

Helena BIS¹, Krzysztof FRĄCZEK¹,
Ewa MĘDRELA-KUDER² and Dariusz ROPEK³

MYCOLOGICAL QUALITY OF WATER WITHIN THE MUNICIPAL LANDFILL AND IN ITS SURROUNDINGS

JAKOŚĆ MIKOLOGICZNA WÓD W OBRĘBIE SKŁADOWISKA ODPADÓW KOMUNALNYCH I NA OBSZARZE PRZYLEGŁYM

Abstract: The aim of this study was to evaluate the effect of a municipal landfill on the number of microscopic fungi in surface water, groundwater and leachate within and in the surroundings of the landfill. Surface water samples were collected from the stream Malinowka and from the stream channel, groundwater samples – from 9 piezometers located around the landfill and a leachate sample – from the leachate reservoir. Water sampling was performed once per month on an annual basis (2011). The number of fungal colony forming units (CFU/cm³) on Malt Extract Agar (MEA, Oxoid, Basingstoke, Great Britain) was determined by plating dilutions method. Based on the conducted study, the presence of microscopic fungi – *Micromycetes* was found in surface water, groundwater and leachate within and in the vicinity of the examined landfill. Large quantitative, but small qualitative differences were detected in various research sites. In terms of seasons, surface and groundwater samples were characterized by the highest abundance of fungi in summer and autumn, while the lowest abundance was recorded in winter. In the leachate samples the highest fungal abundance was recorded in the autumn-winter season, while the lowest – in spring and summer. This demonstrates the need for regular monitoring of water both within and in the areas surrounding the landfill.

Keywords: waste, municipal landfill, surface and groundwater, leachate

Introduction

Intensive development of civilization entails environmental pollution, including water. The concept of water pollution has many definitions due to the complex nature of

¹ Department of Microbiology, University of Agriculture, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 41 81, email: rrfracuse@cyf-kr.edu.pl

² Section of Hygiene and Health Education, University School of Physical Education in Krakow, al. Jana Pawła II 78, 31–531 Kraków, Poland, email: ewa.medrela@awf.krakow.pl

³ Department of Agricultural Environment Protection, University of Agriculture, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 02, email: rropek@cyf-kr.edu.pl

this phenomenon. From the perspective of water management, water pollution is a condition when the chemical composition is changed so that its direct use for domestic or industrial purposes becomes restricted or impossible. The biological context of this definition implies that the water contamination is any toxic agent towards natural water features and water organisms, creating an imbalance in the biocenosis, resulting in the removal of one or more species that are sensitive to a particular type of pollution [1].

Waste generation is inseparable part of human domestic and economic activity. In practice, the majority of waste is landfilled and only a small percentage is processed. No waste storage technology can guarantee the complete protection of the environment from the risk associated with the waste disposal, hence the need for continuous monitoring of environmental impact of landfills. Groundwater around municipal landfills is mostly threatened by leachate. Often, the pollution load in leachate is greater than in municipal sewage [2–5].

Heavy metals are the most difficult leachate contaminants to deal with. Due to their toxic character they disturb the natural biological balance of the aquatic environment and inhibit the self-purification processes of water [6, 7]. It should, however, be noted that also microbiological contamination of leachate is a constant threat to the quality of both surface water and groundwater. The number, type and distribution of waterborne microorganisms depends on numerous physical and chemical factors, including environmental conditions, season, the quality or depth of water, *etc.* These factors also affect the physiology and morphology of bacteria, fungi or cyanobacteria. Too high concentrations of some nutrients, as well as extreme temperature and pH, negatively affect the proliferation of almost all microorganisms, which have a narrow range of tolerance [3, 8].

The aim of this study was to assess the effect of the municipal landfill Barycz in Krakow on the number of microscopic fungi in surface and groundwater as well as in leachate within and in the landfill's surroundings. Identification of fungi was performed based on the analysis of macro- and microscopic features of colonies using the available fungal taxonomic manuals.

Material and methods

To perform mycological studies, the water samples were collected within and in the vicinity of the municipal waste landfill Barycz in Krakow. It is owned by the Krakow municipality and has been entrusted for operation to the Municipal Sanitation Company Ltd. It is about 3.5 km from Wieliczka in sinkholes formed at the depleted water-bearing sands of Bogucice. It is situated in the valley of a small stream Malinowka, which is a tributary of Serafa, which in turn supplies the waters of the Vistula. The area of the municipal landfill Barycz extends from south-west to north-east, its elevation is 250 m a.s.l. in the north-east and 270 m a.s.l. in the south-west. A part of this facility is located on the hill slopes and a part – in the valley. The total area of the landfill is 37 ha. It is divided into three parts; the first one was operated from the end of 1974 to 1992, the second – to February 28th 2005 and currently the third sector – an eleven hectare basin – has been operated.

Surface water samples were collected from the stream Malinowka and from the stream channel located south of the landfill (10–14), groundwater samples were collected from 9 piezometers situated around the 3rd stage of the landfill's operation sector, where waste is being deposited (1–9), while the leachate sample was collected from the leachate reservoir (15). The collection of representative water samples was performed once per month on an annual basis (2011) with a sample scoop (PB1 model, Biosphera, Poland). The location of the research sites depended on the direction of water runoff and reflected the spatial arrangement. The research sites were indicated and described in Fig. 1.



Fig. 1. Schematic layout of the research sites for water sampling at the municipal landfill Barycz in Krakow: 1–9 – groundwater research sites (piezometers), 10–14 – surface water research sites (Malinowka stream), 15 – leachate research site (working leachate reservoir)
Source: Google Earth.

After collection, the samples were transported to the laboratory of the Department of Microbiology, University of Agriculture in Krakow, where microbiological analyzes were conducted concerning the determination of fungal number per one cm^3 of water. The number of *Colony Forming Units* (CFU/cm^3) of fungi on *Malt Extract Agar* (MEA, Oxoid, Basingstoke, Great Britain) was determined by plating dilutions method. Plates with MEA were incubated for 4 days at 30°C and then for 4 days at 22°C . Qualitative research was conducted using the membrane filtration method. The systematic identification of fungi was performed by comparing their morphological characteristics with several taxonomic manuals [9–13].

Results and discussion

Waste deposited in landfill sites may be a source of various pollutants not only for soil but also for groundwater and surface water. Aquatic environments surrounding municipal waste storage facilities are primarily exposed to leachate-transported pol-

lution. Landfills with improper ground sealing become the major threat, as the contaminants can infiltrate even to considerable depth and distance [14, 15]. So far, water samples in the vicinity of municipal landfills were examined only chemically, omitting the microbiological aspect [16, 17].

Recognizing the importance of this issue, the main scientific objective of the comprehensive microbiological analyzes in this study, was to assess the mycological quality of surface water, groundwater and leachate within the municipal landfill and in its surroundings. The complete research results are shown in Fig. 2 and 3. Based on these results it was concluded that the number of fungi in the examined surface water (sites 10–14) ranged from 0 to 72 CFU/cm³. The highest mean number of fungi throughout the study period was found at the site no. 11 (23.0 CFU/cm³) and the lowest – at the site no. 14 (14.0 CFU/cm³). In the remaining research sites the recorded mean numbers ranged from 15 to 20 CFU/cm³. In the case of the examined groundwater samples collected from 9 research sites (piezometers) the number of fungi ranged from 0–83 CFU/cm³. The maximum mean number of fungi was found at the site no. 1 (27 CFU/cm³) and the lowest – at the site no. 8 (12 CFU/cm³). In the remaining research sites the recorded mean numbers of fungi ranged from 15 to 25 CFU/cm³. The authors of numerous publications emphasize that the spread of contamination in groundwater depends primarily on: the water flow velocity, hydraulic gradient of water, concentration of pollutants, filtration coefficient of aquifers and soil sorption capacity. However, the possibility of spreading of microorganisms in groundwater is limited due to the relatively short period of their survival [18–20].

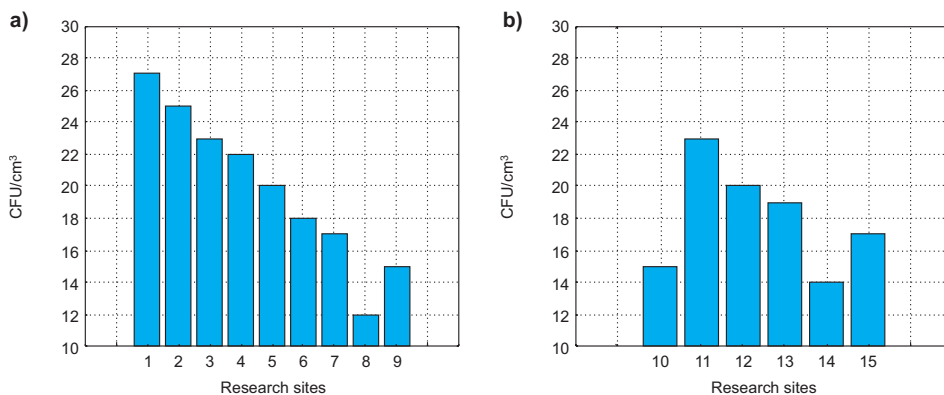


Fig. 2. Mean number of fungi (CFU/cm³) at designated research sites within and in the vicinity of the waste landfill: a) groundwater, b) surface water and leachate

On the other hand, in the leachate sample (site 15) the number of fungi varied from 0 to 41 CFU/cm³ and their mean number throughout the study period was 17 CFU/cm³. Reducing the number of fungal colonies is equal to the reduction of the degrading effect of municipal landfills on the environment. It should, however, be noted that the landfilling of municipal waste inevitably leads to the formation of leachate. The amount of generated leachate depends, among others, on the type of waste, the method of

storage, the period of landfill operation and the amount of precipitation. In turn, the composition of leachate depends on the stage of decomposition and on a substance contained in waste [7].

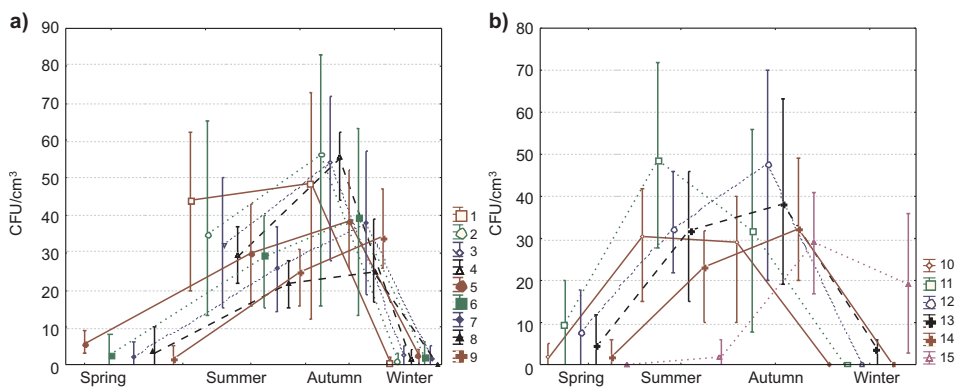


Fig. 3. Seasonal variation in the number of fungi (CFU/cm³) (mean values; whiskers – min-max.) at the designated research sites within and in the vicinity of the waste landfill: a) groundwater, b) surface water and leachate

Figure 3 presents the number of fungi in different research periods (seasons of the year). The data obtained show that the highest mean abundance of the studied microorganisms in surface water samples was found in autumn (36 CFU/cm³) and slightly less in summer (32 CFU/cm³), while the lowest – in winter (1.0 CFU/cm³). In the case of groundwater, the highest mean number of microscopic fungi was recorded in autumn (46 CFU/cm³) and the lowest in winter – 1 CFU/cm³. On the other hand, the highest mean abundance of fungi in leachate sample was recorded in autumn and winter (29 and 19 CFU/cm³, respectively), while in the spring and summer seasons, the occurrence of fungi was rare. The observed relationships may result from the temperature difference and the content of organic compounds, which are one of the most important environmental factors affecting the occurrence of waterborne fungi. It should be emphasized that bacteria or fungi may grow only in a specific range of temperatures. At temperatures below zero (0 °C) water freezes and causes cell, cytoplasm or organelle damage. On the other hand, when the maximum temperature is exceeded, it causes irreversible coagulation of both structural and enzymatic proteins, and as a consequence – thermal death of microorganisms [1, 21].

The occurrence of different genera and species of waterborne fungi was also found in the examined water samples, among which *Actinospora megalospora*, *Campylospora choretocladia*, *Centrospora filiformis* and *Leptomitus lacteus* were the predominant ones. Yeast and yeast-like fungi of the genera: *Candida*, *Cryptococcus*, *Cephalosporium*, *Pichia*, *Hansenula*, *Torulopsis*, *Rhodotorula* and *Saccharomyces* were largely represented. According to Dynowska [22] the above-mentioned yeast-like fungi can tolerate large amounts of pollution and some of their species occur only in highly degraded ecosystems. Many of them are the potential etiological factors of fungal

diseases, mainly in immunosuppressed patients. If these fungi have sufficient amount of nutrients in the environment, they begin to grow vigorously and constitute a very significant source of mycological infections. According to Olanczuk-Neyman [20] the increase in water pollution is accompanied by the decrease in the microbial diversity in this environment, while at the same time the number of pathogenic microorganisms increases. Nevertheless, they cannot grow or proliferate there and die eventually. However, some of them can survive for some time, whose length depends on the type of water and the prevailing conditions.

Table 1

Species composition of microscopic fungi in the water samples at the designated research sites within and in the vicinity of the waste landfill

No.	Genus/species	Surface water	Groundwater	Leachate
1	<i>Actinospora megalospora</i>	+++	+	++
2	<i>Anguillospora longissima</i>	++	++	+
3	<i>Campylospora chaetoclada</i>	+	+	++
4	<i>Controspora filiformis</i>	++	+	+
5	<i>Leptomitius lacteus</i>	+++	+	+
6	<i>Candida</i> sp.	+++	++	+++
7	<i>Cryptococcus</i> sp.	+	++	++
8	<i>Cephalosporium</i> sp.	++	++	+
9	<i>Pichia</i> sp.	+	+	+
10	<i>Hansenula</i> sp.	+	+	++
11	<i>Torulopsis</i> sp.	+	++	+++
12	<i>Rhodotorula</i> sp.	++	+++	+++
13	<i>Saccharomyces</i> sp.	++	+	++
14	<i>Alternaria</i> sp.	+	+	+
15	<i>Aspergillus</i> sp.	+	++	++
16	<i>Botrytis cirenea</i>	++	++	+++
17	<i>Chaetomium</i> sp.	++	+	++
18	<i>Fusarium</i> sp.	+	++	+
19	<i>Penicillium</i> sp.	+	++	++
20	<i>Trichoderma</i> sp.	++	+	+++

Legend: +++ very frequent occurrence; ++ frequent occurrence; + rare occurrence.

Based on the conducted research, it was found that the examined water samples also contain a rich microflora of land saprophytes and ubiquitous fungi such as *Alternaria*, *Aspergillus*, *Botrytis*, *Penicillium*, *Chaetomium*, *Fusarium*, *Trichoderma*. Several of them represent soilborne allochthonous microflora, although some genera (*Botrytis*) may be waterborne. These fungi play an important role in the self-purification process of water. Some of them take part in the decomposition of organic acids, pectin, cellulose, hemicellulose or lignin. Some of them conduct protein ammonification or

participate in the hydrolysis of fats, etc.. By mineralizing organic matter contained in water, they also contribute to the supply of nutrients to detritivorous organisms [22]. Certain species may produce antibiotic substances, probably modifying, to some extent, the quantitative and qualitative composition of waterborne microflora.

Conclusions

The following conclusions may be drawn based on the conducted analyzes of water samples:

1. The presence of microscopic fungi – *Micromyces* was recorded in the examined surface water, groundwater and leachate samples collected within and in the vicinity of the municipal landfill Barycz in Krakow.
2. The distance of the research sites from the active landfill sector affected the number of fungi occurring both in groundwater and in surface water.
3. Large quantitative, but small qualitative differences were detected in various research sites. Depending on the season, surface and groundwater samples were characterized by the highest abundance of fungi in summer and autumn, while the lowest abundance was recorded in winter. In the leachate samples the highest fungal abundance was recorded in the autumn-winter season, while the lowest – in spring and summer.
4. Quantitative and qualitative differentiation of microscopic fungi in the examined water samples indicates the necessity for regular water monitoring both within and in the areas surrounding the landfill.

Acknowledgement

The project was funded by the National Science Centre.

References

- [1] Pawlaczyk-Szpilowa M. Mikrobiologia wody i ścieków. Warszawa: PWN; 1978.
- [2] Frączek K, Ropek D. Municipal waste dumps as the microbiological threat to the natural environment. *Ecol Chem Eng S*. 2011;18:93-110.
- [3] Frączek K, Grzyb J, Ropek D. Microbiological hazard to the environment posed by the groundwater in the vicinity of municipal waste landfill site. *Ecol Chem Eng S*. 2011;18:211-221.
- [4] Slack RJ, Gronow JR, Voulvoulis N. Household hazardous waste in municipal landfills: contaminants in leachate. *Sci Total Environ*. 2005;337:119-137. DOI:10.1016/j.scitotenv.2004.07.002.
- [5] Renou S, Givaudan JG, Poulain S, Dirassouyan F, Moulin P. Landfill leachate treatment: Review and opportunity. *J Hazard Materials*. 2008;150:468-493. DOI:10.1016/j.jhazmat.2007.09.077.
- [6] Li Y, Low GKC, Scott JA, Amal R. Microbial reduction of hexavalent chromium by landfill leachate. *J Hazard Materials*. 2007;142:153-159. DOI:10.1016/j.jhazmat.2006.07.069.
- [7] Salem Z, Hamouri K, Djemaa R, Allia K. Evaluation of landfill leachate pollution and treatment. *Desalination*. 2008;220:108-114. DOI:10.1016/j.desal.2007.01.026.
- [8] Rheinheimer G. Mikrobiologia wód. Warszawa: PWRiL; 1987.
- [9] Raper KB, Fennel DI The Genus *Aspergillus*. Baltimore: Williams and Wilkins Co; 1965.
- [10] Atlas RM. Handbook of Microbiological Media. Boca Raton: CRC Press; 2004.
- [11] Domsch KH, Gams W, Traute-Heidi A. Compendium of Soil Fungi. London: Harcourt Brace Jovanovich Publishers, Academic Press; 1980.

- [12] Fassatiowa O. *Microscopic Fungi in Technical Microbiology*. Warszawa: Scientific and Technical Publishing; 1983.
- [13] Samson RA, Hoekstra ES, Frisvad JC. *Introduction to Food- and Airborne Fungi*. Seventh Edition. Utrecht: Centraalbureau voor Schimmelcultures; 2004.
- [14] Petts J, Eduljee G. *Environmental impact assessment for waste treatment and disposal facilities*. Chichester: John Wiley & Sons Ltd.; 1996.
- [15] Szymańska-Pulikowska A. Wpływ zabiegów rekultywacyjnych na środowisko wodne w otoczeniu składowiska odpadów komunalnych. *Zesz Probl Postęp Nauk Roln.* 2004;501:435-442.
- [16] Bojarska K, Bzowski W, Zawisłak J. Monitoring wód gruntowych i powierzchniowych w rejonie składowiska odpadów komunalnych w Zakopanem. *Gospodarka Odpadami Komunalnymi, Materiały Konferencji Naukowo-Technicznej, Koszalin-Kołobrzeg.* 2001;103-112.
- [17] Golimowski J, Koda E, Mamełka D. Monitoring wód w rejonie rekultywowanego składowiska odpadów komunalnych. *Gospodarka Odpadami Komunalnymi, Koszalin-Kołobrzeg.* 2001;87-102.
- [18] Drzał E. *Fizyko-chemiczne i mikrobiologiczne zagrożenia środowiska przez odpady*. Warszawa; Biblioteka Monitoringu Środowiska; 1995.
- [19] Szynekiewicz Z. *Praca zbiorowa. Mikrobiologia*. Warszawa: Wyd Naukowe PWN; 1975.
- [20] Olańczuk-Neyman K. *Mikroorganizmy w kształtowaniu jakości i uzdatnianiu wód podziemnych*. Gdańsk: Wyd Politechniki Gdańskiej; 2001.
- [21] Smyła A. *Analiza sanitarna wody*. Częstochowa: WSP; 2002.
- [22] Dynowska M, Bieduszkiewicz A. *Grzyby chorobotwórcze jako potencjalne bioindykatory w monitoringu ekosystemów wodnych. Środowiskowe zagrożenia dla społeczeństwa u progu XXI wieku, Tarnów; 2000.*

JAKOŚĆ MIKOLOGICZNA WÓD W OBRĘBIE SKŁADOWISKA ODPADÓW KOMUNALNYCH I NA OBSZARZE PRZYLEGLYM

Katedra Mikrobiologii
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem przeprowadzonych badań była ocena wpływu składowiska odpadów komunalnych na kształtowanie się liczebności grzybów mikroskopowych występujących w wodach powierzchniowych, podziemnych i odciekowych w jego obrębie i obszarze z nim sąsiadującym.

Próbki wód powierzchniowych pobrano z potoku Malinówka i rowu cieku wodnego, wód podziemnych z 9 piezometrów położonych wokół składowiska, a wód odciekowych ze zbiornika na odcieki. Pobranie próbek wody dokonano raz w miesiącu w cyklu rocznym (2011 r.). Liczbę jednostek tworzących kolonie (jtk/cm³) grzybów na agarze słodowym (Malt Extract Agar, MEA, Oxoid, Basingstoke, Wielka Brytania) oznaczano metodą posiewu rozcieńczeń. Na podstawie przeprowadzonych badań stwierdzono, że na składowisku odpadów komunalnych i na terenach przyległych stwierdzono w wodach powierzchniowych, podziemnych i odciekowych występowanie grzybów mikroskopowych – *Micromycetes*. W poszczególnych stanowiskach badawczych stwierdzono duże zróżnicowanie grzybów pod względem ilościowym, natomiast niewielkie zróżnicowanie gatunkowe. W wodach powierzchniowych i podziemnych najwyższe ich liczebności odnotowano w zależności od pory roku w okresie jesiennym i letnim, a najniższe w zimie. W wodach odciekowych najwyższe liczebności grzybów stwierdzono w okresie jesienno-zimowym, natomiast najniższe w okresie wiosny i lata. Świadczy to o konieczności regularnego monitoringu wód znajdujących się na terenie składowiska, jak i na obszarze z nim sąsiadującym.

Słowa kluczowe: odpady, składowisko komunalne, wody powierzchniowe, wody podziemne, odciek