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EXPOSURE TO AIRBORNE FUNGI DURING SORTING OF RECYCLABLE PLASTICS IN WASTE TREATMENT FACILITIES

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ABSTRACT

Background: In working environment of waste treatment facilities, employees are exposed to high concentrations of airborne microorganisms. Fungi constitute an essential part of them. This study aims at evaluating the diurnal variation in concentrations and species composition of the fungal contamination in 2 plastic waste sorting facilities in different seasons. **Material and Methods:** Air samples from the 2 sorting facilities were collected through the membrane filters method on 4 different types of cultivation media. Isolated fungi were classified to genera or species by using a light microscopy. **Results:** Overall, the highest concentrations of airborne fungi were recorded in summer (9.1×10^3 – 9.0×10^5 colony-forming units (CFU)/m³), while the lowest ones in winter (2.7×10^3 – 2.9×10^5 CFU/m³). The concentration increased from the beginning of the work shift and reached a plateau after 6–7 h of the sorting. The most frequently isolated airborne fungi were those of the genera *Penicillium* and *Aspergillus*. The turnover of fungal species between seasons was relatively high as well as changes in the number of detected species, but potentially toxigenic and allergenic fungi were detected in both facilities during all seasons. **Conclusions:** Generally, high concentrations of airborne fungi were detected in the working environment of plastic waste sorting facilities, which raises the question of health risk taken by the employees. Based on our results, the use of protective equipment by employees is recommended and preventive measures should be introduced into the working environment of waste sorting facilities to reduce health risk for employees. Med Pr 2017;68(1):1–9

Key words: occupational exposure, airborne fungi, waste sorting facilities, plastic waste, potential health risk, identification of fungi

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INTRODUCTION

In many working environments of waste management, employees are exposed over long periods to high concentrations of airborne microorganisms. Many studies by different authors have recently pointed out health risks associated with such environments [1–3]. Waste sorting facilities represent one such working environment since waste in sorting facilities is frequently contaminated by organic residues that serve as a nutrient substrate to numerous microorganisms. Fungi make up an important part of these microorganisms and

multitude of their mycelial fragments and other dispersal particles may be released during waste handling into the working environment [4].

In waste sorting facilities, high concentrations of airborne fungi were found varying within a wide range of values depending on the sampling site, sampling method and processing of samples (1.9×10^3 – 1.6×10^4 colony-forming units (CFU)/m³) [5], 0.8 – 2.4×10^4 CFU/m³ [4], 6.5×10^2 – 2.5×10^4 CFU/m³ [6], 0.3 – 1.6×10^5 CFU/m³ [7], 7.8×10^3 – 2.3×10^5 CFU/m³ [8], 1.5×10^3 – 2.9×10^5 CFU/m³ [9]). Generally, the high amounts of airborne fungi particles inhaled by employees in sorting facilities may result in

different health problems such as respiratory diseases (upper airway inflammation, cough, dyspnea, whistling breath, allergic diseases) [10,11] and gastrointestinal problems (diarrhea) [12].

When evaluating the employees' exposure to microscopic fungi, it is also necessary not only to determine airborne fungi concentrations but also to identify their species composition since their harmfulness to humans varies [13]. Fungal species composition in air samples was described only in several studies with genera *Penicillium* and *Aspergillus* often dominating otherwise very broad spectra of detected species [8,9,13–15]. These genera contain species able to produce mycotoxins and pose a direct health risk to employees [16]. Even less is known about how fungal concentrations and species composition vary depending on environmental conditions, such as a seasonal variation and time since the start of the work shift. This study aims at evaluating the airborne fungi contamination levels in 2 waste sorting facilities during the working shift in different seasons of the year. Further, we put emphasis on the identification of common and potentially toxigenic species.

MATERIAL AND METHODS

Sampling sites, sampling design and sample processing

Sampling of airborne fungi was carried out in 2 plastic waste sorting facilities in the Czech Republic. The samples were taken in the breathing zone (approximately at the height of 1.5 m) near to the conveyor belt where employees sort plastic waste. The samples were collected during 2013 and 2014 (October 2013, January 2014, May 2014, and August 2014). In each sampling season, samples of airborne fungi were collected within one work shift. During each work shift (duration 8 h), there were performed 10 measurements, the first one before the beginning of the work shift, then every hour during the shift and the last one an hour after the end of the shift. In total, 1920 samples were taken.

During each measurement, air was sampled by means of a 37-mm Filter Holder (BGI Inc., USA) connected with a portable constant-flow Leland Legacy Sample Pump (SKC Ltd., UK). The pump was calibrated to the flow rate of 5 l/min. The sampling period per 1 sample was 24 min. Thus, the amount of sampled air was 120 l. Sampling and subsequent processing of samples were performed according to methods described by Černá et al. [17].

Four types of cultivation media with added antibiotics were used for the collection and detection of a broader spectrum of airborne fungi from sampled air: dichloran rose-Bengal chloramphenicol (DRBC), yeast glucose chloramphenicol (YGC), Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (100 mg/l) and malt extract agar (MEA) (Oxoid Ltd., UK) supplemented with chloramphenicol (100 mg/l). Petri dishes were incubated at $25\pm 1^\circ\text{C}$ for 72 h. After the incubation, colonies of fungi were counted and recalculated as the number of CFU/m³. All cultivation media were replicated on 6 plates per sample.

Fungal species identification

Colony-forming units of the fungi from the plates were divided into morphotypes. Representative colonies of each morphotype were selected for the identification. These colonies were concurrently cultivated on 3 cultivation media MEA, Czapek Dox Agar (CZA) and Czapek Yeast Extract Agar (CYA) (HiMedia Laboratoires Pvt. Ltd., India) at $25\pm 1^\circ\text{C}$ for 7 days. Then the fungi were classified to genera or species by using a light microscopy. Identification of fungi was achieved through macro- and microscopic characteristics as described by Ellis and Hesseltine [18], Pitt and Hocking [19] and de Hoog et al. [20].

Statistical analysis

The data on the concentration of airborne fungi was analyzed by means of hierarchical ANOVA due to the split-plot structure of the dataset with 4 levels. For the final analysis purposes, the response variable, abundance of CFU/m³ was log-transformed in order to meet the assumption of homogeneity of variance (increasing variance with fitted mean was detected from regression diagnostic graphs of the preliminary analysis). The tested predictors were: season, waste sorting facility, sampling time (both linear and quadratic terms of the considered relationship), cultivation medium and all their interactions. We did not consider interactions of other factors with season since there were only 2 sampling occasions per season. All computations were undertaken in R 3.0.1 (R Core Development Team, Austria) statistical environment under the base installation [21].

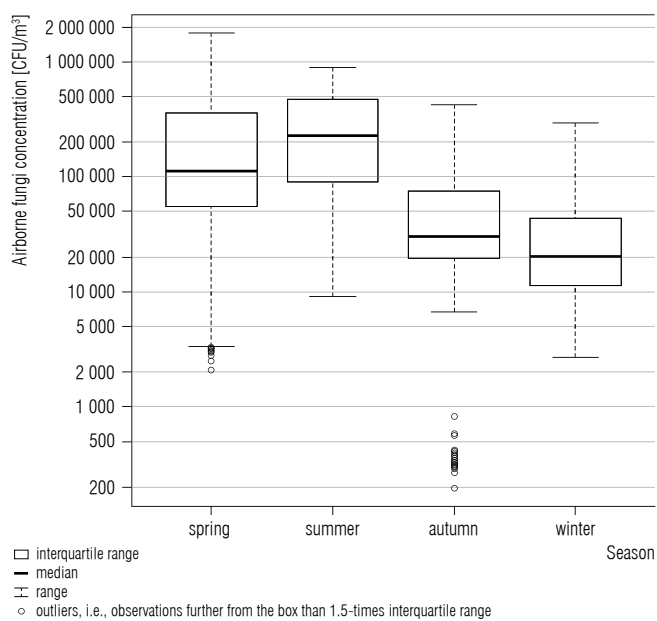
The species composition of the detected fungi was analyzed by means of the canonical correspondence analysis (CCA). The dependent variables, i.e., the numbers of the CFU for each fungal species, were log-transformed prior to the analysis since the dataset was

largely dominated by a few common species, while a multitude of relatively infrequent species was present as well. We tested for differences in fungal species composition among the sampling seasons using the permutation tests with 4999 permutations. A proper number of replicates for testing the effect of sampling season is 8 (2 sorting facilities × 4 sampling seasons), which was achieved by applying the hierarchical design with 8 whole plots containing 80 split-plots each and allowing to permute only the whole-plots (see Lepš and Šmilauer [22] for further argumentation). All multivariate analyses were undertaken in Canoco 5.04 (Microcomputer Power Inc., USA) [23].

RESULTS

Concentrations of airborne fungi

There was a marginally significant trend of summer and spring samples yielding the highest CFU concentrations (Table 1, Figure 1). The concentrations of airborne fungi ranged 2.1×10^3 – 1.8×10^6 CFU/m³ in spring, 9.1×10^3 – 9.0×10^5 CFU/m³ in summer, 2.0×10^2 – 4.2×10^5 CFU/m³ in autumn and 2.7×10^3 – 2.9×10^5 CFU/m³ in winter. Contrary to our expectations, the differences in airborne fungi concentrations among the 2 facilities were not of particular importance (Table 2).



CFU – colony-forming units.

Fig. 1. Airborne fungi concentration in the studied plastic waste sorting facilities in the Czech Republic, 2013–2014, by season

The results of the split-plot ANOVA indicated that the hour of sampling (both linear and quadratic term) and the type of cultivation medium were the only drivers of the detected CFU concentrations (Table 2). The

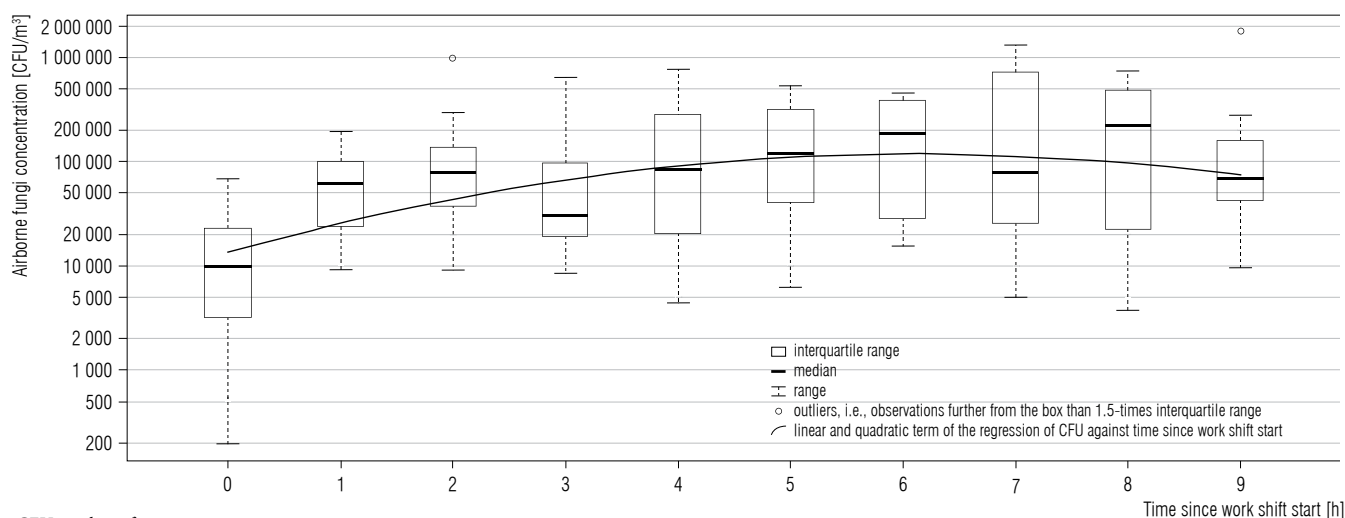
Table 1. Airborne fungi in the studied plastic waste sorting facilities in the Czech Republic, 2013–2014, by season

Season and waste sorting facility	Airborne fungi [CFU/m ³]			
	M	SD	back-transformed M	95% CI
Spring				
A	1.1×10^5	8.6×10^4	9.4×10^4	8.7×10^4 – 1.0×10^5
B	3.5×10^5	3.1×10^5	1.6×10^5	1.3×10^5 – 1.9×10^5
Summer				
A	3.4×10^5	2.6×10^5	2.3×10^5	2.0×10^5 – 2.6×10^5
B	2.4×10^5	2.1×10^5	1.5×10^5	1.3×10^5 – 1.7×10^5
Autumn				
A	2.4×10^4	1.4×10^4	1.6×10^4	1.3×10^4 – 1.9×10^4
B	1.0×10^5	9.6×10^4	6.3×10^4	5.6×10^4 – 7.2×10^4
Winter				
A	5.0×10^4	6.2×10^4	2.5×10^4	2.2×10^4 – 2.9×10^4
B	2.3×10^4	1.6×10^4	1.8×10^4	1.7×10^4 – 2.0×10^4

CFU – colony-forming units, M – mean, SD – standard deviation, back-transformed M – mean on the logarithmic scale (the scale of measurements, where approximation by normal distribution and data analysis is possible), which was back-transformed to the original scale, 95% CI – 95% confidence interval of the back-transformed mean (the asymmetry of confidence intervals on the original scale corresponds to the skewness of the response variable).

initial increase and later stagnation of the CFU concentration during the progressing working shift (Figure 2) explained together 20.8% of the total variation (Table 2).

On the other hand, effects of the cultivation medium were completely marginal given the amount of variation it explained. The highest numbers of the detected CFU



CFU – colony-forming units.

Fig. 2. Airborne fungi concentration in the studied plastic waste sorting facilities in the Czech Republic, 2013–2014, by time elapsed since the work shift start

Table 2. Hierarchical ANOVA of log-number of colony-forming units (CFU) of airborne fungi in the studied plastic waste sorting facilities in the Czech Republic, 2013–2014

Predictor	df	Explained variation* [%]	Sum of squares	p
Variation among seasons and factories				
season	3	35.4	1 547.9	0.088
factory identity	1	0.9	39.9	n.s.
residual variation	3	5.9	259.9	
Variation among hours within sampler type, factory and season				
hour of sampling (linear)	1	13.1	574.0	< 0.001
hour of sampling (quadratic)	1	7.7	335.8	< 0.001
factory × hour (linear)	1	0.7	28.5	n.s.
factory × hour (quadratic)	1	0.6	26.1	n.s.
residual variation	68	33.8	1 476.3	
Variation among mediums within hour, sampler type, factory and season				
medium	3	0.1	6.3	< 0.001
factory × medium	3	0.0	0.4	n.s.
hour (linear) × medium	3	0.0	0.9	n.s.
hour (quadratic) × medium	3	0.0	0.2	n.s.
factory × hour (linear) × medium	3	0.0	0.4	n.s.
factory × hour (quadratic) × medium	3	0.0	0.1	n.s.
residual variation	222	0.9	37.5	
Variation among dishes within medium, hour, sampler type, factory and season				
residual variation	1 600	0.8	34.4	

df – degrees of freedom, n.s. – not statistically significant.

* Amount of total variation (i.e., at all hierarchical levels) explained.

of airborne fungi within all measurements were detected on the DRBC cultivation medium as compared to the other cultivation media (SDA, MEA and YGC).

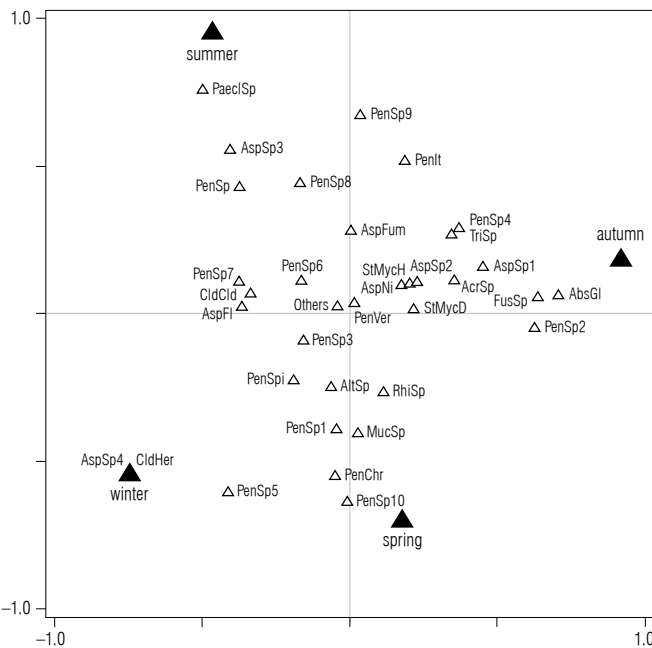
Species composition of fungi cultivated from samples

The exploration of the multivariate data on the fungal species composition indicated that the effect of the season on the species composition of fungi cultivated from samples may only be expected. The canonical correspondence analysis indicated that the season identity explained 11.4% of the variance in the species composition of fungi ($p = 0.010$) (Figure 3).

The dominating airborne fungi detected in this study belonged to the genus *Penicillium* (75.1% of all cultivated fungi) but there was a turnover of particular species among seasons. The next most frequently detected genera were in the decreasing order: *Aspergillus* (11.3%), *Acremonium* (3.1%), *Paecilomyces* (2.6%),

Cladosporium (1.9%), *Rhizopus* (1.1%), *Mucor* (1.0%), *Absidia* (0.5%), *Trichoderma* (0.4%), *Alternaria* (0.1%) and *Fusarium* (0.1%). The highest diversity of fungal species was observed in the samples taken in autumn. The presence of potentially toxigenic fungi *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *Penicillium chrysogenum* was recorded in all seasons in both facilities.

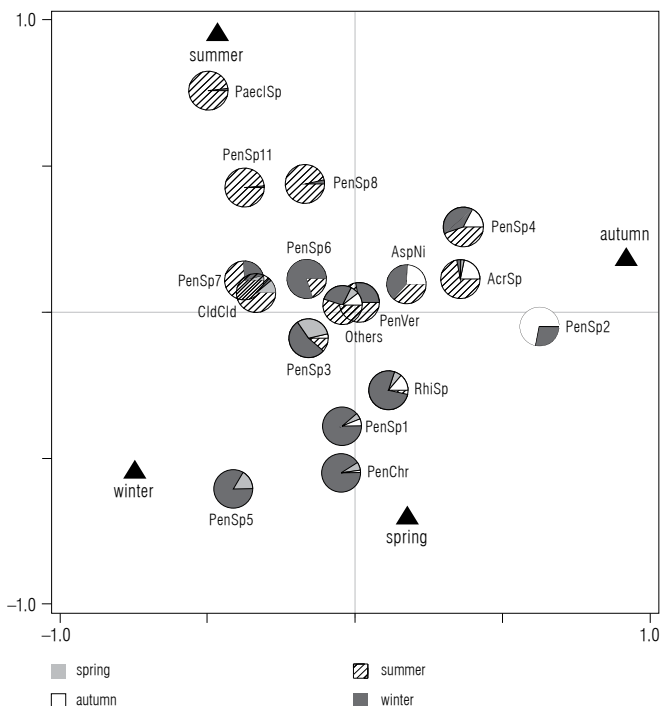
Winter and summer samples were specific by unique abundant fungi species (winter – *Rhizopus* sp., *P. chrysogenum*, *Penicillium* sp. 1, *Penicillium* sp. 5, *Penicillium* sp. 6; summer – *Paecilomyces* sp., *Cladosporium cladosporioides* s. l., *Penicillium* sp. 7, *Penicillium* sp. 8, *Penicillium* sp. 11), while autumn and spring samples hardly contained these species (Figure 4).



AbsGl – *Absidia glauca*, AcrSp – *Acremonium* sp., AltSp – *Alternaria* sp., AspFl – *Aspergillus flavus*, AspFum – *Aspergillus fumigatus*, AspNi – *Aspergillus niger*, AspSp1–4 – *Aspergillus* sp. 1–4, CldCld – *Cladosporium cladosporioides* s.l., CldHer – *Cladosporium herbarum* s.l., FusSp – *Fusarium* sp., MucSp – *Mucor* sp., PaecSp – *Paecilomyces* sp., PenChr – *Penicillium chrysogenum*, PenIt – *Penicillium italicum*, PenSpi – *Penicillium spinulosum*, PenSp1–11 – *Penicillium* sp. 1–11, PenVer – *Penicillium verrucosum*, RhiSp – *Rhizopus* sp., StMycD – dark sterile mycelium, StMycH – hyaline sterile mycelium, TriSp – *Trichoderma* sp., others – unidentified fungi.

* First and second canonical axes explain 5.1% and 4.6% of variation respectively and account for 85.3% of the total 11.4% of variation explained by sampling season.

Fig. 3. Canonical correspondence analysis (CCA) of fungi species composition (log-transformed counts of colony-forming units (CFU)) in the studied plastic waste sorting facilities in the Czech Republic, 2013–2014*



Abbreviations as in Figure 3.

* The pie charts depict proportions of detected CFUs of a given fungal species in a given season.

Fig. 4. Canonical correspondence analysis (CCA) of composition of fungi species occurring in more than 5% of samples in the studied plastic waste sorting facilities in the Czech Republic, 2013–2014*

DISCUSSION

Concentrations of airborne fungi

Our results indicate that the seasonal and diurnal variations in concentrations of airborne fungi need to be taken into account when assessing the load rate of employees in the waste sorting facilities. Their importance

is comparable to other drivers of load rates of employees such as the sorting technology (the open conveyor belt, ventilation system, accumulation of waste in the plant, frequency and quality of cleaning) [7,14] and the quality of the input material (i.e., its contamination by microscopic fungi) [8].

The overall measured exposure of employees to airborne fungi was more or less comparable to that reported in studies from similar waste treatment facilities [4,6–9,14,15,24]. Nevertheless, comparing the results of these studies is complicated due to the different sampling methods, sample processing applied and other sources of variation (see Černá et al. [17], Eduarda and Heederik [25] for discussion of the problem). However, the concentrations of airborne fungi in this study were clearly higher (2–4 orders of magnitude) to those found in other indoor environments [26–29]. It points to the potential health risk for employees.

There was a trend of highest concentrations of airborne fungi being measured in summer and spring, while the lowest ones are reported to occur in winter. On the contrary, Rahkonen [6] measured the highest concentration of airborne fungi in autumn and then in spring and summer. Differences in the measured concentrations may point to the varying microclimate conditions (temperature, relative air humidity) inside the sorting facilities during the year [30]. Higher temperature and air humidity may cause an increased microbial activity and thus a higher concentration of airborne fungi [31]. However, release of fungal particles into ambient air also depends on fungal genus as well as air velocity [32].

The diurnal variation in the airborne fungi concentrations showed a quite expectable pattern, i.e., gradual increase since the start of the working shift, which reaches a plateau after ca 6–7 h of working, however notable is the difference of the order of magnitude between the lowest and highest predicted values. The observed trend could be associated with the increasing amount of sorted waste during the work shift and depletion of its supply for sorting towards the end of the work shift.

Species composition of fungi cultivated from samples

The species composition of airborne fungi cultivated from samples was similar to that referred in studies from similar facilities [8,9,13,14,24], where the dominating species were from the genera *Penicillium* and *Aspergillus* with their proportions varying among studies.

In our study, the dominating fungi were those of the genus *Penicillium* (75.1%) followed by *Aspergillus* (11.3%). Lehtinen et al. (2013) [9] reported the identification of the genus *Penicillium* in 93% of all the cultivated fungi. On the other hand, Viegas et al. (2014) [13] predominantly identified the genus *Aspergillus*, the genus *Penicillium* was not determined. Tolvanen et al. (1999) [24] determined the genus *Aspergillus* in 40% and the genus *Penicillium* in 44% of all the cultivated fungi.

In our study, species composition of airborne fungi in waste sorting facilities changed during the year (Figure 3). The species composition may be influenced by microscopic fungi from waste as well as airborne fungi from the outside environment, that penetrate through doors and windows. The most frequently isolated fungi from outside environment are those of the genera *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* [33–35]. Genera *Penicillium* and *Aspergillus* dominated in this study in all seasons. On the other hand, the next most frequently detected genera *Acremonium*, *Paecilomyces*, *Cladosporium*, *Rhizopus* predominated only in one season (Figure 4). Larsen and Gravesen [33] recorded the same seasonal patterns of genera *Penicillium*, *Aspergillus* and *Cladosporium* in the long-term study which was conducted in an outdoor environment.

In both waste sorting facilities, the potentially mycotoxins-producing fungi *A. niger*, *A. fumigatus*, *A. flavus* and *P. chrysogenum* were detected in all seasons and *A. niger* was even the second most frequently isolated fungal species in all samples. These fungal species were also detected in air samples from waste sorting facilities in several other studies [7,8,13]. However, only a limited number of studies focus on employees' exposure to mycotoxins in waste sorting facilities. Viegas et al. (2015) [36] found high aflatoxin B1 values (produced by *A. flavus*) in blood samples collected from employees of waste sorting facility. This mycotoxin is considered by different International Agencies as a genotoxic and potent hepatocarcinogen. Moreover, other mycotoxins are probably present in the working environment of waste sorting facilities and this aspect should be taken into consideration due to their possible synergistic reactions [37]. However, fungi are still used as an indirect indicator of mycotoxins' presence in working environments [38].

Some fungi detected in our study belong to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium* is assumed to elicit allergic inflammatory reactions and different human infections [27,39,40]. However, there is a little information on clinically sig-

nificant concentrations of these airborne fungi necessary to cause health problems. Bagni et al. [41] reported that 1×10^2 CFU/m³ of the genus *Alternaria* and 3×10^3 CFU/m³ of the genus *Cladosporium* led to allergic reactions. These concentrations were exceeded in our study in both facilities in all seasons.

Several studies showed a relationship between the working activities in waste management and the presence of various health problems in employees, such as respiratory diseases [1,2,10], gastrointestinal problems [1,2] and metabolic syndrome [3]. However, a direct link to fungi cannot be drawn since employees in waste management are exposed besides to fungi also to dust, bacteria and other metabolites [6,9,14,15]. Nevertheless, this aspect should be taken into consideration for the risk assessment process due to possible synergistic effects on human health.

Based upon our results, we recommend the use of the protective equipment (thick rubber gloves, respiratory mask, working clothes) by employees and the introduction of preventive measures in working environment of waste sorting facilities. We especially recommend to raise employees' awareness of health risks that they may be exposed to and to disseminate information about preventive methods applicable during the work. Furthermore, adequate ventilation system in the working environment should be installed, frequency and quality of the wet cleaning phase should be increased and regular and detailed medical examinations of employees should be introduced. These recommendations could lead to minimizing of risks to employees' health in waste sorting facilities.

CONCLUSIONS

We performed a general evaluation of the occupational exposure of workers employed in the plastic sorting plant to airborne fungi during the work shift in four seasons of the year. Overall, high concentrations of airborne fungi and the presence of potentially toxigenic fungal species in the work environment were detected in all measurements with some of the harmful taxa (e.g., *Aspergillus niger*) being among the most frequently species. A trend of higher airborne fungi concentrations was found in summer and spring when compared to autumn and winter. The lowest airborne fungi concentrations were found at the beginning of the work shift followed by the quick increase reaching a plateau (sometimes followed by a slight decrease towards the end of the shift). This study shows that the sorting plant

is the working environment with increased concentrations of airborne fungi and corresponding preventive measures need to be taken in order to decrease the employees' exposure to harmful agents.

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