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FUNGAL CONTAMINATION IN POULTRY WASTE DURING THE INDUSTRIAL PROCESSING

ZANIECZYSZCZENIE MYKOLOGICZNE W PRZEROBOWEJ MASIE ODPADOWEJ Z PRZEMYSŁU DROBIARSKIEGO

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Streszczenie. Przemysł drobiarski produkuje duże ilości odpadów, które obejmują odpady stałe i ścieki. Przeprowadzono liczne badania nad zanieczyszczeniem mikrobiologicznym w różnych etapach hodowli i przetwórstwa drobiu. Niewiele jest jednak doniesień dotyczących zanieczyszczeń mykologicznych w różnych etapach zagospodarowania odpadów z przemysłu drobiarskiego. Celem badań było analiza mykologiczna różnych odpadów drobiarskich, m.in. rodzajów piór po uboju, szlamu, osadu biologicznego i kompostu. Próbkę pobierano w kilku terminach w latach 2015–2016. Izolację grzybów przeprowadzono metodą seryjnego rozcieńczenia i posiewu na podłożu RBA, uzupełnionym antybiotykiem. Identyfikację grzybów przeprowadzono mikroskopowo i genetycznie (gen RNA z rybosomalnym genem 18S). Liczba grzybów (jtk · g⁻¹ s.m.) wyizolowanych z piór kurcząt i gęsi wynosiła 10³–10⁴, z piór kaczyc i indyczych – 10⁶–10⁷, z kompostu – 10³–10⁵, ze szlamu – 10⁴, a z osadu biologicznego – 10⁴–10⁶. W wyniku przeprowadzonej analizy stwierdzono zależność między liczebnością grzybów z różnych materiałów odpadowych, pobranych w zmiennych warunkach, a miejscem ich pochodzenia.

Key words: fungi, poultry waste.

Słowa kluczowe: grzyby, odpady drobiowe.

INTRODUCTION

Poultry is one of the most intensively reared domesticated species and one of the most developed and profitable animal production industry (Al-Jamaien et al. 2013; Opara et al. 2014). The poultry industry produces large amounts of waste that includes solids and liquids. The solid waste consists of bedding materials, excreta (manure), feed, feathers, hatchery waste (empty shells, infertile eggs, dead embryos and late hatchlings), sludge, abattoir waste (offals, blood, feathers and condemned carcasses) and dead birds (Moreki and Chiripasi 2011; Adedayo 2012). All poultry wastes have to be well managed to minimize environmental hazards originated from the poultry operations. Especially, evaluation of the chicken manure is one of top priority issues for poultry production sector (Unal et al. 2015).

Poultry waste materials, including litter and dead birds, must be properly managed to ensure beneficial use and to prevent adverse effects on the environment and poultry health. Litter

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should not be reused when a disease outbreak occurs in a flock (Anne 2007). Poultry litter may contain pathogenic microorganisms (Kim et al. 2012), drug residues and hard or metallic objects that are injurious to crops, poultry, humans and other domestic animals. Poultry waste is usually a combination of poultry bird feces, urine, saw dust and remnants of animal feeds, drugs and pesticides (Asaniyan 2007; Musa et al. 2009; Wilkinson 2011; Adedayo 2012; Musa et al. 2012).

Fungal infections are common in all kinds of poultry, but are less prevalent as compared to bacterial and viral infections. Fungi are eukaryotic organisms, comprising of both yeasts and moulds. They cause significant economic losses to the poultry industry either due to their direct infectious nature or due to production of mycotoxins resulting in high morbidity and mortality, especially in young birds and cause stunted growth, diarrhea, and fatal encephalitis (Singh et al. 2012; Grenier and Applegate 2013). Seasonal variation play important role in spread of fungal infections with predominance during summer and presence of fungi in the poultry litter material during autumn make the eradication difficult (Soliman et al. 2009; Ashwathanarayana and Raja 2016). Exposure to certain fungi is known to produce adverse human health effects through three specific mechanisms the first generation of a harmful immune response such as allergy or hypersensitivity pneumonitis, second direct infection by fungal organism, and third toxic irritant effects from mold by-products such as mycotoxins (Adams et al. 1993; Bush et al. 2006). Fungi are major causes of morbidity and mortality in the world today. Yeasts and molds are found in meat and poultry products before spoilage. Mold species include *Cladosporium*, *Sporotrichum*, *Geotrichum*, *Penicillium* and *Mucor* while yeasts species include *Candida* spp., *Cryptococcus* spp. and *Rhodotorula* spp. (Dave and Ghaly 2011). Fungi are found in diverse places depending on environmental conditions and nutritional availability in the soil. Most of the fungi are saprophytic in nature, some are symbiotic and some are parasitic causing infections in plants and animals. Fungi can cause a number of different types of illness through the production of spores. The fungal species most often encountered with intoxications belong primarily to five genera: *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium*. Other genera, including *Chaetomium*, *Claviceps*, *Diplodia*, *Myrothecium*, *Phoma*, *Phomopsis*, *Pithomyces* and *Strachybotrys*, also contain mycotoxic fungi. These molds produce many different toxic compounds but not all isolates of the same species produce toxins (Cole et al. 2003; Brase et al. 2009).

MATERIAL AND METHODS

The present paper deals with various waste materials from poultry industry at different stages of their management subjected to analysis of mycological contamination (Table 1): feathers from slaughtered poultry (chicken, duck, goose, and turkey), sediment from the centrifuge, sludge from liquid waste reservoir, and poultry compost. These materials originated from three poultry slaughter houses localized in Western Poland. Samples were collected during 12 months at different time intervals. Due to restricted availability and uneven supplies, combinations of analyzed samples varied. The analysis was done in 3 replications. The RBA medium (Rose Bengal Agar) with streptomycin was used to count the moulds (Downes and Ito 2001). Cultures were incubated at 23–24°C for 4–5 days, and then grown colonies were

counted and results were expressed in cfu per 1 g of waste dry matter. Identification method of the most common units was preceded by microscopic and genetic analysis 18S ribosomal RNA gene. Achieved results were computed applying Statistica 12 software.

Table 1. Data about sampling termines, localizations and waste materials

Tabela 1. Dane ogólne dotyczące terminów poboru prób, obiektów oraz rodzaju materiału

Localization Lokalizacja	Objects (kind of waste materials) Obiekty (rodzaje materiałów odpadowych)	Date Data
1 (B)	chicken feathers – pióra kurze	30.01.2015
2 (P)	turkey feathers – pióra indycze	05.03.2015
3 (D)	duck feathers – pióra kacze	13.04.2015
	goose feathers – pióra gęsie	11.05.2015
	slime – szlam	21.05.2015
	biological sludge – osad z wirówki	20.06.2015
	compost – kompost	25.06.2015
		23.11.2015
		15.01.2016

RESULTS AND DISCUSSION

Based on achieved results it can be found that both tested materials and their origin are contaminated with microscopic fungi (Tables 2 and 3). The contamination amounted from about $160 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ to $23 \cdot 10^6 \text{ cfu} \cdot \text{g}^{-1} \text{ d.m.}$ average about $18 \cdot 10^5 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ According to many authors (Asaniyan et al. 2007; Wilkinson et al. 2011; Adedayo 2012), the poultry waste can contain pathogenic microorganisms, remains of drugs etc., which are harmful for crops, poultry, and for people and pests.

Table 2. Independence tests for variables: sampling time, object and localization

Tabela 2. Testy niezależności dla zmiennych: data pomiaru, obiekt oraz ubojnia

Variable Zmienna	Df df	Chi-square Chi-kwadrat	p-value Wartość p	G-square G-kwadrat	p-value Wartość p
Data – Data	8	22.50000	0.004069	28.82659	0.000340
Object – Obiekt	6	13.97143	0.029957	17.17288	0.008668
Localization – Ubojnia	2	20.06250	0.000044	24.52436	0.000005

Table 3. Descriptive statistics for investigated localizations (number in $\text{cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$)

Tabela 3. Statystyki opisowe dla badanych lokalizacji (liczebność w $\text{jtk} \cdot 1 \text{ g}^{-1} \text{ s.m.}$)

Localization Ubojnia	N cases N przypadków	Mean Średnia	Minimum Minimum	Maximum Maksimum	Standard deviation Odchylenie standardowe
1 (B)	8	2 928 745	159	23 138 410	8 166 392
2 (P)	2	7 919 145	2711	15 835 580	11 195 529
3 (D)	15	361 349	1857	4 457 953	1 141 652

Scatter of results related to fungal contamination of materials was not uniform. In the case of feathers, it was the largest for duck and turkey, while small for chicken and goose. Considering other materials, the centrifuge sediment was characterized by quite large result scatter (Fig. 1 and 2). When analyzing particular localizations (slaughter houses), a great differentiation of results in slaughter houses No. 2 (P) and No. 1 (B) was recorded, whereas in

slaughter house No. 3 (D), it was much lower. Results achieved in subsequent test dates were characterized by very uniform scatter, except from two last ones, when both fungal contamination level and result scatter were considerably higher (Fig. 3).

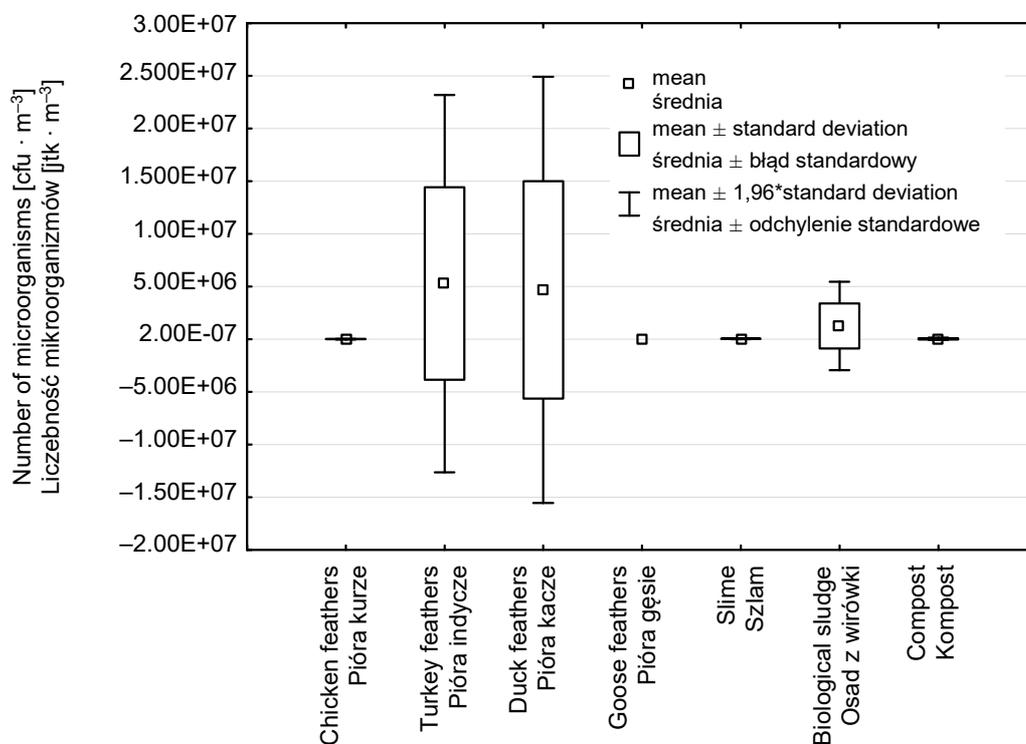


Fig. 1. Charakterystyka rozkładu liczebności grzybów w badanych odpadach
Ryc. 1. Charakterystyka rozkładu liczebności grzybów w badanych odpadach

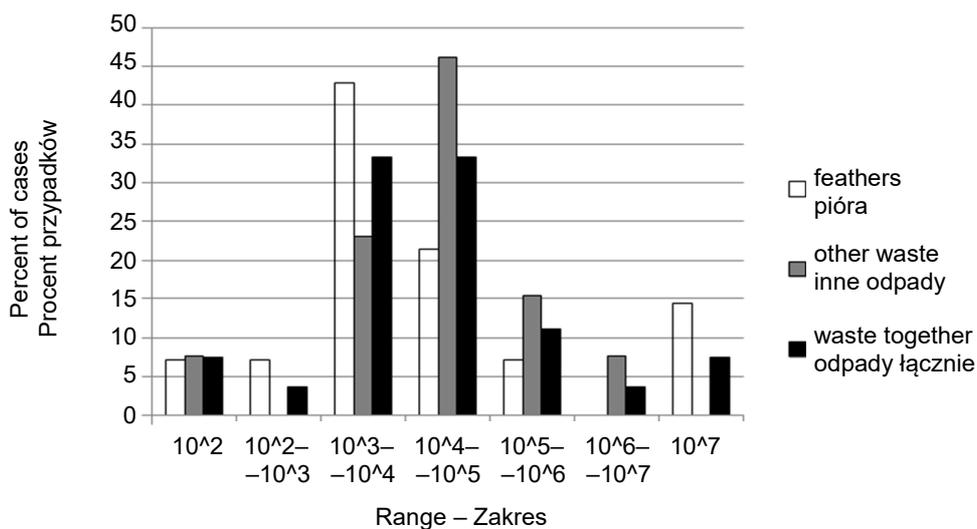


Fig. 2. Histogram for the results of measurements of the contamination of investigated materials with moulds
Ryc. 2. Histogram dla uzyskanych wyników zanieczyszczenia grzybami badanych materiałów

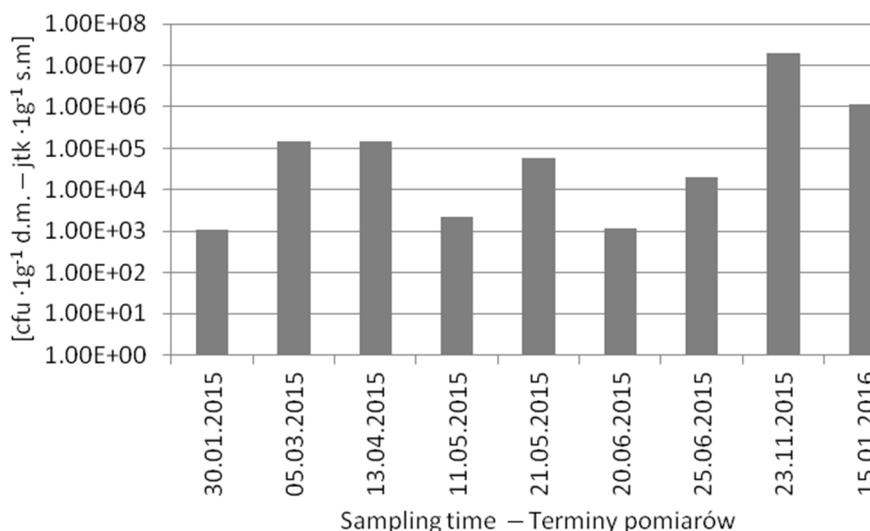


Fig. 3. Contamination of moulds samples in individual thermines
Ryc. 3. Zanieczyszczenie próbek grzybami w poszczególnych terminach

Highly significant differences in contamination degree values due to fungi were found. The independence tests also revealed the presence of significant differences between contamination values for dates, objects, and sampling localization (Tables 2 and 3).

The distribution of values of tested samples fungal contamination corresponded to Gauss curve: the largest number of measurements (20–45%) reached values 10^3 – 10^4 and 10^4 – 10^5 . Lower and higher values were recorded for much smaller number of cases (Fig. 2), which related both to contamination of feathers and other materials. However, for feathers, the largest number of results was grouped within the range of 10^3 – 10^4 , while other materials within 10^4 – 10^5 . It can be evidence that the latter were stronger contaminated with fungi. Fungal contamination is common at all poultry species, although less widespread than bacterial and viral ones. They cause remarkable economic loss in poultry industry due to their direct infectious nature or due to production of mycotoxins, which makes significant morbidity and mortality, namely at young birds, as well as growth inhibition, diarrhea, and fatal encephalitis (Singh et al. 2012; Grenier and Applegate 2013).

The largest fungal contamination was recorded for turkey feathers, average of about $8 \cdot 10^6$ cfu · 1 g⁻¹ d.m. (Fig. 4.) (from $3 \cdot 10^3$ cfu · 1 g⁻¹ d.m. to $15 \cdot 10^6$ cfu · 1 g⁻¹ d.m.). It exceeded results achieved at the initial step of measurements by almost four times.

The duck feathers contained from about 160 cfu · 1 g⁻¹ d.m. to about $23 \cdot 10^6$ cfu · 1 g⁻¹ d.m., with mean value of almost $6 \cdot 10^6$ cfu · 1 g⁻¹ d.m. Degree of the contamination decreased during the year with significant increase (about 100-fold) at the later stages.

Fungal contamination of chicken feathers was within the range from about $16 \cdot 10^2$ cfu · 1 g⁻¹ d.m. to $33 \cdot 10^3$ cfu · 1 g⁻¹ d.m., average $16 \cdot 10^3$ cfu · 1 g⁻¹ d.m., although at the initial period till March 2015, the contamination level was over one order lower than in further measurements. For goose feathers, the result was $2 \cdot 10^3$ cfu · 1 g⁻¹ d.m.

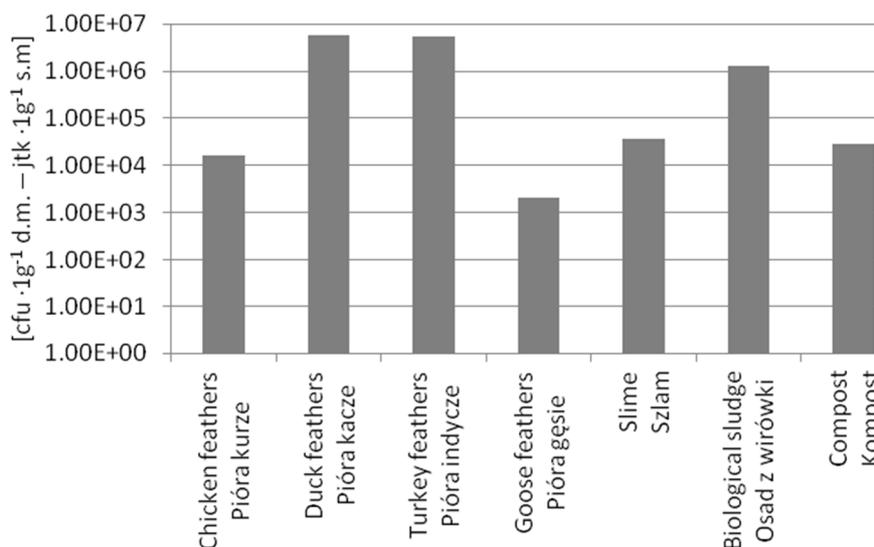


Fig. 4. Contamination of moulds samples in examined objects (materials and wastes)
Ryc. 4. Zanieczyszczenie grzybami poszczególnych obiektów (materiałów i odpadów)

Among other materials, the largest fungal contamination degree was observed in sediment from centrifuge: from $26 \cdot 10^3 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ to about $45 \cdot 10^5 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ with average value of almost $13 \cdot 10^5 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ The lowest contamination was recorded in the middle of measurement period. Sludge and compost contained much less count of fungi, respectively $23 \cdot 10^3 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ to $6 \cdot 10^4 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ (mean $37 \cdot 10^3 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$) and $2 \cdot 10^3 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ to $1 \cdot 10^5 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ (mean $38 \cdot 10^3 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$). Nowadays, fungi are one of the most important cause of worldwide morbidity and mortality. The mould species include *Cladosporium*, *Sporotrichum*, *Geotrichum*, *Penicillium* and *Mucor*, while yeast species contain *Candida spp.*, *Cryptococcus spp.*, and *Rhodotorula spp.* (Dave and Ghaly 2011). Fungi were present at various sites depending on environmental conditions. They can cause a spectrum of diseases due to spores production. Fungal species the most often found during intoxication are mainly of five genera: *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium*. Other ones, including *Chaetomium*, *Claviceps*, *Diplodia*, *Myrothecium*, *Phoma*, *Phomopsis*, *Pithomyces* and *Strachybotrys*. These forms produce various toxic compounds, but not every isolate of the same species produce toxins (Cole et al. 2003; Brase et al. 2009). The analysis most often revealed the isolation of the following moulds: *Aspergillus sp.*, *Penicillium sp.*, *Scopulariopsis sp.*, *Trichoderma sp.*, *Acremonium sp.*, *Cephalosporium sp.*, *Alternaria sp.*, *Pithomyces sp.*, *Eurotium sp.*, and yeast-like fungi: *Rhodotorula sp.*, *Candida sp.*, *Yarrowia sp.*, and *Saccharomyces sp.*

Considering average values on the measurement dates (Fig. 3), the least fungal contamination was recorded on dates 1, 4, 6, and 7, in range slightly over $1 \cdot 10^3 \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$ The largest number of fungi was isolated on dates 8 and 9 – about $2 \cdot 10^{6-7} \text{ cfu} \cdot 1 \text{ g}^{-1} \text{ d.m.}$

The change in seasons plays an important role in widespread of fungal contamination. Prevalence of infections in summer and presence of fungi in poultry litter in autumn make that eradication is difficult (Soliman et al. 2009). Exposure to some fungi is harmful for human due to three specific mechanisms of the first generation of immune response such as allergy or

pneumonia, second direct infection by fungal organism, and third toxic effect of irritant action on mold by-products such as mycotoxins (Adams et al. 1993; Bush et al. 2006).

Tested localization can be lined up referring to the increasing contamination of materials with fungi in a sequence: slaughter house No. 3 (D) ($40 \cdot 10^4$ cfu $\cdot 1$ g⁻¹ d.m.), No. 1 (B) ($3 \cdot 10^6$ cfu $\cdot 1$ g⁻¹ d.m.), No. 2 (P) ($8 \cdot 10^6$ cfu $\cdot 1$ g⁻¹ d.m.). Differences between slaughter houses result from conditions, animal slaughter technology, and waste management differences. Slaughter house No. 3 was the largest and the most modernized, while others were much smaller and more traditional.

CONCLUSIONS

1. Fungal contamination was found in all tested materials, in all slaughter houses, and on all measurement dates. Its variation was not subject to univocal dependencies during the year.
2. Scatter of results related to contamination of tested feathers was the largest for turkey and duck, and in the case of other materials, for sediment from centrifuge. The largest result scatter was recorded in slaughter houses No. 1 (B) and 2 (P), while in No. 3 (D) it was slight.
3. Distribution of achieved contamination results corresponded to normal distribution with maximum at 10^3 – 10^4 for feathers and at 10^4 – 10^5 for other materials. Feather contamination with fungi increased in the sequence: goose, chicken, duck, and turkey. For other materials, it was: compost, sludge, centrifuge sediment.
4. Contamination of various origin materials differed between slaughter houses. The largest contamination of test materials with fungi was recorded in slaughter house No. 2 (P), the smallest in No. 1 (B), while the lowest level of contamination occurred in slaughter house No. 3 (D).

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Abstract. The poultry industry produces large amounts of waste that include solid waste and waste water. Although, numerous studies have been conducted on microbial associates with various stages related to poultry and meat products processing. Only a few studies have been reported on fungal exists of poultry wastes. The aim of this study investigation of fungi associated with different poultry wastes. The sample of poultry wastes included feathers, slime, biological sludge and compost. The samples were taken at various times in peroid from 2015–2016 from different poultry slaughter houses. Isolation of fungi was performed by serial dilution and plating methods on Rose Bengal Agar (RBA), medium supplemented with antibiotic. Identification of fungi was carried out by microscopic and genetic analysis 18S ribosomal RNA gene. The number of fungi (cfu · g⁻¹ d.m.) were isolated from chicken and goose feathers 10³–10⁴, from duck and turkey feathers even 10⁶–10⁷, compost 10³–10⁵, slime – 10⁴ and biological sludge – 10⁴–10⁶. From the overall analysis, the dependence between the number of fungi and various type of samples and the season of taking the samples were found and type of poultry slaughter houses.