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Impact on the Environment**

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Toxicological Assessment of Closed Municipal Solid-waste Landfill Impact on the Environment

Jūratė Žaltauskaitė, Iveta Vaitonytė

Vytautas Magnus University, Department of Environmental Science, Faculty of Natural Sciences
Vileikos st. 8, LT-44404, Kaunas, Lithuania

Corresponding author: jurate.zaltauskaite@vdu.lt

J. Žaltauskaitė, Vytautas Magnus University, Department of Environmental Science, Faculty of Natural Sciences
Vileikos st. 8, LT-44404, Kaunas, Lithuania

A large number of municipal solid waste landfills in Lithuania pose a serious environmental threat to the quality of soil, surface and ground water. The physicochemical characteristics and toxicity of closed Panevėžys municipal solid-waste landfill leachate and its impact on soil and surface water were assessed. Landfill leachate is a complex mixture of various inorganic and organic compounds. The toxicity of municipal solid waste landfill leachate and surface water was evaluated using bioassays with aquatic plants (*Lemna minor* L.) and micro-invertebrates (*Daphnia magna*). The leachate was shown to be toxic to *D. magna*, and it also reduced the growth and biomass of *L. minor*. The toxicity has been linked with ammonium and heavy metal content as the prime contributors to the toxicity.

Keywords: landfill leachate, *Lemna minor*, *Daphnia magna*, toxicity.

Introduction

Landfills are of great environmental importance in Lithuania, which currently disposes approximately 80% of its waste in landfill sites. Landfilling accounts for more than 70% of waste treatment in Lithuania (Eurostat, 2014). Municipal landfills are the sources of a wide range of chemicals and may pose ecological and human health

risks. The chemicals are released into the environment in the form of leachate, gas and particulate matter.

Landfills affect soil quality in the surroundings, and usually increased concentrations of chlorides, sulphates, nitrates and heavy metals are detected in the vicinity of a landfill (Hernández et al., 1998). However, the ma-

major potential environmental threat related to landfills is leachate. The exposure of landfill leachate to environment and biota may occur in different ways, such as uncontrolled overflow, rainfall run-off and infiltration.

Leachate is generated by percolation of precipitation and moisture through waste layers in a landfill. Landfill leachate is a complex mixture containing dissolved organic matter, inorganic macro-components, heavy metals and xenobiotic organic compounds (Kjeldsen et al., 2002). Moreover, landfill leachate is a source of new and emerging pollutants, such as perfluorinated compounds, alkylphosphates, personal care products and pharmaceuticals (Eggen et al., 2010).

The composition of leachate is highly variable and depends on numerous factors, such as age of a landfill, degree of solid waste stabilisation, solid waste characteristics, composition of waste, size of a hill, moisture and degree of rainwater infiltration, temperature and landfill geometry. Since it is a complex and highly variable composition, the hazard assessment of leachate is difficult. Traditionally, landfill leachate hazard assessment has been based on the physicochemical parameters, e.g., biological oxygen demand (BOD), pH, chemical oxygen demand (COD) and total dissolved solids. However, these parameters do not give an indication on the toxic potential of leachate to biota. Moreover, the majority of chemicals in leachate are not subject of routine monitoring and, therefore, biological effects resulting from the exposure of a living organism to these numerous pollutants may be underestimated. Toxicity bioassays integrate the biological effects of all compounds present in leachate and other factors, such as bioavailability, toxicant interaction and others. Toxicity bioassay, using species that represent different trophic levels, is the best approach to evaluate the toxicity of leachate. The impact of leachate on living organisms is very high and governed by several factors, such as high load of organic compounds, high content of ammonium, alkalinity and heavy metals (Thomas et al., 2009).

The majority of old Lithuanian landfills were built without engineered liners and leachate collection and treatment systems. To implement EU Landfill Directive (Council Directive 1999/31/EC), Lithuania has closed old, unsafe and/or small landfills and only those meeting the EU requirements have remained in operation or new, modern engineered landfills have been implemented.

Closed landfills require post-closure maintenance, and a period of at least 30 years of aftercare is specified in 1999/31/EC. Aftercare management of closed landfills typically includes monitoring of emissions and environmental quality (air, soil, surface water and groundwater), maintenance of the final cover and collection systems of leachate and gas (Sizirici et al., 2011, Laner et al., 2012). The main aim of this paper was to determine the impact of Panevėžys municipal solid waste (MSW) landfill leachate on the soil and surface water quality and toxicity to aquatic macrophyte *Lemna minor* and micro-invertebrate *Daphnia magna*.

Materials and methods

Sampling site characteristics

Panevėžys municipal solid waste (MSW) landfill is situated in the north-eastern part of Lithuania (55° 42' 7.82", 24° 29' 16.36"). The landfill is situated 8 km southeast from Panevėžys city centre. Panevėžys MSW landfill was established in 1975 and closed in 2009. The area of the landfill is 52.53 ha. During the operation, the landfill received more than 1.5 million m³ of municipal and non-hazardous industrial wastes. The mean annual temperature at the study site is 6.5°C, and the mean precipitation amount is approximately 650 mm y⁻¹.

The samples of the soil, the leachate and the surface water were collected in November, 2015. The leachate was sampled from a landfill leachate storage reservoir. The samples of the leachate and the surface water were collected in 2 L flasks and transported to the laboratory. Filtered (0.45 µm) (for chemical analysis) and non-filtered samples (for bioassays) were stored in a refrigerator prior to performance of chemical analysis and bioassays.

In order to determine the impact of the landfill on the surface water quality, the surface water samples were collected from Aulamas stream flowing through the landfill. Aulamas stream water samples were collected from 2 sampling sites: upstream (HP2) and downstream (HP1) of the landfill.

The soil samples were taken from the topsoil layer (0–15 cm) within a 25 m radius of the landfill waste dumping site. In the laboratory, the soil samples were dried,

ground and sieved prior to chemical analysis. The soil samples were dried for 48 h at 70°C temperature in an oven. Soil $\text{pH}_{\text{H}_2\text{O}}$ was detected potentiometrically. The soil samples for heavy metal analysis were digested using the Milestone Ethos One closed-vessel microwave system.

For the quantitative determination of metals (Cu, Cd, Ni, Pb) in the soil and the leachate, a Shimadzu AA-6800 atomic absorption spectrometer was used. All the reagents were of the analytical reagent grade. Analysis of anions and cations in the water was performed by ion chromatography with conductivity detection (Dionex 1100). pH was measured potentiometrically. Concentrations of ammonia (NH_3) were estimated from the measured concentrations of ammonium (NH_4^+) and taking into account a Kb of 1.8×10^{-5} (Pablos et al., 2011).

$$\text{NH}_3 = \text{NH}_4^+ \times 10^{(\text{pH}-14)} / 1.8 \times 10^{-5} \quad (1)$$

Toxicity assessment

The leachate and surface water toxicity assessment was conducted using micro-invertebrates *Daphnia magna* and aquatic macrophytes common duckweed (*Lemna minor* L.). The standardised growth inhibition test with common duckweed *Lemna minor* was performed according to OECD Guideline 221 (OECD 2004). The stock culture of *L. minor* was grown in modified Steinberg medium (ISO/DIS20079) in growth chambers at $24^\circ\text{C} \pm 2^\circ\text{C}$ with a light/dark cycle of 16/8 h. Ten double-fronded healthy *L. minor* colonies were transferred to Petri dishes containing different concentrations of the leachate (100%, 50%, 25%, 12.5%, 6.25%, 3.125%), the surface water (Aulamas stream) and the control. The dilutions of the leachates were made with the control Steinberg growth medium. The assays were performed in 3 replicates per treatment and lasted 7 days.

The toxicity was recorded as percent inhibition of growth (fronds number and biomass) (relative to control) of *L. minor* as a result of 7-day exposure. The specific growth rate was calculated from the following equation with fronds number at the end (N_{t_1}) and the start of the test (N_{t_0}):

$$r = \frac{\ln(N_{t_1}) - \ln(N_{t_0})}{t_1 - t_0} \quad (2)$$

The acute 24–48 h *Daphnia magna* immobilisation test was performed according to OECD Guideline 202 (1984). The control was the growth medium of *D. magna*. Five organisms (less than 24 h old) were exposed to each dilution sample in 3 replicates. After 24-h and 48-h incubation in the light/dark cycle 16/8 h at $20 \pm 1^\circ\text{C}$, the number of immobilised individuals was recorded.

The results of the toxicity tests were expressed as the concentration of the sample that produced a 50% effect (e.g., growth inhibition or mortality) (EC_{50} or LC_{50}). EC_{50} (LC_{50}) values were expressed as a percentage of the leachate tested. The toxicity values were converted to toxic units (TU), i.e., inverse of EC_{50} (LC_{50}) expressed in %:

$$\text{TU} = \frac{1}{\text{EC}_{50}} \times 100 \quad (3)$$

The toxicity classification is reported as follows (Persoone et al., 2003): $\text{TU} < 0.4$ – no acute toxicity; $0.4 < \text{TU} < 1$ – slight acute toxicity; $1 \leq \text{TU} < 10$ – acute toxicity; $10 \leq \text{TU} < 100$ – high acute toxicity; $\geq \text{TU} 100$ – very high acute toxicity.

A one-way analysis of variance (ANOVA) was used to assess the leachate concentration and the surface water effect on the estimated end points. Significant differences between the control and the treatment samples were determined by the Dunnett test, and $p < 0.05$ was considered to be significant. Significant differences between the treatments were determined by the Student test, and $p < 0.05$ was considered to be significant. The statistical analysis was carried out using Statistica software.

Results and discussion

Chemical characteristics of leachate and surface water

Chemical characteristics of Panevėžys MSW landfill leachate and Aulamas stream water are presented in Table 1. Generally, in the analysis of MSW landfill leachates' chemistry, the focus is on the concentrations of nutrients, especially nitrogen. The concentration of nitrates in the leachate met the Lithuanian legislative

limits for nitrates in the leachate (Standards for effluent quality, 1996). The concentrations of ammonium exceeded the permitted limits by 47.9 times. The ammonia (ionized NH_4^+ and un-ionized NH_3) content in leachates, wastewaters and surface waters has previously been shown to explain a significant amount of toxicity to plants and invertebrates (Clément et al., 1997, Ward et al., 2002, Svensson et al., 2005, Žaltauskaitė and Čypaitė, 2008, Thomas et al., 2009). Long-term studies indicate that the concentration of ammonia in leachate does not decrease with time, and it often constitutes a major long-term pollutant in leachate even after the closure (Kjeldsen et al., 2002).

Analysis of the heavy metal concentrations in the leachate revealed that Pb was the most abundant metal in the leachate and the other metals were ranked in decreasing order $\text{Pb} > \text{Ni} > \text{Cu} > \text{Cd}$ (Table 1). The heavy metal concentrations in the leachate exceeded the legislation limits. High heavy metal levels could be partially explained by the fact that both household and industrial waste was disposed during the operation in Panevėžys MSW landfill. Such leachate chemistry parameters are typical of the leachate of municipal landfills, and the concentrations of

pollutants were in the same order as those measured in the leachates from other landfills in Lithuania (Jaskelevičius and Lynikienė, 2009, Svecevičius et al., 2013).

The impact of the landfill leachate on the quality of stream water was observed. The concentrations of oxidised and reduced nitrogen in the samples collected downstream the landfill were higher than in the samples collected upstream the landfill (Table 1). The concentrations of ammonium in Aulamas stream water exceeded the permitted levels both upstream (3.64 times) and downstream (4.06 times) the landfill. The elevated nutrient concentrations have also been found in groundwater and surface waters in the municipal solid waste landfill area in Lapės (Juodkazis and Urbanavičiūtė, 2002, Česonienė and Lukenskienė, 2006). An increased heavy metal content has been recorded in the stream flowing through Gdansk landfill (Poland) (Melnyk et al., 2014).

The concentrations of heavy metals in the soil collected in the vicinity of the landfill were detected in a wide range (Figure 1). The concentrations of Cu and Pb exceeded the Lithuanian legislation limits (Order for approval of the requirements for chemicals, 2008) by twofold, and

1	$\text{NO}_3\text{--N}$	$\text{NH}_4\text{--N}$	NH_3^*	pH	Cu	Pb	Cd	Ni
2	3	4	5	6	7	8	9	
Leachate	8.35	719.16	513.68	9.00	2.65	4.00	0.40	3.03
Surface water HP2	0.03	2.84	0.075	7.57	N.A.**	N.A.	N.A.	N.A.
Surface water HP1	0.06	3.17	0.082	7.56	N.A.	N.A.	N.A.	N.A.

* concentration estimated from measured pH and NH_4^+ values

** N.A. not analysed

Table 1

Chemical characteristics of landfill leachate (mg L^{-1}) and surface water (Aulamas stream)

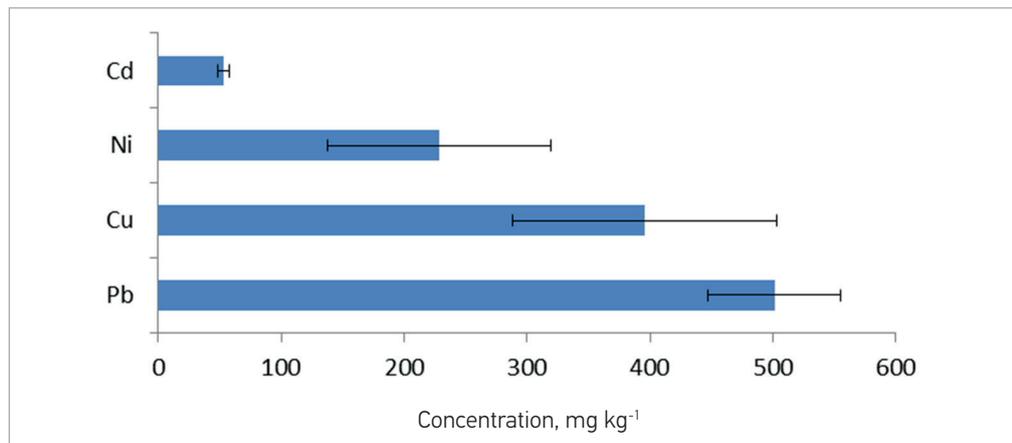


Fig. 1

Heavy metal soil concentrations (mg kg^{-1}) in Panevėžys MSW landfill

Cd by up to 17.7 times. No significant relationship was found between heavy metal soil concentration and distance from the waste dumping site. After leachate application on the vegetated landfill cover in Ottawa County (Michigan), a trend toward gradually increasing metal concentrations in the leachate irrigated plots has been observed (MacDonald et al., 2008). Calace et al. (2001) have pointed out that landfill leachate application led to an increased capacity to retain heavy metals. Chemical analysis of soils and waters in the vicinity of 15 closed solid waste landfills in the province of Madrid (Spain) have revealed the presence of elevated levels of heavy metals (Pastor and Hernández, 2012).

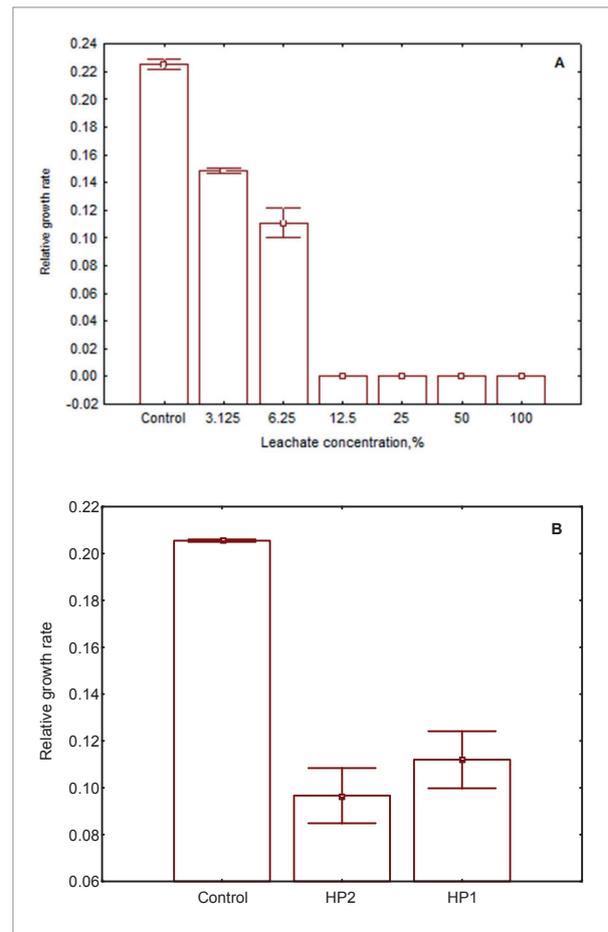
Toxicity to *Lemna minor* and *Daphnia magna*

The relative growth rate of *L. minor* during the whole 7-day exposure period was significantly affected by the leachate (ANOVA, $F = 459.41$, $p < 0.001$) (Figure 2A). The breakdown of all colonies of *L. minor* was observed after their exposure to the solutions containing more than 12.5% of the leachate. The exposure to the lowest (3.125%) leachate concentration in the medium led to a decrease of the growth rate by 34.07% in comparison with the control plants ($p < 0.05$). The calculated EC_{50} , effective concentration resulting in the relative growth rate reduction by 50%, was 4.7% for Panevėžys MSW leachate, i.e., TU 21.78. The results indicate that the landfill leachate could be considered as highly acute toxic for the growth of aquatic plants.

L. minor grown in Aulamas stream water produced fewer new fronds than *L. minor* grown in the control medium. The relative growth rate of *L. minor* in Aulamas stream water was by 45.5–52.96% lower than that of the control plants ($p < 0.05$) (Figure 2B). The relative growth rate of *L. minor* exposed to Aulamas surface water downstream the landfill (HP1 site) was by 15.86% higher than that exposed to Aulamas surface water upstream the landfill (HP2 site); however, the difference was not statistically significant (U-test, $p = 0.38$). The higher new frond production in HP1 treatment could be explained by higher nutrient concentrations downstream the landfill (Table 1). The study indicates that the landfill affects the surface water quality, which may lead to reduced aquatic plant growth. Several studies have observed that even groundwater collected near a landfill might be phytotoxic and reduce algae growth (Baun

Fig. 2

Relative growth rate of *L. minor* exposed to leachate and surface water for 7 days



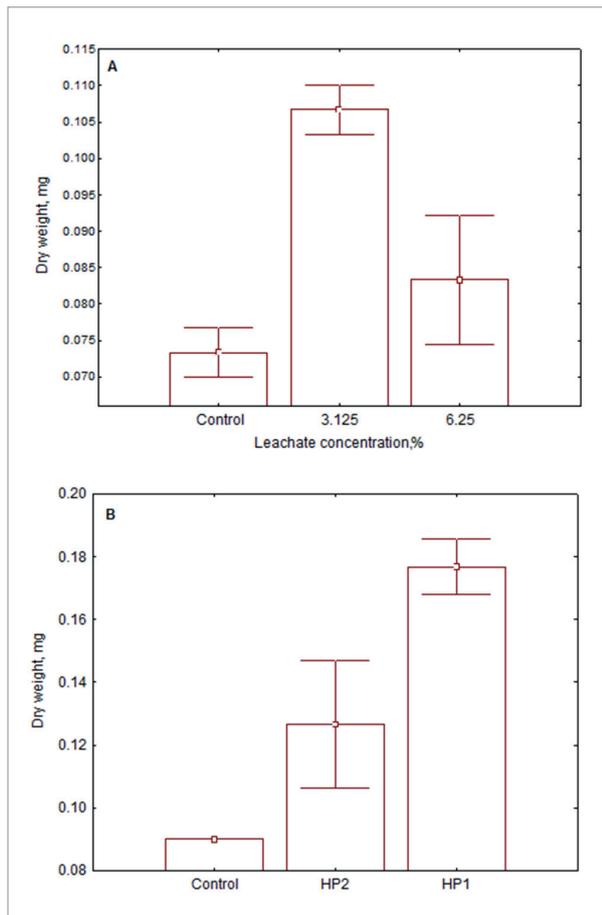
et al., 2000). Our results correspond well with those obtained in other studies indicating that leachate concentrations higher than 10% exhibit extremely high toxicity to the growth of *L. minor* (Clément and Bouvet, 1993, Devare and Bahadir, 1994). Untreated landfill leachate has been shown to reduce the growth rate, frond area, chlorophyll content and fluorescence of 4 strains of *L. minor* (Mackenzie et al., 2003).

Since the leachate concentrations higher than 12.5% induced the death of *L. minor* colonies, the dry weight of *L. minor* was analysed only for 3.125% and 6.25% treatments (Figure 3A). Both treatments with low leachate concentrations (3.125% and 6.25%) resulted in higher dry weight of plants in comparison with the control

ones. The stimulatory effect was more pronounced in the treatment with the lowest concentration, which may be due to a high content of nutrients. This stimulatory effect could be explained by a high content of nutrients in the diluted leachate (Table 1). The second possible explanation for higher biomass in the low concentrations of the leachate could be that these colonies produced very few new fronds (Figure 2A), but they were of higher biomass. The stimulatory effects of low leachate or wastewater concentrations on the growth of plants have been recorded in several studies (Clément and Bouvet, 1993, Žaltauskaitė and Vaisiūnaitė, 2010, Žaltauskaitė and Čypaitė, 2008, Žaltauskaitė et al., 2014). This stimulation effect could be explained by sufficiently high concentrations of nutrients and relatively low concentrations of other toxic substances.

Fig. 3

Dry weight of *L. minor* exposed to leachate and surface water for 7 days

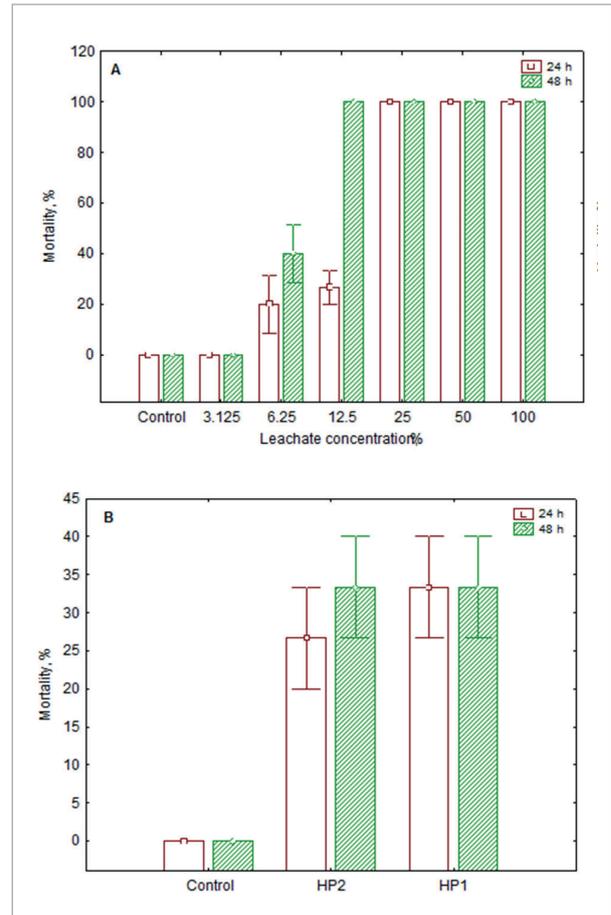


The similar stimulatory effect on the biomass growth was also observed in the treatment with Aulamas stream water. The dry weight of *L. minor* exposed to the surface water collected upstream the landfill was by 40.22% higher than the dry weight in the control. *L. minor* plants exposed to the surface water collected downstream the landfill were almost twofold higher than the control plants ($p < 0.05$).

The leachate had a very significant effect on *D. magna* (ANOVA, $F_{24} = 91.50$, $F_{48} = 122.00$, $p < 0.001$) survival (Figure 4A). Only the lowest tested leachate concentration (3.125%) produced no mortality. The 6.25% and 12.5% treatments led to the death of more than 20% of *D. magna* after 24 h of exposure, and the toxicity had increased after one more day of exposure, i.e., the mortality reached 40% and 100%, respectively. An increase

Fig. 4

Mortality of *D. magna* exposed to leachate (A) and surface water (B) for 24 h and 48 h



in the leachate concentration from 12.5% to 25% in solution resulted in a sharp increase in mortality, which reached 100%. The results of logistic regression show that the risk of death of *D. magna* increases with the concentration of the leachate in the test solution (24h: $x^2 = 104.65$, 48h: $x^2 = 118.34$; $p < 0.001$). The calculated LC_{50} value was 11.93% for 24 h and 6.92% for 48 h. The results indicate that the leachate toxicity increased with the time of exposure. The calculated LC_{50} corresponds to 8.38 TU and 14.45 TU for 24 h and 48 h, respectively. Based on the calculated toxic units, Panevėžys MSW landfill leachate is classified as highly acute toxic to *D. magna*. The determined TU using the *D. magna* immobilisation test were in accordance with the results of a study in France when 27 landfill leachates were studied (Clément et al., 1997). High acute toxicity of landfill leachates to aquatic micro-invertebrates, such as *Daphnia magna* and *Thamnocephalus platyurus*, has been recorded in previous studies (Matejczyk et al., 2014, Melnyk et al., 2014).

Aulamas stream water had a significant effect on *D. magna* survival (ANOVA, $F > 10.5$, $p < 0.05$) (Figure 4B). The stream water taken upstream the landfill had no acute toxicity for *D. magna* after 24 h (< 0.4 TU), but after 48 h it exhibited slight acute toxicity to *D. magna*. The mortality of *D. magna* exposed to Aulamas stream water downstream the landfill reached 33.33%, which corresponds to the slight acute toxicity class. Melnyk et al. (2014) have tested the stream water collected upstream and downstream Gdansk landfill and observed that stream water collected downstream the landfill exhibited high toxicity to *Thamnocephalus platyurus* and *Sorghum saccharatum*. Moreover, high *T. platyurus* mortality was observed in the samples with low values of total organic carbon (TOC), the parameter of routine monitoring indicating that physicochemical analysis is insufficient to assess the ecological status of surface water bodies.

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D. magna was more sensitive to the leachate and the surface water than *Lemna minor*. Higher sensitivity of micro-invertebrates to the leachate compared with the sensitivity of primary producers (plants or algae) has been observed in several previous studies (Tsarpali et al., 2012).

Conclusions

Chemical analysis conducted on the soils and the surface water around the closed landfill showed that nutrients and heavy metals may be present in relatively high concentrations even after the closure of the landfill. It indicates that survey and monitoring of the closed landfill is of particulate concern. The study revealed that leachate might be classified as highly acute toxic to aquatic plants. The leachate severely inhibited biomass and new frond production of *Lemna minor*. Surface water flowing through the landfill area inhibited the growth rate of *Lemna minor*, though a slight stimulation of biomass due to relatively high concentrations of nutrients was observed. It can be presumed that high concentrations of nutrients in water may mask the effects of other toxicants. *Daphnia magna* was extremely sensitive to the leachate and surface water. The obtained results indicate that the leachate could be classified as highly acute toxic. In summary of the results of chemical analysis and bioassays, it could be pointed out that the closed landfill remains a serious source of permanent pollution and might affect the biota in the surroundings of the landfill.

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Toksikologinis uždaryto municipalinių atliekų sąvartyno poveikio aplinkai vertinimas

Jūratė Žaltauskaitė, Iveta Vaitonytė

Vytauto Didžiojo Universitetas, Gamtos mokslų fakultetas, Aplinkotyros katedra, Kaunas, Lietuva

Didelis municipalinių kietųjų atliekų sąvartynų skaičius Lietuvoje kelia rimtą grėsmę dirvožemio, paviršinio ir požeminio vandens kokybei. Uždaryto Panevėžio buitinių atliekų sąvartyno filtrato poveikis dirvožemiui ir paviršiniam vandeniui ir jo toksiškumas buvo vertinamas fizikocheminiais metodais bei biotestais. Sąvartyni filtratas tai įvairių organinių ir neorganinių junginių mišinys. Sąvartyno filtrato toksiškumas buvo vertinamas taikant biotestus su vandens augalais (*Lemna minor* L.) ir mikrobestuburiais (*Daphnia magna*). Sąvartyno filtratas buvo toksiškas *D. magna*, neigiamai veikė *L. minor* augimo greitį ir biomasės prieaugį. Didelis filtrato toksiškumas sietinas su aukšta amonio jonų ir sunkiųjų metalų koncentracija.

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