounds, including those found in landfills and in leachates generated there, may be absorbed through ingestion (e.g., of contaminated water), inhalation, or through the skin. Each of the considered routes poses a significant threat to human health [48]. However, it should be remembered that potentially toxic substances/mixtures reach tissues further away from the site of direct contact and generate various forms of damage, stimulating, among others, the process of carcinogenesis. That is why we decided to analyze, among others, glioma cells and colorectal adenocarcinoma. Orescanin et al. also used the colorectal adenocarcinoma line—Caco-2—as a model, with which they demonstrated extremely high cytotoxicity of untreated sludge [49]. We also observed an obvious stimulation of cancer cell proliferation as a result of exposure to LL, which was similar to an increase observed in our previous study [12]. This may be due to an increase in the level of oxidative stress in these cells, which usually translates into increased tumor growth. In turn, very high concentrations of LL caused the death of cancer cells, most likely by necrosis.

Taking everything together, it can be assumed that in such a complex environmental matrix as landfill leachates, various reactions undoubtedly occur between its components, which translates into the effect observed in plant tissues, bacterial and fungal cells, as well as in human cells.

5. Conclusions

Regardless of the biological models used, the toxic effect of LL was confirmed in our studies. Any release of such leachates into the environment without prior appropriate treatment is potentially extremely dangerous. Therefore, both landfills and leachates generated there should be constantly monitored to exclude the possibility of their entering ground or surface waters. Research such as ours is extremely valuable from the point of view of the need to implement new methods and technologies for leachate treatment and landfill control to prevent possible environmental contamination. Such studies indicate the need to develop toxicological analyses in order to implement the fastest and most effective research methods possible in the future.

The area where the waste landfill is located, where the samples were taken, is not an industrialized area. It is a typical municipal waste landfill, and the areas of the Podlaskie Voivodeship are agricultural rather than industrial areas. Therefore, taking into account the results obtained indicating the toxicity of the tested leachates, we conclude that even the smallest landfills, in areas where particularly toxic waste is not generated, should not be underestimated. According to Zuo et al., if the CR index is lower than 1×10^{-4}, it is an acceptable value and does not indicate a high level of risk [50]. Our results indicate that only heavy metals such as Cd, Pb and Cr may pose a danger to humans (Table 4). The analyzed PAHs did not exceed acceptable standards; therefore, we conclude that the tested leachates do not generally pose an unacceptable health risk to the average exposure population. The exceptions are some heavy metals. When comparing our results with the data obtained by Kalka J. [46], attention should be paid to the origin of the tested leachates. Differences in the composition and toxicity of leachates may result from different geographical locations, and also from different levels of industrialization of a given region. Although our results, including the analysis of CDI and CR indicators, do not indicate significant toxicity of the tested leachates, we believe that they should be constantly and carefully monitored. Concentrations of 25% and higher turned out to be toxic in the bacterial model, while even very low concentrations in the range of 0.1–1.5% stimulated the growth of cancer cells, which clearly indicates their potential carcinogenic properties.

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