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Ewa ROBACZEWSKA-CHRYCZYK, Krzysztof FIJAŁKOWSKI, Tomasz KAMIZELA

Czestochowa University of Technology, Faculty of Infrastructure and Environment
Institute of Environmental Engineering
ul. Brzeźnicka 60A, 42-201 Częstochowa
e-mail: tkamizela@is.pcz.czest.pl

The Suitability of the *Lepidium* Test for Assessing the Toxicity of Leachate from a Municipal Landfill

Przydatność testu *Lepidium* do oceny toksyczności odcieków ze składowiska odpadów komunalnych

Sealing of landfill and treatment of leachate is a very important issue in the operation of the landfill, part of which is monitoring of leachate. In addition to the assessment of quantitative and qualitative characteristics, monitoring of leachate should also involve the assessment of leachate phytotoxicity. This is particularly important in the case of migration of leachate and its impact on the soil and water environment. The aim of this study was to determine phytotoxicity of leachate from a municipal landfill based on the plant stress test (the *Lepidium* test). The *Lepidium* test was considered as useful in the assessment of toxicity of landfill leachate, resulting from changes in the plant growth depending on leachate concentration in the solution. The use of the *Lepidium* test allowed for the determination of an approximate threshold volume of leachate which leads to the inhibition of test plant growth. Undisturbed plant development was achieved in the environment with leachate concentrations below 1÷2%. It is also suggested that the test methodology should be extended in order to measure protein concentration. The usefulness of measuring peroxidase was not demonstrated. Based on the values of physico-chemical indicators of leachate and the content of metals, no significant toxic factors were found to inhibit the growth of the test plant.

Keywords: **landfill leachate, phytotoxicity, *Lepidium* test, municipal waste, peroxidase activity**

Introduction

Leachates from municipal landfills are characterized by high concentrations of organic and inorganic components, including heavy metals and many hazardous organic substances. The indicators of leachate contamination differ greatly in ranges of values, depending on the age of the landfill. Data obtained from several landfills located in Poland showed that BOD_5 ranges from 100 to 50,000 mg/L, COD ranges from 5,000 to 60,000 mg/L and particulate organic matter ranges from 300 to 50,000 mg/L. Leachate effluents are also considered to be a potential source of pathogenic microorganisms. Significant amounts of bacteria in the first months of operating a landfill are observed in leachates, including faecal *Escherichia coli* and *Streptococcus* [1].

An operating landfill should be equipped with an installation for collecting and treatment of leachate to such an extent that the effluent can be received by municipal treatment plant or discharged into water or soil. There are several physical, chemical, biological and combined methods of treatment. The choice between various methods of pre-treatment or treatment and the effectiveness of removal of contaminants depend on qualitative and quantitative characteristics of the leachate [2-5]. It should be emphasized that leachate is the contaminant with the longest emissions as its emission occurs not only during landfill operation but also during its rehabilitation.

Therefore, both qualitative and quantitative characteristics of the leachate should be monitored. However, it is likely that monitoring the phytotoxicity of leachate may be of equal importance. It is particularly important if leachate migrations occur and affect soil and water environments, including plants. Leachate migration constitutes a potential stress and toxic factor to plants and can limit or inhibit their growth. A number of test plants have been used in the assessment of toxicity of environmental samples. Plant tests are based on measuring the number of germinated seeds and the growth of roots. For example, *Lepidium* test measures, among other things, the sensitivity of *Lepidium sativum* roots to the presence of mutagenic and carcinogenic compounds in the soil. The *Lepidium* test has been used to evaluate the inhibition of meristematic cell division in roots by cytotoxic compounds, leading to developmental arrests [6, 7].

The aim of this study was to establish the phytotoxicity of leachate from a municipal landfill. The study discusses the results obtained from the *Lepidium* test. The aim of the study was to see whether the standard *Lepidium* test, besides the measurement of the length of roots and the number of germinated seeds, is capable of adequate biochemical response. This required establishing the changes in protein concentration and activity of guaiacol peroxidase (POD) which become activated when the plant is under stress. The research determined the likelihood of development or inhibition of the growth of the plant subjected to a stress factor using various leachate doses.

1. Material and methods

1.1. Leachate

The samples were obtained from a collector pipe supplying leachate to the buffer tank and then to a treatment system based on membrane technology. The samples were collected in the spring season at a municipal landfill in the Silesian Voivodeship in Poland. The basic characteristics of the leachate are presented in Table 1. The assay was performed for a sample of supernatant liquid after centrifugation and filtration of leachate (centrifugation: 12,100 RCF, 15 minutes, filtration: qualitative filter paper, basis weight: 65 g/m^2). Chemical oxygen demand (COD) was assayed by means of cuvette tests using HACH DR 5000 (LCK 114) spectrophotometer. Five-day biochemical oxygen demand (BOD_5) was evaluated with a manometric WTW Oxi-Top test set. Total organic carbon (TOC) was assayed by means of an Analytik Jena multi N/C 3100 using non-purgeable Organic Carbon (NPOC) method. Alkalinity was measured using the potentiometric method whereas ammoniacal nitrogen was establish by means of the titrimetric method.

Table 1. **Characteristics of the analysed leachate**

Indicator	Measurement unit	Mean	Deviation
pH	–	8.35	0.23
Alkalinity	mg $CaCO_3$/L	2158	94.7
COD	mg O_2/L	1840	201.3
BOD_5	mg O_2/L	174	54.8
TOC	mg C/L	321.3	36.8
Ammoniacal nitrogen	mg N-NH_4^+/L	250.2	25.4

Additionally, atomic emission spectroscopy was used to evaluate the concentration of selected metals in accordance with the PN-EN ISO 1185:209 standards (Table 2).

Table 2. **Concentration of metals in leachate from the municipal landfill analysed in the study**

Element	Unit	Mean	Deviation
Cd	mg/L	Not detected	–
Cr		1.69	0.03
Cu		0.22	0.1
Ni		0.30	0.01
Pb		Not detected	–
Zn		0.25	0.01
Ca		300.10	1.54
Fe		0.68	0.31
K		1 531.0	18.8
Mg		257.7	2.2
Mn		1.58	0.09
P		12.4	0.18

1.2. The *Lepidium* test

The toxicity tests were performed using the garden cress (*Lepidium sativum* L., Brassicaceae). The test was based on the method presented by Walter et al. [8]. Question proposed in the research concerned the dilution of leachate which would not interfere with the plant growth. For this purpose, 18 groups of solutions were prepared, each with different volumetric ratio of leachate and distilled water (Table 3).

Petri dishes used in the study were lined with blotter paper, with each dish containing 10 seeds of cress. The seeds were drenched in 3 ml of concentrated solution. For each concentration of leachate, 10 repetitions were performed (giving the total of 100 seeds used).

In order to compare the plant growth, 5 dishes (50 seeds) from each group were incubated for 48 hours and the remaining 5 dishes (50 seeds) were incubated for 72 hours. The incubation temperature in a fitotron chamber was 25°C and remained unchanged throughout the study. After the incubation period, germinated seeds were counted and the length of roots was manually measured.

Table 3. **Comparison of dilutions used in the study**

No.	Group	1	2	3	4	5	6	7	8	9
1	Leachate, %	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
2	Distilled water, %	100	99.9	99.8	99.7	99.6	99.5	99.4	99.3	99.2
No.	Group	10	11	12	13	14	15	16	17	18
3	Leachate, %	0.9	1.0	2.0	3.0	4.0	5.0	10.0	15.0	20.0
4	Distilled water, %	99.1	99.0	98.0	97.0	96.0	95.0	90.0	85.0	80.0

The results of *Lepidium* test were presented with following indicators: relative seed germination (RSG), relative root growth (RRG) and germination index (GI). The respective indicators were calculated from the following formulae:

$$RSG = \left(\frac{S_E}{S_K}\right) \cdot 100 \quad (1)$$

where:
RSG - relative seed germination, %,
S_E - number of seeds germinated in an extract,
S_K - number of seeds germinated in distilled water.

$$RRG = \left(\frac{R_E}{R_K}\right) \cdot 100 \quad (2)$$

where:
RRG - relative root growth, %,
R_E - length of roots after germination in an extract, mm,
R_K - length of roots after germination in distilled water, mm.

$$GI = (RSG \cdot RRG)/100 \quad (3)$$

where GI - germination index.

1.3. Assaying the protein concentration and peroxidase

The protein concentration was evaluated by means of the Lowry method [9]. Activity of guaiacol peroxidase was assayed using the method presented by Neves et al. [10-12].

1.4. Statistical analysis

Pearson correlation coefficient was calculated to establish the strength of correlations between variables. The nonparametric Mann-Whitney U test was used to assess the variance of RSG, RRG and GI values, protein concentration, and POD activity between 48-hour and 72-hour incubation periods. Statistica 12 PL software was used to perform the statistical analysis.

2. Results and discussion

From the standpoint of physicochemical properties, leachate is a complex and variable mixture of organic and inorganic substances. Leachate composition depends on the type of waste discharged to a landfill and reflects changes in landfill's microbiological activity. The age of the landfill is a critical determinant of leachate composition. It was found based on physicochemical indicators, especially BOD_5/COD ratio of 0.1, that the landfill analysed in the study was stable.

Elemental analysis of leachate did not detect the most toxic metals such as Cd or Pb (see Table 2). These elements are highly toxic to plants because of their role in the formation of reactive oxygen species (ROS). Leachate contains significant concentrations of basic biogenic elements such as N, P and K, which play a crucial role in plant growth, and, in the case of potassium, in increasing resistance to stress and pathogens. The concentration of active forms of potassium (ions) was detected to be at 1.5 g/L of leachate, which is equal to 1% of the recommended dose of potassium in leaf fertilizers. The leachate was also found to contain other microelements necessary for plant growth: Cu, Zn, Fe, Mn, and ions of Ca and Mg, with all of them present in non-toxic concentrations. Chrome and nickel concentrations (1.7 and 0.3 mg/L, respectively) did not exceed the permissible levels defined by Polish law for organic and mineral fertilizers (Cr - 100 mg/L, Ni - 60 mg/L) [13, 14].

It was observed that after 48 and 72-hour incubation periods, the seeds germinated only in the samples where the leachate accounted for no more than 15% of the mixture (Table 4). Significant differences related to the incubation period used were found in the length of cress roots (Fig. 1). After 48-hour incubation, roots were shorter by 20÷50% from those found in the seeds after 72-hour incubation. Roots after the longer incubation period were found to be tender and fragile. Other morphological changes such as sprain were not observed. Data analysis showed that concentration which does not exceed 1% represents a threshold which ensures uninhibited growth of the plant. Leachate concentration of over 15% leads to total inhibition of plant growth.

Table 4. **Number of germinated seeds after 48 and 72-hour incubation periods**

No.	Incubation period	Leachate, percentage by volume, %								
		0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
1	48 h	50	50	50	50	50	50	49	50	49
2	72 h	50	50	50	50	50	50	48	50	49
No.	Incubation period	Leachate, percentage by volume, %								
		0.9	1.0	2.0	3.0	4.0	5.0	10.0	15.0	20.0
3	48 h	47	47	48	48	50	47	45	36	0
4	72 h	47	47	48	48	50	47	45	36	0

Changes in relative seed germination, derived from the number of germinated seeds, do not reveal significant differences for the experimental variable of the incubation period (Figs. 2 and 3). Leachate concentration was found to be a key factor in RSG changes. Solutions containing up to 3% of leachate showed a linear trend for RSG. Leachate concentration of 5÷10% led to the test plant response in the form of a limited number of germinated seeds and a declining RSG. Germination process would break down at 10÷15% leachate concentrations and the absence of germination was observed for the concentration of 20%.

Due to the method of calculation and value substitution, relative seed germination and germination index are mutually overlapping measurement points. Changes in RRG and GI depended on both the incubation period and leachate concentration (Figs. 2, 3). In the case of the 48-hour incubation period, the uninhibited plant growth was achieved in the environment with leachate concentration of 1÷2%. With the 72-hour incubation period, threshold leachate concentration value was established at 0.9%. RRG and GI values were observed to fall almost linearly when those levels of concentration were exceeded. Both indicators reached zero when the leachate concentration in the solution was 20%.

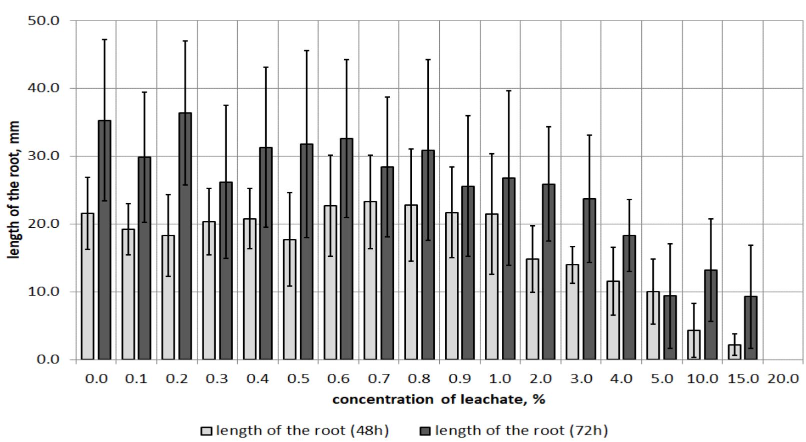

Fig. 1. Changes in the length of *Lepidium* sp. roots depending on the leachate concentration and incubation period

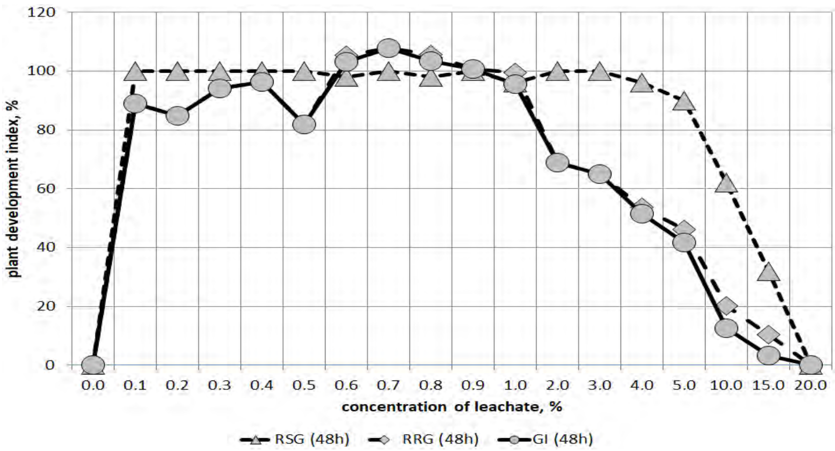

Fig. 2. Values of plant growth indicators (RSG, RRG and GI) after 48-hour incubation period

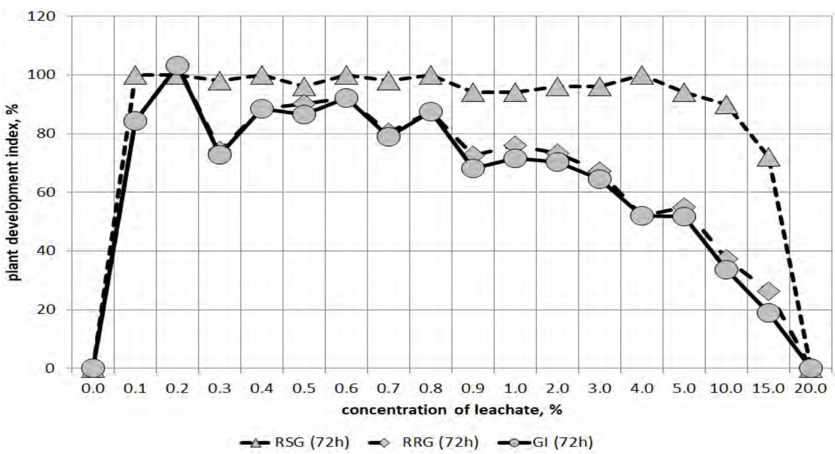

Fig. 3. Values of plant growth indicators (RSG, RRG and GI) after 72-hour incubation period

Protein concentration assayed in the samples from 48 and 72-hour incubation periods did not show any significant differences caused by incubation period or the leachate concentration in the solution (Fig. 4). Leachate concentration ranging from 0.1 to 4÷5% did not modify protein concentration, which remained constant at 2.5 mg/L. When leachate concentration reaches the levels of 5 to 20%, protein concentration declines until the total absence of proteins. It should be noted that protein concentration faded away when leachate concentration reached 15÷20%.

The correlation between the concentrations of peroxidases and leachate was characterized by a set of irregular changes (Fig. 5). The chart revealed that in the case of the 48-hour incubation period, the highest level of stress for cress coincided with low leachate concentrations of up to 0.4%. In the case of the 72-hour incubation period, there was virtually no plant response to stress induced by the presence of leachate, regardless of its level.

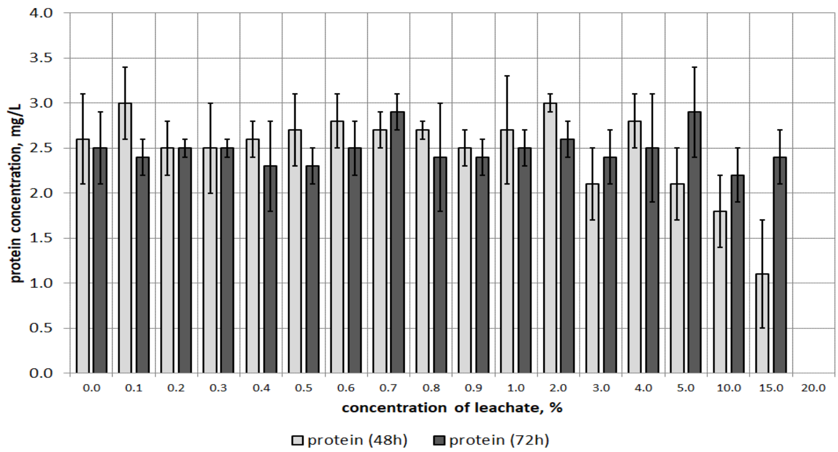

Fig. 4. Protein concentration and leachate concentration after 48 and 72 hours

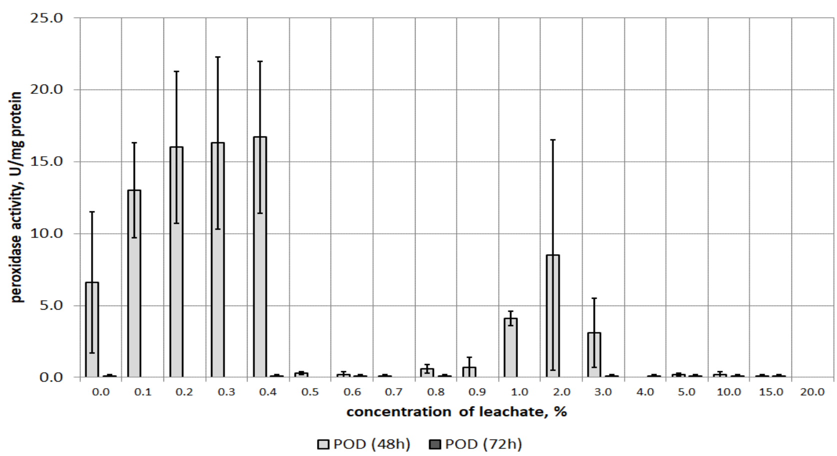

Fig. 5. Peroxidase activity and leachate concentration after 48 and 72 hours

In the group of indicators designated in the 48-hour experiment, most showed strong correlations r = 0.7 – 0.9 (Table 5). POD activity was the only indicator which was not correlated with changes in leachate, RSG, RRG, GI and protein concentrations. It is worth noting that the correlation coefficient between leachate concentration and protein concentration was r = –0.94. This significant correlation shows that protein concentration can be a good indicator of test plant response to the inhibiting effect of leachate. Furthermore, protein concentration showed weaker correlation with the plant growth indicators (RSG, RRH and GI) that constitute the actual results of the *Lepidium* test.

The results obtained for tests based on the 72-hour incubation period also point to statistically significant correlations between leachate concentrations, *Lepidium* test indicators and protein concentrations (Table 6). In general, strength of these correlations falls to the range of r = 0.5÷0.7, suggesting a moderate relationship.

Similar to correlation matrix for indicators in the 48-hour *Lepidium* test, the activity of peroxidases did not show statistically significant correlations with other indicators.

Table 5. **Correlation matrix for variables of the 48-hour *Lepidium* test**

No.	L.	L.	RSG (48 h)	RRG (48 h)	GI (48 h)	Proteins (48 h)	POD (48 h)
1	L.	–	**–0.69**	**–0.74**	**–0.75**	**–0.94**	–0.36
2	RSG (48 h)		–	**0.87**	**0.86**	**0.71**	0.17
3	RRG (48 h)			–	**1.00**	**0.69**	0.19
4	GI (48 h)				–	**0.70**	0.21
5	Proteins (48 h)					–	0.32
L. - leachate concentration, %							

Table 6. **Correlation matrix for variables of the 72-hour *Lepidium* test**

No.	L.	L.	RSG (72 h)	RRG (72 h)	GI (72 h)	Proteins (72 h)	POD (72 h)
1	L.	–	**–0.53**	**–0.71**	**–0.72**	**–0.72**	0.13
2	RSG (72 h)		–	**0.85**	**0.83**	**0.64**	–0.04
3	RRG (72 h)			–	**1.00**	**0.51**	–0.28
4	GI (72 h)				–	**0.49**	–0.27
5	Proteins (72 h)					–	0.19
L. - leachate concentration, %							

General observation based on the comparison of the strength of correlations in the two groups of the experiments (48 hours and 72 hours) shows that extending the *Lepidium* test is not statistically beneficial. Limitation in the strength of correlations is a premise for assuming inhomogeneous changes of indicators, depending on the postulated experimental conditions, or for possible flaws in inference.

The evaluation of variability for mean values of RSG, RRG, GI, protein concentration and POD activity between 48 and 72 hours of incubation periods was performed using the nonparametric Mann-Whitney U test. The respective test probability values are presented in Table 7.

Table 7. **Test probability values for the Mann-Whitney U test**

No.	Variable	Test probability
1	RSG	0.4890
2	RRG	0.3839
3	GI	0.3503
4	Proteins	0.1899
5	POD	0.0003
Null hypothesis: variation of mean variable levels during 48 and 72-hour incubation shows statistically significant differences. The level of significance set at $\alpha = 0.05$		

The results of the Mann-Whitney U test showed that there was no statistical variation for RSG, RRG, GI and protein concentrations between the 48 and 72-hour incubation periods. Magnitude of the variation was presented using means and standard deviations (Table 8).

The analysis of means and standard deviations confirms statistical results. However, it was found that the incubation period was a significant variable in the *Lepidium* test. This was based on two premises, i.e. the observed but not parametrized changes in the fragility of the roots and, as observed by authors, statistically significant differences in relative root growth (RRG). It should be noted that the study aimed to establish the phytotoxicity and adaptive potential of plants, and both elements can change as the experiment duration is extended.

Table 8. **Means and standard deviations of individual indicators for different incubation periods**

No.	Incubation period	RSG		RRG		GI		Proteins		POD	
		x	σ	x	σ	x	σ	x	σ	x	σ
1	48 h	81.8	34.5	68.3	37.9	66.6	38.9	2.3	0.7	3.9	5.8
2	72 h	84.9	31.6	64.4	30.5	62.5	30.9	2.3	0.6	0.1	0.1
x - mean, σ - standard deviation											

Conclusions

The results obtained in the study lead to the following conclusions:

- The *Lepidium* was considered as useful and reliable tool for assessment of toxicity of landfill leachate, resulting from changes in the plant growth depending on leachate concentration in the solution.
- The use of the *Lepidium* test allowed for determination of an approximate threshold volume of leachate which leads to the inhibition of test plant growth. Undisturbed plant growth was found for the environment with a leachate concentration of below 1÷2%.
- The *Lepidium* test results were correlated with the changes in protein concentrations. The measurement of protein concentrations was considered to be a more reliable determinant of plant response to a stress factor than the number of germinated seeds or relative root growth. It was suggested that in addition to the *Lepidium* test, protein concentration should also be measured.
- It was found that *Lepidium sativum* is not a suitable plant for establishing the level of stress induced by leachate toxicity. It should be confirmed that *Lepidium* test should only be used in assessing growth inhibition. In the biochemical test, there was no correlation between the *Lepidium* test indicators and POD activity used for the evaluation of stress levels. The results were inconclusive, with both inhibition and activation of guaiacol peroxidase observed.
- It was found that duration of tests was an important factor and provided evidence for adaptive capabilities of plants. Extending the test duration led to an increase

in root length and it made roots tender and fragile. However, statistical analyses showed no significant differences with regard to the incubation period.
- Metal concentrations did not demonstrate the toxic effects of leachate.

Acknowledgments

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References

[1] Rosik-Dulewska C., Podstawy gospodarki odpadami, WN PWN, Warszawa 2010.

[2] Neczaj E., Okoniewska E., Kacprzak M., Treatment of landfill leachate by sequencing batch reactor, Desal. Water Treat. 2005, 185, 357-362.

[3] Yang D., Englehardt J.D., Electrochemical oxidation for landfill leachate treatment, Waste Manag. 2007, 27, 380-388.

[4] Salem Z., Hamouri K., Djemaa R., Allia K., Evaluation of landfill leachate pollution and treatment, Desal. Water Treat. 2008, 220, 108-114.

[5] Mariam T., Nghiem L.D., Landfill leachate treatment using hybrid coagulation-nanofiltration processes, Desal. Water Treat. 2010, 250, 677-681.

[6] Gouider M., Feki M., Sayadi S., Bioassay and use in irrigation of untreated and treated wastewaters from phosphate fertilizer industry, Ecotoxicol Environ Saf. 2010, 73, 932-938.

[7] Khan A.D., Libby M., Winnick D., Palmer J., Sumarah M., Ray M.B., Macfie S.M., Uptake and phytotoxic effect of benzalkonium chlorides in Lepidium sativum and Lactuca sativa, J. Environ. Manage. 2018, 206, 490-497.

[8] Walter I., Martınez F., Cala V., Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses, Environ. Pollut. 2006, 139, 507-514.

[9] Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., Protein measurement with the Folin phenol reagent, J. Biol. Chem. 1951, 193, 265-275.

[10] Neves V.A., Lourenço E.J., Peroxidase from reach fruit: thermal stability, Braz. Arch. Biol. Technol. 1998, 41, 179-186.

[11] Flocco C.G., Giulietti A.M., In vitro hairy root cultures as a tool for phytoremediation research, Phytoremediation: Methods and Reviews, ed. N. Wiley, Wiley Humana, Totowa, NJ, 2007, 23, 161-173.

[12] Kumar P., Kamle M., Singh J., Rao D.P., Isolation and characterization of peroxidase from the leaves of Ricinus communis, Int. J. Biotechnol. Biochem. 2008, 4, 283-292.

[13] Ustawa z dnia 10 lipca 2007 r. o nawozach i nawożeniu, Dz.U. Nr 147, poz. 1033.

[14] Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 18 czerwca 2008 r., Dz.U. Nr 119, poz. 765.

Streszczenie

Wody odciekowe ze składowisk odpadów komunalnych charakteryzują się wysoką koncentracją składników organicznych i nieorganicznych, w tym metali ciężkich i wielu niebezpiecznych substancji organicznych. Wody odciekowe są również uważane za potencjalne źródło mikroorganizmów chorobotwórczych. Uszczelnienie składowiska oraz oczyszczanie wód odciekowych stanowi bardzo istotne zagadnienie eksploatacyjne składowiska odpadów, którego częścią jest monitoring odcieków. Monitoring wód odciekowych, oprócz charakterystyki ilościowo-jakościowej, powinien opierać się również na ocenie fitotoksyczności odcie-

ków. Ma to szczególne znaczenie w przypadku migracji wód odciekowych i ich wpływu na środowisko gruntowo-wodne. Celem przeprowadzonych badań było ustalenie fitotoksyczności wód odciekowych ze składowiska odpadów komunalnych na podstawie roślinnych testów stresowych. W pracy przedstawiono wyniki badań uzyskanych w teście *Lepidium* oraz zawartości białek i peroksydazy występujących w roślinie testowej. Badania określały możliwość rozwoju bądź zahamowania wzrostu rośliny, która została poddana czynnikowi stresowemu, jakim były różne dawki wód odciekowych. Test *Lepidium* został uznany za przydatny test w ustalaniu toksyczności odcieków ze składowisk odpadów. Zastosowanie tego testu pozwoliło ustalić przybliżoną progową objętość odcieku, która wpłynęła na hamowanie rozwoju roślin testowych. Niezakłócony rozwój roślin osiągnięto w środowisku o stężeniu odcieków nieprzekraczającym 1÷2%. W celu pełniejszego badania toksyczności odcieków proponuje się zastosowanie testu *Lepidium* wraz z równoległym pomiarem stężenia białek. Nie stwierdzono przydatności pomiaru peroksydaz. Na podstawie wartości wskaźników fizykochemicznych odcieków i zawartości metali nie stwierdzono istotnych czynników toksycznych hamujących rozwój badanej rośliny.

Słowa kluczowe: wody odciekowe, fitotoksyczność, test *Lepidium*, odpady komunalne, aktywność peroksydazy