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Microbial contamination of money sorting facilities

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Abstract

Introduction. Money is the most common item with which we have daily contact. Circulated banknotes and coins can become microbiologically contaminated and act as both a source and a means of spreading such pollutants.

Materials and method. The study was carried out in three money sorting facilities in Poland. Bioaerosol samples were collected using a 6-stage Andersen impactor, and microorganisms deposited on tabletop surfaces were sampled using the swab method. Bacterial and fungal concentrations were calculated and all isolated species were taxonomically identified.

Results. The study confirmed that means of payment are active sources of microbial emission in money sorting facilities. The bioaerosol concentrations did not exceed the threshold limit values proposed for this type of office premises. It confirms that ventilation systems in these facilities worked efficiently, protecting them from the migration of microbial contaminants present in both indoor and outdoor (atmospheric) background air. On the other hand, the average concentrations of bacteria and fungi on tabletop surfaces in banknote and coin sorting rooms were above the proposed purity levels for indoor surfaces and should be treated as microbiologically contaminated. Microbiota isolated from the air and surfaces were very diverse and among those strains were bacterial and fungal pathogens that can pose a health threat to exposed individuals.

Conclusions. The results showed that employees in money sorting facilities were exposed to microorganisms that may contribute to the development of adverse health outcomes. To protect them, highly efficient hygienic measures should be introduced in this working environment, to prevent both unwanted pollution and subsequent secondary emission of microbial contaminants from sorted means of payment and tabletop surfaces.

Key words

money sorting, banknotes, coins, bacteria, fungi, bioaerosol, surface contamination, size distribution

INTRODUCTION

Money is the most common item with which we have daily contact. Banknotes and coins in circulation can become contaminated and therefore become both a source and a means of spreading microbial contamination. The possibility that banknotes can act as environmental vectors for transmitting pathogenic microbes was recognized as a result of research already carried out in the 1970s [1]. The current study shows that practically all currency (especially banknotes) serves as an ideal nutrition source for microorganisms for several reasons. Firstly, any folds or intentionally designed (e.g. to reduce the number of counterfeits) and roughness of banknote or coin surfaces serve as places where organic debris are deposited, allowing the microorganisms to maintain their viability for a long period of time. Secondly the means of payment accompany people's lives for many years, and the age and denomination are directly related to the degree of pollution (i.e. usually older banknotes and coins are more contaminated than newer ones) [2]. Finally, it is a fact that persons living in unsanitary conditions or having unhygienic habits also contaminate the means of payment with microorganisms (by improper hand washing, e.g. after using the toilet or handling food, soil, etc., and licking fingers while counting banknote, and transferring microorganisms on their hands after coughing, sneezing, from wounds, or body secretions and excretions). A source of contamination may also be bad currency handling practices, such as spilling money during traditional ceremonies [3–7]. Regardless of the socio-economic status, everyone has certain amounts of money at their disposal at any given time. By participating in the circulation, people contribute to the spread of microbial (including infectious) pollutants in the population, which should be considered as a significant threat to public health [8–15]. Khin et al. [16] found high levels of intestinal pathogens in samples taken from banknotes obtained from butchers and fish traders selling their products at a market. Uraku et al. [17], examining the microbial contamination of banknotes, found the largest number on denominations collected from beggars (2.4×10^5 cfu), followed by hawkers (1.8×10^5 cfu), women selling on markets (1.6×10^5 cfu), bus conductors (1.5×10^5 cfu), and bankers (1×10^5 cfu).

The degrees of contamination and the types of microorganisms present on circulating money depend on the sanitary and microclimatic conditions and microbiota composition in a given environment, as well as on the material structure of a given currency. Numerous studies have shown that paper currency is more contaminated than polymer varieties [e.g. 18–21], and the longer a currency remains in circulation, the greater the risk of its pollution,

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and the lower the denomination of money, the higher the exchange frequency between people and the higher its microbial contamination [22].

In the scientific literature, there are numerous studies devoted to the quantitative and qualitative assessment of microbial contamination of various types of means of payment. Evidence of microbiological contamination of banknotes has so far been presented by researchers from all continents [16, 23]. Such investigations showed that microbial pollution can affect 60% – 100% of banknotes in circulation. From microorganisms transported on banknotes, the most frequently isolated were bacteria from *Klebsiella* (incl. *K. pneumoniae*), *Staphylococcus* (incl. *S. aureus*, *S. epidermidis*), *Escherichia* (incl. *E. coli*), *Salmonella* (incl. *S. typhi*), *Proteus* (incl. *P. mirabilis*), *Pseudomonas* (incl. *P. putida*), *Serratia*, *Citrobacter*, *Shigella*, *Listeria*, *Bacillus* (incl. *B. subtilis*, *B. megaterium*), *Enterobacter* (incl. *E. aerogenes*), *Aeromonas* (incl. *A. hydrophila*), *Streptococcus*, and *Micrococcus* genera, filamentous fungi from *Aspergillus* (incl. *A. niger*, *A. flavus*, *A. nidulans*), *Penicillium* (incl. *P. citrinum*), *Alternaria* (incl. *A. tenuis*), *Curvularia* (incl. *C. pallescens*), *Cladosporium* (incl. *C. cladosporioides*), *Rhizopus* (incl. *R. stolonifer*), *Fusarium*, *Colletotrichum* (incl. *C. truncatum*, *C. gloesporoides*), and *Trichoderma* (incl. *T. viride*) genera, and yeasts from *Candida* genera [e.g. 1, 8–15, 18, 24–28]. Their degree of contamination may reach 10^9 colony forming units (cfu) per one banknote [29].

Compared to the research on banknote contamination, there are only a few reports on the microbiological quality of coins. In 1999, Jiang and Doyle [30] suggested that coins could serve as potential carriers of pathogens such as *E. coli* and *Salmonella* spp. There is also very little information about the isolation of potentially pathogenic fungal conidia from coins. The first report of this type was made by Kuria et al. [31], who confirmed the presence on coins of filamentous fungi (from *Penicillium*, *Aspergillus*, *Fusarium*, *Rhizopus*, and *Alternaria* genera) and yeasts. Research by Abirami et al. [28] on coins from Maharashtra State in India also revealed the presence of numerous species of filamentous fungi, including *A. niger*, *A. flavus*, *A. nidulans*, *P. citrinum*, *A. tenuis*, *C. pallescens*, *C. cladosporioides*, *R. stolonifer*, *Fusarium* spp., and *T. viride*. As noted by several research groups, a greater number of microorganisms occur on the surfaces of banknote rather than on coins, which may suggest that coins are safer in circulation within population than banknotes [32, 33].

There is a high probability of isolating microbiota with a taxonomic composition similar to that of banknotes and coins, both in the rooms where these means of payment are sorted and from machines used for this purpose, e.g. banknote sorters and counters. A study by Eneumor et al. [34] carried out in such rooms in four Nigerian banks showed that 70% of the banknote counters were contaminated with bacteria, and 25% with fungi. Among the most frequently isolated microorganisms from counters there were cocci of *Staphylococcus*, *Enterococcus*, and *Streptococcus* (incl. *S. pyogenes*) genera, Gram-negative bacilli, *S. typhi*, *E. coli*, and *Proteus* spp., as well as fungi of *Aspergillus*, *Mucor*, *Rhizopus*, and *Penicillium* genera. Since all these microorganisms have a pathogenic potential, the contaminated elements of counting and sorting machines, as well as worktop surfaces, may become sources of infection spreading among bank employees and in the general population, creating a risk for both employees and clients of these facilities.

While the studies devoted to the quantitative and qualitative evaluation of microbial contamination of banknotes and coins are available in the scientific literature, knowledge about the hygienic conditions of the premises in which they are sorted and subsequently packed before their redistribution, is practically non-existent. Hence, the aim of this study was to assess the bacterial and fungal contamination of the air and surfaces in money sorting facilities and, subsequently, evaluate the hygienic quality of this specific environment for employees occupationally exposed to harmful microbiological agents.

MATERIALS AND METHOD

This study was carried out in three money sorting facilities located in central (1) and southern (2) Poland. Both banknotes and coins were sorted at workplaces in the studied premises. Polish currency notes are made of special 80 g/m^2 white cotton banknote paper with the surface refined with polyvinyl alcohol. Surprints and overprints are made of monochrome inks using the techniques of steel engraving (used to print the main graphic motifs on both sides of the banknote, digital and word markings of the nominal value, name of the issuer as well as being responsible for angular effect), typography (used to print series markings and numbering) and offset (applied to create the underprint), supplemented with silk-screen printing and flexography. In turn, the coins used in Poland are minted mainly from copper, nickel, tin and aluminum alloys. The most common alloy is copper-nickel with small additions of other elements, such as aluminum, iron, silicon or manganese. It is used to produce a few Polish monetary units, e.g. 10 groszy, 50 groszy and 1 zloty coins. The alloy is used in 2 and 5 zlots coins, the core of which is made of cupro-nickel and the surrounding ring of aluminum bronze with small admixtures of nickel, manganese and iron. The currencies with the lowest denominations, i.e. 1, 2 and 5 groszy coins, were initially struck from brass (an alloy of copper and zinc) with the addition of manganese, while now they are minted from steel coated with a layer of manganese brass. An additional technique used in Polish mints to increase the resistance of coins is oxidation, i.e. the process of covering the coin surface with a layer of oxides from the raw material from which it is made. This treatment additionally increases the resistance of the coin to corrosion.

For the study, measurements were carried out in the summer season, defined as the period from May – September, when the average outdoor air temperature was above 10 °C on at least seven consecutive days. All studied premises were located in multi-story buildings located in urban areas. The sorting rooms were equipped with ventilation systems enabling air-conditioning of the tested interiors. Their cubic capacities ranged from 50 m^3 – 294 m^3. In none of the examined buildings, neither in their history nor current state, were visible signs of moisture damage indicating conspicuous microbial growth were noted. During the measurements, there were several to a dozen people in the rooms. The bioaerosol samples were collected using stationary sampling methods at workplaces during typical occupational activities, in corridors neighbouring the sorting rooms in order to determine the indoor background level of contamination, and in the direct surrounding of the building (in the ambient air) in order to check both the outdoor

background level of contamination and the degree to which microorganisms migrate to the studied premises. During the measurements, all sampling instruments were placed at a height of 1–1.5 m above the floor or ground level to simulate aspiration from the human breathing zone. Viable (understood in this study as culturable) bioaerosol samples were taken using a six-stage Andersen impactor (model WES-710, Westech Instrument, Upper Stondon, UK) at a flow rate of 28.3 l/min. At the beginning of each measurement cycle, bacterial aerosol was collected on blood trypticase soy agar (TSS 43001, bioMérieux, Marcy l'Etoile, France), and after impactor reloading, fungi were aspirated on malt extract agar (MEA, Oxoid Ltd., Basingstoke, UK). The sampling time was five minutes for both bacterial and fungal aerosol, and their concentrations were expressed as colony forming units in 1 m^3 of sampled air (cfu/m^3).

The bioaerosol measurements were complemented by evaluation of bacterial and fungal content in the dust settled on tabletop surface, on which banknotes and coins were sorted. Each time, the settled dust was taken using a sterile nylon flocked applicator equipped with Amies liquid (eSwabÔ, Copan Italia S.p.A., Brescia, Italy) from the surface of 100 cm^2 (a square shaped 10×10 cm sterile template was applied), and subsequently immersed in Amies liquid for transport to the laboratory. After sampling, to extract the collected microorganisms, the swabs were vortexed for 10 min using a programmable rotator-mixer (model Multi RS-60, Biosan, Riga, Latvia) in 5 ml distilled water. The spread plate method was applied in which 0.2 ml of the resulting suspension was spread evenly over the same media as that used for bio-aerosol sampling.

In total, 15 money sorting rooms were tested from which 46 air and 30 surface samples were collected. All impactor and settled dust samples were incubated at the following temperatures: bacteria – one day at 37 °C, followed by three days at 22 °C and three days at 4 °C; fungi – four days at 30 °C followed by four days at 22 °C. After incubation, the viable microbial concentrations in the air and dust were calculated as colony forming units per 1 m^3 (cfu/m^3) and per 1 cm^2 (cfu/cm^2), respectively. Bacterial and yeast strains were identified by Gram staining (111885 Gram-color stain set, Merck KGaA, Darmstadt, Germany) [35], their morphology and, finally, by the biochemical API tests (bioMérieux). Filamentous fungi were identified according to their morphology using several identification keys [36–40]. The isolated pathogens were additionally analyzed by molecular methods (polymerase chain reaction (PCR) followed by random amplification of polymorphic DNA-RAPD typing). In total, seven bacterial and four fungal species were analyzed in that way.

DNA was isolated from pure bacterial/fungal cultures grown on TSS/MEA plates using QIAmp DNA (Qiagen, Hilden, Germany) or Fungi DNA (Syngen Biotech, Wrocław, Poland) Mini-Kits. The isolated bacterial DNA was used as a template in PCR with BAK11w (5′-AGTTTGATCMTGGCTCAG-3′) and BAK2 (5′-GGACTACHAGGGTATCTAAT-3′) primer sets which allow amplification of bacterial 16S rRNA gene fragment corresponding to *Escherichia coli* 16S rRNA gene positions from 10 – 806. The isolated fungal DNA was used as a template in PCR with ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primer sets which allow amplification of the fungal genome fragment located between 18S and 28S rRNA genes, covering the ITS1, 5.8S rRNA, and ITS2 fragments. The amplified PCR products were purified, sequenced using DNA analyzer (model 3730, Applied Biosystems, Waltham, MA, USA), and compared to the GenBank database (National Center for Biotechnology Information, US National Library of Medicine, Bethesda, MD, USA) using the BLAST (Basic Local Alignment Search Tool) algorithm [41–43].

During the collection of microbial samples, the air temperature and relative humidity as major microclimate parameters influencing the growth of studied microbiota, were measured using a hytherograph (model Omniport 20, E+E Elektronik GmbH, Engerwitzdorf, Austria).

All bioaerosol, settled dust, and microclimate parameter measurements were performed in triplicate. The collected data were statistically elaborated using analysis of variance (ANOVA) followed by Fisher LSD tests, *t*-test, and Pearson correlation analysis, using Statistica (data analysis software system) version 10. (StatSoft, Inc., Tulsa, OK, USA). Probability values were treated as statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The concentrations of bacterial and fungal aerosols measured using an Andersen impactor at workplaces in money sorting rooms, in neighbouring corridors (indoor background), and in the direct vicinity of the buildings (outdoor background) are presented in Table 1. In all tested indoor premises and in ambient air, the bacterial and fungal aerosol concentrations were below 5,000 cfu/m^3. The airborne bacterial concentration in banknote as well as coin sorting rooms exceeded 1.2 and 1.8 times, as well as 6.9 and 10 times more than those observed in the indoor and outdoor backgrounds, respectively. In the case of fungal aerosol, the concentrations recorded in the banknote sorting rooms were similar to those observed in ambient air, whereas the levels noted in the air of coin sorting rooms and in indoor background were five and 2.2 times lower than those in the atmospheric air, respectively. Although the average concentrations of bacterial and fungal aerosols were different in the individual groups of the studied rooms and environments, the analysis of variance confirmed a statistically significant difference (ANOVA: $p<0.01$) only between the indoor (sorting rooms and nearby corridors) bacterial concentrations and those measured in the outdoor background (Fisher LSD: $p<0.05$).

Table 1. Concentrations of bioaerosols (cfu/m^3) in money sorting facilities and in indoor and outdoor background air

Sampling points		Bacterial aerosol		Fungal aerosol	
		Average	Range	Average	Range
Work-places	Banknote sorting rooms	1,125	183–4,382	500	7–4,000
	Coin sorting rooms	1,632	424–2,840	95	14–176
Indoor background		924	63–3,696	213	14–367
Outdoor background		163	92–211	477	133–1,222

The observed relationships are consistent with the current state of knowledge about the sources of origin of the studied bioaerosols. In the case of bacterial aerosol, humans are considered the main source and constantly active indoor emission. Hence, the gathering of people in a limited office space resulted in obtaining much higher concentrations of

bacterial aerosol indoors than outdoors. Moreover, the nature of occupational activities carried out in the examined rooms and the release of microbial pollutants into the air from the sorted banknotes and coins were also obviously responsible for the observed bacterial contamination levels. In turn, the most productive sources of fungal aerosol (i.e. soil, plants, water reservoirs etc.) are present outdoor, and the constant, although of varying intensity, ambient air migration into the indoor environment is the major process causing biological contamination of interiors with this type of bioaerosol. While banknote sorting provides an additional load of fungal particles into the air of the examined premises, in the case of coin sorting the performed occupational activities resulted in a significantly lower environmental burden with such particles.

Unfortunately, no data regarding concentrations of microbial pollutants in the air of money sorting facilities exist in the scientific literature. Also, interpretation of the results of quantitative bioaerosol measurements performed in both indoor and outdoor environments is difficult due to the lack of widely acceptable hygienic standards and/or threshold limit values (TLV) for harmful microbiological agents. Determination of the degree of microbial air pollution expressed by the number of colony forming units (cfu) in 1 m^3 of the air to-date is the best known and most frequently used measure of exposure to harmful microbiological agents [44]. In the hygienic assessment of the environments examined in this study, the TLVs elaborated based on volumetric sampling methods and proposed by the Expert Group on Biological Agents at the Polish Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environmen, was used (Tab. 2 and 3) [45, 46]. Comparison of bioaerosol concentrations with hygienic standards showed that both bacterial and fungal levels in the air did not exceed the TLV proposals established for indoor premises and ambient air.

All bacterial and fungal species isolated from the air and tabletop surfaces in the money sorting facilities and from the indoor and outdoor background air are presented in Table 4. The whole airborne microbiota was analyzed using biochemical tests, supplemented in the case of pathogens by molecular methods. Compared to GenBank database, the sequence similarity of 99–100% was achieved for those strains. In total in the air, 42 bacterial species belonging to 20 genera and 45 fungal species belonging to 21 genera (including 40 mould species belonging to 17 genera and five yeast species belonging to four genera) were identified. Of these 19 bacterial and 18 fungal species were solely present in the air and, as such, the sorted currency was probably not their source of origin. Among the isolated airborne microbiota, 12 bacterial and 17 fungal species have cellulolytic properties. Taking into account the material dealt with in money sorting facilities, their ubiquity is probably related to the presence of banknotes in this specific working environment. These microorganisms may treat the paper structure of the banknote material as a potential source of nutrients, causing its bio-deterioration. Banknotes thus polluted may become a secondary source of indoor microbial emissions. Moreover, among the identified airborne microbiota, the presence of six bacterial and three fungal pathogens was confirmed (by PCR technique). All these strains are classified in Group 2 according to their level of risk of infection and, as such, posed a health threat to the employees exposed to direct contact with them or their inhalation [47, 48].

Table 2. Recommended threshold limit values for microorganisms in indoor air [46]

Microbial agents	Residential and public utility premises
Mesophilic bacteria	5×10^3 cfu/m^3
Gram-negative bacteria	2×10^2 cfu/m^3
Thermophilic actinomycetes	2×10^2 cfu/m^3
Fungi	5×10^3 cfu/m^3

Table 3. Proposals for assessment of microbial contamination of outdoor air [46]

Microbial agents	Level of microbial contamination	
	Acceptable	Unacceptable
Bacteria (together)	≤5×10^3 cfu/m^3	>5×10^3 cfu/m^3
Gram-negative bacteria	≤2×10^2 cfu/m^3	>2×10^2 cfu/m^3
Thermophilic actinomycetes	≤2×10^2 cfu/m^3	>2×10^2 cfu/m^3
Fungi	≤5×10^3 cfu/m^3	>5×10^3 cfu/m^3

The percentage distributions of individual identified groups of microorganisms in relation to the total microbiota isolated from the air and tabletop surface in the studied money sorting facilities, and in the indoor and outdoor background air, are shown in Figure 1. The qualitative composition of airborne microbiota differed significantly between the banknote and coin sorting rooms, and the air of both these premises were qualitatively different from indoor and outdoor backgrounds in this aspect (ANOVA: $p<0.01$).

Gram-positive cocci were dominant in the air of all indoor premises. While in coin sorting rooms their percentage reached 85%, in banknote sorting and internal background premises their presence was on a similar level (47% and 55%, respectively). In the latter mentioned environments, the levels of the remaining groups of isolated microorganisms were also very similar to each other. Gram-positive cocci are described in the scientific literature as commonly occurring both in the indoor (air) and outdoor (air, soil, water) environments [49]. As shown by the obtained results, the major air pollution sources with cocci seems to be people

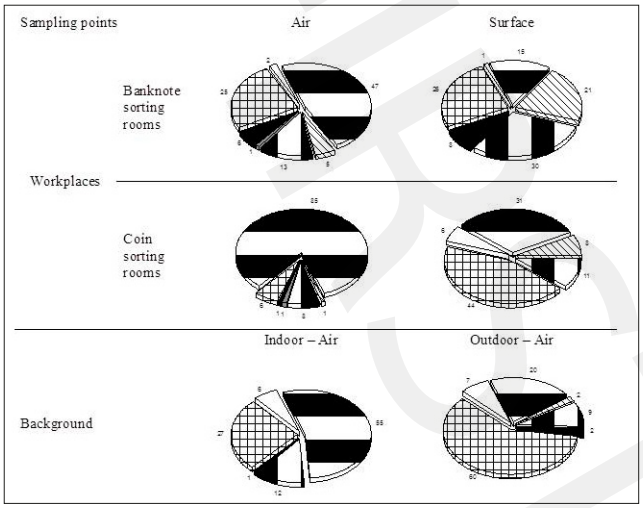

Figure 1. Percentage distribution of bacteria and fungi isolated from the air and tabletop surfaces in money sorting facilities and in the indoor and outdoor background air. The respective patterns on slices represent: ▲ Gram-positive cocci, ◬ nonsporing Gram-positive rods, ⩓ endospore forming Gram-positive rods, △ Gram-negative rods, ▲ mesophilic actinomycetes, ⩓ filamentous fungi and △ yeasts

Table 4. Microorganisms isolated from the air and tabletop surfaces in money sorting facilities and from the indoor and outdoor background air. In each environment, the species constituted at least 50% of isolated microbiota in at least one collected sample are given in squares

Microorganisms		Banknote sorting rooms		Coin sorting rooms		Indoor background	Outdoor background
Bacteria		Air	Surface	Air	Surface	Air	Air
Gram-positive cocci	*Aerococcus* spp.	×		×			
	Aerococcus viridans						×
	Kocuria kristinae	×	×	×	×	×	×
	Kocuria spp.					×	×
	Kocuria varians	×				×	×
	Micrococcus luteus	×	×	×	×	×	×
	Micrococcus spp.#	[×]	×	[×]	×	[×]	×
	Staphylococcus capitis					[×]	×
	Staphylococcus chromogenes	×		×		×	×
	Staphylococcus cohnii subsp. *cohnii*	×		×		×	×
	Staphylococcus epidermidis#	×	×		×	×	×
	Staphylococcus haemolyticus	×	×	×	×	×	
	Staphylococcus hominis	×	×	×	×	[×]	
	Staphylococcus lugdunensis					×	
	Staphylococcus saprophyticus		×	×	×	×	
	Staphylococcus sciuri#	×				×	×
	Staphylococcus warneri#	×	×	×	×	×	×
	Staphylococcus xylosus	×	×	×		×	×
	*Streptococcus mutans**	×					
Non-sporing Gram-positive	*Arthrobacter globiformis**	×	×	×	×	×	
rods	*Brevibacterium linens*# *	×	×	×	×		×
	Cellulomonas spp.#	×	×	×	×	×	×
	Corynebacterium propinquum		×				
	Corynebacterium spp.	×	×	×		×	×
	Corynebacterium striatum	×			×	×	
	Microbacterium spp.	×		×		×	×
	Rothia mucilaginosa	×	×	×	×		×
Endospore forming Gram-	*Bacillus cereus*#	×		×		×	×
positive rods	*Bacillus circulans*#				×		
	Bacillus firmus				×		
	Bacillus laterosporus						×
	Bacillus licheniformis#		×		×	×	
	Bacillus megaterium	×		×			×
	Bacillus mycoides		×		×		×
	Bacillus pumilus#		×		×		
	Bacillus subtilis# *		×				
	Bacillus spp.#	×	×			×	×
	Paenibacillus macerans		×		×	×	
	Paenibacillus polymyxa		×				
Gram-negative rods	*Ochrobactrum anthropi*					×	×
	Pseudomonas alcaligenes					×	
	Pseudomonas spp.	×					×
	Pseudomonas stutzeri	×		×		×	
	Sphingomonas paucimobilis			×			
Mesophilic actinomycetes	*Actinomyces gerencseriae**		×		×	×	×
	Nocardia asteroides# *			×			×
	Rhodococcus spp.	×	×		×		
	Streptomyces albus# *		×		×	×	×

Microorganisms		Banknote sorting rooms		Coin sorting rooms		Indoor background	Outdoor background
Fungi		Air	Surface	Air	Surface	Air	Air
Filamentous fungi	*Acremonium* spp.#		×		×		×
	Acremonium strictum#	×		×			
	Alternaria alternata#	×	×		×	×	×
	Alternaria spp.#		×		×	×	×
	Aspergillus flavus# *	×	×		×		×
	Aspergillus fumigatus# *		×		×	×	×
	Aspergillus ochraceus#				×		
	Aspergillus oryzae		×				
	Aspergillus sydowii					×	
	Aspergillus spp.# *			×			
	Aspergillus terreus#		×		×		
	Cephalosporium charticola	×		×		×	×
	Chaetomium funicola#			×	×		
	Chrysosporium spp.					×	
	Cladosporium cladosporioides#			×	×	×	×
	Cladosporium herbarum	×				×	×
	Cladosporium sphaerospermum#			×	×		
	Cladosporium spp.#						×
	Fusarium oxysporum						×
	Fusarium sporotrichoides			×	×		×
	Geotrichum spp.		×				
	Heterocephalum auranticum			×			
	Mucor hiemalis			×	×		×
	Mucor plumbeus	×					
	Mucor racemosus	×		×			×
	Mucor spp.#						×
	Oidiodendron citrinum			×			
	Oidiodendron spp.						×
	Penicillium aurantiogriseum	[×]	×	×		×	×
	Penicillium brevicompactum#	×	×	×	×		
	Penicillium carneum						×
	Penicillium chrysogenum#	×	×	×	×	×	
	Penicillium commune#	×	×	×	×		
	Penicillium crustosum	×	×	×	×		×
	Penicillium expansum						×
	Penicillium italicum						×
	Penicillium spp.	×	×	×	×	×	×
	Penicillium verrucosum	×	×	×	×		
	Rhizopus spp.#	×	×	×	×		×
	Rhizopus stolonifer	×	×		×		×
	Scopulariopsis fusca	×	×	×	×		×
	Sporotrichum spp.#	×	×	×	×		×
	Stachybotrys chartarum# *		×		×		
	Trichoderma viride#		×		×		×
	Ulocladium chartarum	×	×	×	×		
Yeasts	*Candida guilliermondii*		×		×		
	Candida spp.		×		×	×	
	Cryptococcus albidus	×				×	
	Cryptococus laurentii	×	×	×	×		
	Geotrichum candidum	×				×	×
	Saccharomyces cerevisiae	×				×	×

* pathogens from risk group 2 according to Commission Directive (UE) 2019/1833 [47] and Ordinance of Polish Minister of Health [48]; # cellulolytic microorganisms [39, 51, 52, 68]

themselves, and the means of payment with which they have contact during occupational activities. The presence of both of these potential emission sources is the most likely cause of their visible quantitative advantage over other microbiota components. The percentage distribution analysis also clearly confirms that the sorted currency is an active source of microbial emission indoors. Representatives of all seven identified groups of microorganisms were found in the air of the banknote sorting rooms. In the coin sorting rooms, only yeasts were absent from the air, and no microorganisms representing non-sporing Gram-positive rods were found in the air of indoor background premises. Considering the fact that in all the studied interiors professional activities were constantly performed during the bioaerosol measurements, they both suggest that the above microbial emission sources unequivocally shape the hygienic quality of this working environment.

In turn, the atmospheric air was dominated by filamentous fungi, mainly from *Penicillium*, *Aspergillus*, *Alternaria*, *Cephalosporium*, and *Paecilomyces* genera. They accounted for 60% of the isolated microbiota. Although in the scientific literature these fungi are often mentioned as being widespread and isolated from the air and dust in the indoor environment, the main area of their existence is the outdoor environment from which their conidia can migrate to the premises, along with the air supplied by the ventilation system, employees on their bodies (e.g. hair, hands) or clothes [40, 45, 50–52], and the sorted means of payment. The quantitative and qualitative analyses of airborne microbiota revealed that the ventilation systems of the buildings were working efficiently, especially in eliminating the coarse microbial particles with larger aerodynamic diameters (such as *Alternaria*). Such systems protect the money sorting rooms from the migration of microbial pollutants with atmospheric air, and thus reduce the exposure of employees to harmful microbiological agents. On the other hand, banknotes and coins are delivered for sorting in bags made of polypropylene, linen or jute fabric. Often, the way they are transported results in the accumulation of moisture inside the transport bag, which may in turn favour the development of the mycobiota. The existence of this phenomenon is confirmed by isolation from the air (and tabletop surfaces – see below) of a few hydrophylic species (e.g. *Aspergillus fumigatus*, *Alternaria alternata*, *Trichoderma viride*, *Stachybotrys chartarum*, *Mucor plumbeus*, *Rhizopus* spp.). Moreover, it should be remembered that in the indoor environment, fungal conidia can live for a long time on equipment and different materials, including elements of heating and ventilation (air-conditioning) installations, maintaining their ability to survive even for several dozen years [49]. Filamentous fungi (mainly of *Stachybotrys*, *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, *Trichoderma*, and *Acremonium*/*Cephalosporium* genera) can also be a source of mycotoxins that are harmful to human health [50, 53]. For almost all the above listed fungi, IgE-mediated allergy has also been demonstrated and noted in the scientific literature [e.g. 50]. Therefore, when analyzing the health risks of these microorganisms in such a specific working environment, their allergenic and toxic effects should always be taken into account.

When particle-lung interactions are studied for the purpose of exposure assessment, the aerosol is most frequently characterized by its particle size distribution. From a mechanical point of view, the human respiratory tract is a structure allowing separation of inhaled aerosol particulates into fractions with different penetration abilities depending, among others, on their aerodynamic diameters. The use in this study a six-stage Andersen impactor allowed obtaining data on particle size distribution of microbial aerosols in money sorting facilities, and in the atmospheric air around them (Fig. 2). For the four studied environments, i.e. banknote and coin sorting rooms, as well as indoor and outdoor backgrounds, the analysis of variance revealed significant differences in bacterial (ANOVA: $p<0.0001$) and fungal (ANOVA: $p<0.001$) aerosol size distributions. In the case of airborne bacteria, the highest differences were noted for size distribution curves between coin sorting rooms and outdoor environment (Fisher LSD: $p<0.01$). In the case of fungal aerosol, the highest differences were visible between the banknote and coin sorting rooms. Moreover, size distribution analysis showed that bacteria in the air of the examined premises appeared mainly in the form of single cells/spores and/or their aggregates with dust particles in the aerodynamic size range of 1.1–3.3 μm, while fungi appeared mainly as single conidia in the aerodynamic size range of 2.1–3.3 μm.

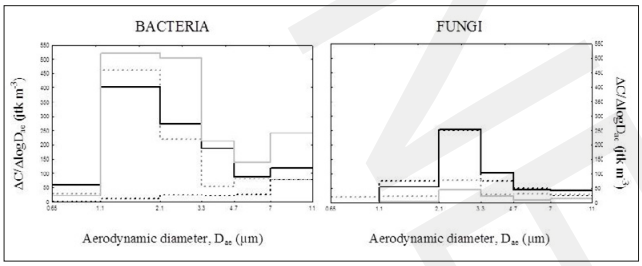

Figure 2. Size distribution of bacterial and fungal aerosols in money sorting facilities and in the indoor and outdoor backgrounds. The respective lines represent: dashed black – outdoor background, dashed grey – indoor background, solid black – banknote sorting rooms and solid grey – coin sorting rooms

Based on the above-described results, it can be concluded that in the case of bioaerosol composed of both bacteria (mainly mesophilic Gram-positive cocci) and fungi, its highest 'load' may reach into the secondary and terminal bronchi. As interactions between aerosol particulates and respiratory tract cells largely depends on the place of their deposition, both these groups of microorganisms penetrating into the lower airways may be responsible for the occurrence of adverse health effects in exposed individuals in the form of allergic inflammation. This observation is consistent with the results of qualitative analysis of the air samples which confirmed the presence of six bacterial and three fungal pathogens in the studied money sorting facilities. With the exception of *Actinomyces gerencseriae* and *Nocardia asteroides*, all other pathogens (i.e. *Streptococcus mutans*, *Arthrobacter globiformis*, *Brevibacterium linens*, *Streptomyces albus* as well as *Aspergillus* species including *A. flavus* and *A. fumigatus*) revealed the highest adverse biological activity after inhalation of dust particles and/or droplets containing them [54].

The results of the quantitative and qualitative analysis of bacteria and fungi collected from tabletop surfaces using the swab method in money sorting facilities are presented in Table 5 and Figure 1. The average concentrations (with standard deviations) of bacteria and fungi in banknote as well

Table 5. Microbial concentrations (cfu/cm²) on tabletop surfaces in money sorting facilities

Workplaces	Bacteria		Fungi	
	Average	Range	Average	Range
Banknote sorting rooms	9	0–22	7	1–20
Coin sorting rooms	5	1–12	3	2–6

Table 6. Viable fungal hygiene guide for indoor surfaces [55]

Viable surface fungal conidia concentration (cfu/cm²)	Hygiene rating
<0.5	Low
0.5–1	Normal
>1–2.5	Elevated
>2.5–12.5	Contaminated
>12.5	Extreme contamination

as coin sorting rooms were 9 (6) cfu/cm² and 7 (6) cfu/cm² as well as five (6) cfu/cm² and three (2) cfu/cm², respectively. The observed differences in microbial concentrations between studied premises were not statistically significant (*t*-test: in both cases $p>0.05$). Correlation analysis of microbial contamination levels in the air and on surfaces revealed a statistically significant relationship for fungal concentrations in banknote sorting rooms only ($r^2=0.34$; $p<0.05$). As in the case of microbial airborne contaminants, interpretation of the quantitative data for bacterial and fungal surface pollutants is hindered by lack of widely acceptable cleanliness standards and/or TLVs. Nevertheless, in the scientific literature in this field, there are standard proposals enabling hygienic condition assessment of surfaces contaminated with fungal conidia in residential or public utility premises (Tab. 6) [55]. Taking into account those levels of mycological purity for indoor surfaces, the tested tabletops in both banknote and coin sorting rooms should be treated as microbiologically contaminated.

Microbiota of the settled dust was less diverse than that of the air (Tab. 4, Fig. 1). In total on the tabletop surfaces, 28 bacterial species belonging to 13 genera and 31 fungal species belonging to 17 genera (including 28 muld species belonging to 15 genera and three yeast species belonging to two genera) were identified. Of these, six bacterial (*C. propinquum*, *B. circulans*, *B. firmus*, *B. pumilus*, *B. subtilis*, and *P. polymyxa*) and six fungal (*A. ochraceus*, *A. oryzae*, *A. terreus*, *Geotrichum* spp., *S. chartarum*, and *C. guilliermondii*) species were solely present on the tabletops and the sorted currency were probably their major source of origin. Also, among the microbiota isolated from the examined surfaces, 12 bacterial and 16 fungal species had cellulolytic properties. Moreover, the qualitative analysis of surface microbiota revealed that the microbiological contamination of tabletops in the banknote sorting rooms differed from that in coin sorting premises. In the banknote sorting rooms, among the most abundant surface contaminants were endospore forming and nonsporing Gram-positive rods as well as filamentous fungi, whereas in the coin sorting rooms, filamentous fungi and Gram-positive cocci formed 75% of surface microbiota.

Comparative analysis of percentage contributions of individual microbial groups in the air and on surfaces in the studied money sorting facilities showed that the qualitative structure of airborne microbiota differed from that observed on tabletops. It is very probable that the sedimentation process of microbial removal from the air did not play a key role in the contamination of tabletop surfaces, and the main influence on their pollution certainly had the means to contaminate through having direct contact with the tabletops during routine work activities. The microorganisms deposited on the surfaces in that way were also probably resistant to disinfectants, which may be indirectly confirmed by the widespread and numerous presence of endospor-forming Gram-positive rods and fungal conidia. Furthermore, the studied surfaces were free from the presence of Gram-negative rods. This can be explained by the low moisture level maintained as a technological requirement in money sorting premises (see below). Moreover, among the identified surface microbiota, the presence of five bacterial and one fungal pathogenic species was confirmed using molecular methods. All these strains are classified in Group 2 according to their level of risk of infection and, as such, they pose a health threat to people exposed to direct contact with or inhaling them [47, 48]. Of these, *B. subtilis* and *S. chartarum* were isolated from studied surfaces only, which once again confirmed that the sorted means of payment are able to carry numerous health important germs.

Inanimate surfaces are often perceived as an active source of infection. The studies conducted so far revealed that the spread of infectious diseases *via* means of payment is important from both the epidemiological and medical points of view [18]. The isolation of harmful microbial agents from different currencies has confirmed that they can play an important role not only in the transmission of pathogens, but also in the spread of drug-resistant microbial strains. Antimicrobial resistance is a worldwide phenomenon that causes high morbidity and mortality. The studies by Elsharief et al. [56] showed that from among the strains isolated from paper currency notes, Gram-positive cocci of *Staphylococcus* (incl. *S. aureus*) and *Streptococcus* genera revealed 100% resistance to amoxicillin, linezolid, clindamycin and erythromycin, and Gram-negative rods of *Escherichia* (incl. *E. coli*), *Pseudomonas*, *Klebsiella*, *Proteus* and *Salmonella* genera demonstrated 100% resistance to linezolid and clindamycin. This example reveals that microbial contamination of money with antibiotic-resistant strains is probably very common. Due to the high circulation of banknotes and coins within population, this phenomenon is of great importance for the control of infectious disease transmission.

Not only bacteria, but many fungal species isolated from banknotes and coins can also cause a variety of opportunistic infections. The common mould *A. niger* can cause pulmonary aspergillosis [57], other serious lung diseases, and otomycosis [58, 59]. In patients with AIDS or other immunocompromised syndromes, such infections can even lead to death [16, 60]. Some *Penicillium* species can induce infections in humans, including pneumonia [61]. *Rhizopus* species can cause mucormycosis and may be responsible for zygomycosis and eye infections [62]. *Fusarium solani* can be responsible for invasive mycoses and opportunistic infections in immunocompromised patients [63]. Also yeasts isolated from contaminated means of payment can provoke numerous adverse health effects, for example, *Candida* species can be responsible for severe endocarditis [64, 65], whereas *Cryptococcus* strains for opportunistic infection in immunocompromised patients [66].

It is known that diseases such as diphtheria, trachoma, gastroenteritis, whooping cough and diarrhea-causing

pathogens can be transmitted through banknotes and coins. It has also been reported that pathogens associated with throat infections, pneumonia or tonsillitis, peptic ulcers, urogenital tract infection, and lung abscess have spread through this route [18]. Ahmed et al. [3] also suggested that banknotes contaminated with pathogenic microbes could play a significant role in cholera, diarrhea and skin infection transmission. The data collected over the last 20 years on the contamination of coins and banknotes with microorganisms indicate that simultaneous contact with money and food can cause many health ailments and diseases [18, 19, 30]. These findings have very important epidemiological and economic implications, especially in underdeveloped and developing countries. Knowledge about the presence of pathogens on means of payment should increase the level of public awareness as this type of risk is by no means limited to the inhabitants of a given region, and may even be greater for emigrants, tourists and visitors from other countries who are typically less resistant to local pathogenic strains [18, 67].

The ranges and average values of air temperature and relative humidity (RH) in the money sorting rooms as well as in the indoor and outdoor backgrounds are shown in Table 7. All these parameters were within the ranges that are normally observed in these types of studied environments and did not significantly differ between them (ANOVA: $p>0.05$). The kind of microorganism that will colonize particular spaces or surfaces in an indoor environment depends on the physical and chemical characteristics of the construction and finishing materials used in a given building, and on the nutrients that the individual components of these materials can become for the microorganisms. Above all, however, it depends on the extent to which a given material in a given environment is able to meet the requirements of specific microorganism in terms of the amount of moisture necessary to initiate its growth and maintain subsequent development. In environmental microbiology, this value is established as 60% of relative air humidity and maintaining the room RH below this level may keep materials fairly dry in the majority of cases. In the studied sorting facilities, the measured RH values were always below 60%. Hence, as could be expected, the correlation analyses did not reveal statistically significant influences of both microclimate parameters on the microbial concentrations in the air and on tabletop surfaces in the examined money sorting premises.

Table 7. Values of temperature and relative humidity in money sorting facilities and in indoor and outdoor background air

Sampling points		Temperature (°C)		Relative humidity (%)	
		Average	Range	Average	Range
Work-places	Banknote sorting rooms	21.2	20.6–21.8	55	51–57
	Coin sorting rooms	23.1	19.4–23.8	54	50–59
Indoor background		22.2	20.1–24.2	52	48–58
Outdoor background		18.8	16.5–21.3	47	41–53

CONCLUSIONS

This study confirmed that means of payment are active sources of microbial emission in money sorting facilities. In both banknote and coin sorting rooms, a total of 48 bacterial species belonging to 20 genera and 51 fungal species belonging to 23 genera were identified in the collected air and surface samples. Among the isolated microbiota, 15 bacterial and 20 fungal species have cellulolytic properties. Taking into account the material dealt with in money sorting facilities, their ubiquity is probably related to the presence of banknotes in this specific working environment. The concentrations of bacterial and fungal aerosols did not exceed the level of 4.4×10^3 cfu/m^3 and, as such, were below the threshold limit values proposed for this type of office premises. It also confirms that the ventilation systems in these facilities worked efficiently protecting the sorting premises from the migration of microbial contaminants present in both indoor and outdoor (atmospheric) background air. On the other hand, however, the average concentrations of bacteria and fungi on tabletop surfaces in banknote and coin sorting rooms reached 9 cfu/cm^2 and 7 cfu/cm^2, respectively. Taking into account the proposed levels of mycological purity for indoor surfaces, the studied tabletops should be treated as microbiologically contaminated. Moreover, among the identified air and surface microbiota, the presence of seven bacterial and four fungal pathogens was confirmed. All these pollutants are classified in group two according to their level of risk of infection and, as such, they pose a health threat to exposed individuals.

All of the above reveals that the employees in money sorting facilities are exposed to microbial contaminants that may contribute to the development of numerous adverse health outcomes. To protect them, highly efficient hygienic measures should be introduced in this working environment to prevent both unwanted pollution and subsequent secondary emission of microbial contaminants from sorted means of payment and tabletop surfaces.

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