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Abstract

Yeasts possess a range of environmental adaptations that allow them to colonize soil and sand. They can circulate seasonally between different components of lake ecosystems, including beach sand, water, and the coastal phyllosphere. The accumulation of people on beaches promotes the development and transmission of yeasts, posing an increasing sanitary and epidemiological risk. The aim of this study was to determine the species and quantitative composition of potentially pathogenic and pathogenic yeasts for humans present in the sand of supervised and unsupervised beaches along the shores of lakes in the city of Olsztyn (north-eastern Poland).

The study material consisted of sand samples collected during two summer seasons (2019; 2020) from 12 research sites on sandy beaches of 4 lakes located within the administrative boundaries of Olsztyn. Standard isolation and identification methods used in diagnostic mycological laboratories were applied.

A total of 259 yeast isolates (264, counting species in two-species isolates separately) belonging to 62 species representing 47 genera were obtained during the study. Among all the isolates, 5 were identified as mixed (two-species). Eight isolated species were classified into biosafety level 2 (BSL-2) and risk group 2 (RG-2). The highest average number of viable yeast cells was found in sand samples collected in July 2019 (5,56 x 10^2 CFU/g), August, and September 2020 (1,03 x 10^3 CFU/g and 1,94 x 10^3 CFU/g, respectively). The lowest concentrations were in samples collected in April, September, and October 2019, and October 2020 (1,48 x 10^2 CFU/g, 1,47 x 10^2 CFU/g, 1,40 x 10^2 CFU/g, and 1,40 x 10^2 CFU/g, respectively).

The results indicate sand contamination with yeasts that may pose etiological factors for human mycoses. In light of these findings, continuous sanitary-epidemiological monitoring of beach sand and further studies on its mycological cleanliness are warranted, along with actions leading to appropriate legal regulations.

Keywords: microfungi; yeasts; sand; beaches; lake ecosystems; sand pollution

Abbreviations: BSL – biosafety level; CFU – colony-forming unit; PCR – polymerase chain reaction; RBC – Rose Bengal Chloramphenicol Agar, RG – risk group

1. Introduction

Yeasts exhibit high physiological and ecological plasticity, and show a wide tolerance to various environmental factors such as temperature, UV radiation, light, humidity, salt content, and conditions of limited availability of nutrients and water (Buczyńska et al. 2007; Krajewska-Kułak et al. 2010; Ejdys et al. 2013; Tedersoo et al. 2014; Wrzosek et al. 2017; Kowalski and Pastuszka 2018; Yurkov 2018; Bałabański and Biedunkiewicz 2023). These properties have allowed microfungi to achieve ecological success and colonize most ecosystems of the biosphere, including water and soil. Studies have demonstrated the presence of yeasts in a variety of environments, including marine, deep-sea, and freshwater reservoirs, aquatic ecotone habitats, ontocenoses of aquatic organisms, the surfaces of coastal phyllosphere, bottom sediment layers of water bodies, as well as sand from beaches and coastal areas (Nagahama 2006; Biedunkiewicz and Góralska 2016; Libkind et al. 2017; Biedunkiewicz et al. 2020; Brandão et al. 2020, 2021; Monapathi et al. 2020; Gouka et al. 2022; Fedorova and Kurakov 2023; Sultana et al. 2024). Yeasts ability to thrive in such a broad range of habitats, from extreme marine conditions to terrestrial and freshwater ecosystems, highlights their versatile metabolic pathways and resilience to fluctuating environmental factors (Tedersoo et al. 2014; Wrzosek et al. 2017; Yurkov 2018; Bałabański and Biedunkiewicz 2023).

Yeasts can not only inhabit but also circulate between different components of lake ecosystems, including beach sand, water, and coastal phyllosphere (Fig. 1). They migrate from soil or sand into water through surface and subsurface runoff, temporarily settling on coastal phyllosphere, which acts as a natural filter for the water body (Fig. 1). Humans, as beach and swimming area users, can serve as temporary vectors for the transmission of microfungi, transferring allochthonous species for which lake ecosystem components are not natural reservoirs (Botha 2011; Kurtzman et al. 2011; Biedunkiewicz et al. 2013, 2020; Biedunkiewicz and Góralska 2016; Nowacka et al. 2018; Yurkov 2018).

Soil and beach sand, in particular, represent environments of interest. Yeasts are a significant component of the taxonomic structure of microbiological soil biomass. Their adaptive capabilities enable them to thrive in soil ecosystems, which includes sand, where yeasts can adhere to granules in suspension or as biofilms (Schoeman et al. 2009; Botha 2011; Maciel et al. 2019; Brandão et al. 2021).

The yeast nutrition method based on primary osmotrophy is a crucial factor in shaping the above-mentioned adaptive abilities. They secrete adaptive exoenzymes into the environment to break down nutritional substrates, with the resulting products then being absorbed into the cells via osmosis. When even small amounts of a new substrate appear or the proportions of existing ones change, yeasts stimulate their enzymatic apparatus to produce new exoenzymes. These properties make microfungi, including yeasts, key players in essential ecological processes such as the biogeochemical cycling of elements in nature (Walker et al. 2016; Wrzosek et al. 2017; Dynowska 2018).

A negative aspect of such advanced adaptive capabilities is the ability to cause diseases in humans. Research shows a continuous increase in the occurrence of yeasts in the human body, including species not previously associated with ontocenoses of the human body (Dynowska et al. 2001; Kurnatowska and Kurnatowski 2018; Bałabański and Biedunkiewicz 2023; Gregorczyk-Maga et al. 2023; Menu et al. 2023).

Mycological studies of lake ecosystems involve determining the species, taxonomic, and quantitative structure of microfungi and examining their biochemical and ecological activities. These parameters provide an overview of the sanitary and ecological condition of the water reservoir and the processes occurring within it, which play an important role in analyzing the dynamics of these ecosystems. Detailed analysis of these factors allows for the assessment of the sanitary-epidemiological status of the studied environments, related risks, and the development of rational water management plans (Dynowska and Biedunkiewicz 2013; Biedunkiewicz et al. 2020).

The aim of the study was to determine the species and quantitative composition of the population of potentially pathogenic and pathogenic yeasts to humans present in the sand of supervised and unsupervised beaches along the shores of lakes in the city of Olsztyn (north-eastern Poland)

2. Materials and Methods

2.1. Research area and material

The research material consisted of yeasts isolated from sand during two summer seasons (April to October 2019 and 2020) from eight research sites located on supervised and unsupervised sandy beaches along the shores of lakes within the administrative borders of Olsztyn (northern Poland, Europe) (Fig. 2). Four lakes were selected for the study: Kortowskie, Skanda, Tyrsko, and Ukiel, whose bathing waters undergo routine bacteriological testing by authorized state institutions (Provincial Sanitary and Epidemiological Station and County Sanitary and Epidemiological Station). Four of the beaches included in the study are supervised by authorized municipal institutions.

2.2. Sampling

Sampling sites were designated at the midpoint of the shoreline of each beach, 3 meters from the water's edge. Fifty-gram sand samples were collected from two depths (10 cm and 50 cm) using a soil auger (Fiskars) sterilized with a 70% alcohol solution. For sample unification, a 1m x 1m square was marked at the designated location, and 5 soil cores were taken (one from each corner of the square and one from the intersection of the diagonals). From each core, 10 grams of soil were collected into a sterile ziplock bag, sealed, and mixed to obtain a unified 50-gram sample. Samples were collected during the first week of each month of the research season, twice a day (in the morning—8:00-10:00 a.m., and in the evening—8:00-10:00 p.m.). During two research seasons, a total of 448 50-gram sand samples were collected from 8 study sites. During sample collection, air temperature and humidity were measured, and the UV index was recorded (Tab. 1). Until yeast isolation, the collected sand samples were stored under refrigeration (4°C) (Wójcik et al. 2013).

Table 1. Values if meteorological parameters during sand sampling

SAMPLING PERIOD	TEMPERATURE		AVERAGE TEMPERATURE	AIR HUMIDITY		UV INDEX
	MORNING	EVENING		MORNING	EVENING	
APRIL / 2019	6°C	14°C	D 12°C N 5°C	36%	32%	3/10
MAY / 2019	8°C	15°C	D 15°C N 8°C	73%	29%	4/10
JUNE / 2019	17°C	25°C	D 25°C N 15°C	64%	45%	7/10

JULY / 2019	20°C	21°C	D 24°C N 15°C	75%	70%	6/10
AUGUST / 2019	16°C	19°C	D 22°C N 14°C	85%	51%	5/10
SEPTEMBER / 2019	13°C	16°C	D 18°C N 11°C	75%	51%	4/10
OCTOBER / 2019	12°C	9°C	D 13°C N 7°C	91%	91%	2/10
APRIL / 2020	4°C	12°C	D 13°C N 5°C	69%	29%	3/10
MAY / 2020	6°C	6°C	D 17°C N 9°C	93%	94%	4/10
JUNE / 2020	12°C	18°C	D 21°C N 12°C	64%	42%	6/10
JULY / 2020	19°C	23°C	D 22°C N 14°C	85%	59%	6/10
AUGUST / 2020	19°C	24°C	D 23°C N 15°C	39%	39%	5/10
SEPTEMBER / 2020	13°C	16°C	D 18°C N 11°C	92%	90%	0/10
OCTOBER / 2020	11°C	8°C	D 13°C N 6°C	88%	88%	2/10

D – average temperature of the month during the day
N – average temperature of the month at night

2.3. Yeast isolation and cultivation

The yeast isolation procedure was carried out within 48 hours of sand sample collection. Yeasts were isolated from the sand samples using a modification of methods described in previous studies (Wójcik et al. 2013, 2016). From the 50-gram sand samples, a 10-gram sand samples were placed in 90 ml of sterile tap water (1:10 dilution) and shaken for 15 minutes. Yeasts were isolated by surface inoculation of 0.1 ml of the shaken suspension on Bengal Rose Agar with chloramphenicol (RBC) (Wójcik et al. 2013, 2016; Echevarría and Iqbal 2021). Each inoculation on the RBC medium was performed in triplicate. Given that 448 sand samples were tested, the total number of inoculations was 1344. In pilot studies, the tested media included RBC agar, Sabouraud agar with chloramphenicol, PDA (potato dextrose agar), and Czapek-Dox agar. The highest efficiency in yeast isolation from the studied sites was achieved using RBC medium. This medium was selected for further research due to the selective properties of Rose Bengal. Pilot studies demonstrated that RBC medium effectively inhibits the growth of filamentous fungi, particularly rapidly growing species such as *Rhizopus* and *Mucor*, thereby facilitating the growth and isolation of yeasts. Inoculated media were incubated at 25°C for 48-72 hours. In pilot studies conducted to validate the method, no growth was obtained at 37°C, so a 25°C incubation temperature was adopted for effective isolation in further studies. After incubation, macroscopic and microscopic evaluations of the colonies were conducted to differentiate yeast colonies from bacterial and filamentous fungal colonies. The yeast colonies were counted and passaged onto Sabouraud agar slants with chloramphenicol and incubated at 25°C for 48 hours. After incubation the cultures were stored under refrigeration (4°C) for further studies.

2.4. Yeast count assessment

To assess yeast population density in the sand, the plate technique using solid media was employed (Wójcik et al. 2013). According to the method, the number of viable cells in the sample is equal to the number of observed colonies. The results were calculated as colony-forming units (CFU) per 1 g of dry soil mass.

2.5. Yeast identification

A macromorphological description of the verified yeast colonies was made, including features such as shape, color, consistency, surface prominence, edge structure, transparency, smell, and growth into the medium. The ability of the isolated yeasts to ferment a basic carbohydrate series (glucose, galactose, maltose, sucrose, lactose) was tested using a liquid medium for carbohydrate zymogram (tested sugar 20 g; peptone 0.5 g; distilled water 1000 ml; bromothymol blue with NaOH to achieve a blue color). Suspensions of the colonies of the tested strains were prepared in sterile tap water with turbidity of 3° on the McFarland scale. A 40 µl drop of the suspension was added to the zymogram medium tubes and incubated at 25°C for 48 hours. Fermentation ability was indicated by a change in the color of the solution from blue to yellow (lowering of the medium's pH due to fermentation) and/or medium turbidity (increased yeast biomass).

To analyze micromorphological features, microcultures were prepared on Nickerson's agar (Dynowska and Ejdys 2011; Dynowska and Biedunkiewicz 2013; Kulesza et al. 2021; Bałabański and Biedunkiewicz 2023). Microcultures were set up in sterile moist chambers by passaging colony inoculum onto the surface of Nickerson's agar, followed by covering it with 20 µl of rabbit serum solution with bacteriological broth (1:1). The inoculum was then covered with a sterile coverslip. To maintain appropriate humidity, 1 ml of sterile distilled water was placed at the bottom of the chamber. The microcultures were incubated at 25°C for 144 hours to reveal diagnostically significant features, such as the size, shape, location, and formation of blastospores, the ability to produce chlamydospores, pseudohyphae, and true hyphae, as well as their size, shape, and arrangement (Dynowska and Ejdys 2011; Biedunkiewicz and Góralska 2016; Biedunkiewicz et al. 2020; Kulesza et al. 2021; Bałabański and Biedunkiewicz 2023). Microscopic observations of the microcultures were made at 48, 72, and 144 hours of incubation, with photographic documentation at each stage..

For strains requiring additional data for identification, Schaeffer-Fulton staining was performed to demonstrate the presence of asci with ascospores and to classify the strain as either ascomycetous or non-ascospore-forming yeasts (Ergül and Çalişkan 2018).

Final species identification and classification of strains into biosafety levels (BSL) and risk groups (RG) were performed using diagnostic keys and data from various sources, including the MycoBank database (de Hoog et al. 2000, 2019a, b; Kurtzman et al. 2011). .Due to the high species diversity, multiple references were consulted to ensure accurate classification. It is important to note that BSL and RG classifications can differ between countries and are subject to change as new data and regulations emerge.

To confirm identifications made using phenotypic methods (taking into account both macro- and microcultures), genomic DNA was isolated using the Genomic Mini AX Yeast Spin kit. Based on the convergence and overlap of phenotypic traits and the results of selected biochemical analyses, it was decided to send 49 of the most representative samples (the most frequently occurring species identified by phenotypic methods) for molecular analysis in order to avoid sending multiple strains of the same species for testing. PCR (polymerase chain

reaction) was performed, followed by purification and sequencing of the ITS-1 and ITS-4 regions of DNA for selected yeast isolates. The resulting nucleotide sequences were compared with the GenBank database using the Nucleotide BLAST tool. The sequences used for identification have been deposited in the GenBank database in the accession number range PQ882533:PQ882625.

2.6. Statistical analysis

Statistical analysis was performed using STATISTICA 13.3 software (StatSoft). The following tests were used in the analysis: nonparametric Mann-Whitney U test, nonparametric Kruskal-Wallis H test, one-way ANOVA (analysis of variance), Tukey's b test, and Spearman's rank correlation coefficient. For all tests, the significance level was set at $p<0.05$.

3. Results

During the study, a total of 259 yeast isolates were obtained, representing 62 species from 47 genera (Tab. 2). Thirty-two of the identified genera belonged to ascomycetes (Ascomycota, 68.1%), and the remaining 15 to basidiomycetes (Basidiomycota, 31.9%) (Tab. 2). Among all the isolates, five were two-species isolates (19.3% of all isolates) (Tab. 2, Tab. 3). Species from two-species isolates were counted separately in both quantitative and statistical analyses (Tab. 2, Tab. 3). Of the 62 species, 8 were classified as BSL-2 and RG-2 (12.9%) (Tab. 2). Preliminary macroscopic selection, including the macromorphological features of the colonies, indicated the need to distinguish different isolates, which were later classified as the same species during further diagnostic stages.

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184 Table 2. List of species isolated in the study, including division, BSL and RG indicator values, and the number of isolates obtained, categorized by research
185 parameters

ON.	GATUNEK	DV	BSL	RG	SUM	M	E	d10	d50	S	US	s19	s20	LK	LS	LT	LU
1	*Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud 1918	A	1	1	**5**	4	1	2	3	1	4	1	4	1	0	3	1
2	*Sporobolomyces xanthus* (Nakase, G. Okada & Sugiy.) Boekhout 1991	B	1	1	**1**	1	0	1	0	0	1	0	1	0	0	0	1
3	*Barnettozyma californica* (Lodder) Kurtzman, Robnett & Bas.-Powers 2008	A	1	1	**12**	7	5	11	1	8	4	8	4	2	0	1	9
4	***Candida albicans** (C.P. Robin) Berkhout 1923	A	**2**	**2**	**1**	1	0	1	0	1	0	0	1	0	1	0	0
5	***Nakaseomyces glabratus** (H.W. Anderson) Sugita & M. Takash 2022	A	**2**	**2**	**12**	6	6	8	4	7	5	3	9	0	5	1	6
6	*Pichia pseudolambica* (M.T. Sm. & Poot) H.Y. Zhu, X.Z. Liu & F.Y. Bai 2024	A	1	1	**5**	4	1	3	2	0	5	1	4	0	3	0	2
7	*Citeromyces matritensis* (Santa María) Santa María 1957	A	1	1	**8**	5	3	8	0	6	2	2	6	2	5	0	1
8	***Clavispora lusitaniae** Rodr. Mir. 1979 (anamorfa*: Candida lusitaniae*)	A	**2**	**2**	**1**	1	0	0	1	1	0	0	1	0	0	0	1
9	*Cryptococcus amylolentus* (Van der Walt, D.B. Scott & Klift) Golubev 1981	B	1	1	**4**	1	3	3	1	0	4	0	4	3	0	1	0
10	*Cryptococcus uniguttulatus* (Wolfram & Zach) Phaff & Fell 1970	B	1	1	**1**	1	0	1	0	0	1	0	1	0	1	0	0
11	***Cutaneotrichosporon jirovecii** (Frágner) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout 2015	B	**2**	**2**	**16**	8	8	12	4	4	12	15	1	4	6	1	5
12	***Cutaneotrichosporon moniliiforme** (E. Guého & M.T. Sm.) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout 2015	B	**2**	**2**	**28**	18	10	17	11	6	22	25	3	8	9	3	8
13	*Cyniclomyces guttulatus* (C.P. Robin) Van der Walt & D.B. Scott 1971	A	1	1	**2**	2	0	0	2	1	1	0	2	0	0	1	1
14	***Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij 1984	A	1	1	**24**	7	17	10	14	13	11	5	19	4	7	2	11
15	***Geotrichum albidum* (Lagerh.) H.Y. Zhu, X.Z. Liu & F.Y. Bai 2024	A	1	1	**3**	2	1	3	0	2	1	0	3	1	2	0	0
16	*Geotrichum galactomycetum* H.Y. Zhu, X.Z. Liu & F.Y. Bai 2024	A	1	1	**2**	1	1	1	1	2	0	0	2	0	1	0	1
17	*Dothiora sorbi* (Wahlenb.) Fuckel 1870	A	1	1	**1**	0	1	0	1	1	0	0	1	0	0	0	1
18	***Exophiala bergeri** Haase & de Hoog 1999	A	**2**	**2**	**1**	0	1	0	1	0	1	0	1	0	0	1	0
19	***Exophiala castellanii** Iwatsu, Nishim. & Miyaji 1984	A	**2**	**2**	**2**	1	1	2	0	0	2	0	2	0	0	2	0
20	***Exophiala jeanselmei** (Langeron) McGinnis & A.A. Padhye 1977	A	**2**	**2**	**3**	3	0	0	3	3	0	0	3	0	3	0	0
21	*Hanseniaspora osmophila* (Niehaus) Phaff, M.W. Mill. & Shifrine 1956	A	1	1	**1**	1	0	1	0	0	1	1	0	0	0	1	0
22	*Isabelozyma rhagii* (Diddens & Lodder) Q.M. Wang, Yurkov, Boekhout & F.Y. Bai 2024	A	1	1	**1**	1	0	1	0	0	1	1	0	1	0	0	0
23	*Komagataella pastoris* (Guilliерm.) Y. Yamada, M. Matsuda, K. Maeda & Mikata 1995	A	1	1	**3**	2	1	2	1	2	1	1	2	0	1	0	2
24	*Kondoa malvinella* (Fell & I.L. Hunter) Y. Yamada, Nakagawa & I. Banno 1989	B	1	1	**4**	2	2	2	2	2	2	2	2	0	2	2	0
25	*Kregervanrija fluxuum* (Phaff & E.P. Knapp) Kurtzman 2006 (anamorfa: *Candida vini*)	A	1	1	**1**	1	0	1	0	1	0	0	1	0	1	0	0
26	*Leucosporidium scottii* Fell, Statzell, I.L. Hunter & Phaff 1970	B	1	1	**1**	0	1	1	0	0	1	0	1	0	0	0	1
27	*Cryptococcus lipoferus* (Den Dooren) C.E. Skinner 1970	A	1	1	**2**	2	0	2	0	2	0	0	2	0	0	0	2
28	*Lodderomyces elongisporus* (Recca & Mrak) Van der Walt 1971	A	1	1	**1**	0	1	1	0	1	0	0	1	0	0	0	1
29	*Metschnikowia pulcherrima* Pitt & M.W. Mill. 1968	A	1	1	**1**	1	0	1	0	0	1	1	0	0	1	0	0
30	*Moniliella spathulata* (de Hoog) C.A. Rosa & Lachance 2009	B	1	1	**2**	1	1	2	0	0	2	0	2	2	0	0	0
31	*Mycogloea nipponica* Bandoni 1998	B	1	1	**6**	2	4	4	2	4	2	0	6	1	3	0	2
32	***Nadsonia commutata* Golubev 1973	A	1	1	**3**	3	0	1	2	1	2	2	1	0	0	0	3
33	*Nadsonia fulvescens* var. *elongata* (Konok.) Golubev & M.T. Sm. 1989	A	1	1	**5**	3	2	3	2	0	5	4	1	3	2	0	0
34	*Naganishia albida* (Saito) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout 2015	B	1	1	**3**	1	2	3	0	2	1	1	2	0	1	0	2
35	*Ogataea angusta* (Teun., H.H. Hall & Wick.) S.O. Suh & J.J. Zhou 2010	A	1	1	**2**	1	1	2	0	0	2	2	0	2	0	0	0
36	*Ogataea minuta* (Wick.) Y. Yamada, K. Maeda & Mikata 1994	A	1	1	**1**	0	1	1	0	0	1	0	1	0	1	0	0

37	*Oosporidium margaritiferum* Stautz 1931	A	1	1	**6**	5	1	3	3	1	5	0	6	2	3	0	1
38	*Papiliotrema laurentii* (Kuff.) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout 2015	B	1	1	**4**	3	1	3	1	2	2	0	4	0	2	0	2
39	*Papiliotrema pseudoalba* (Nakase & M. Suzuki) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout 2015)	B	1	1	**3**	3	0	0	3	0	3	0	3	3	0	0	0
40	***Pichia fermentans* Lodder 1932	A	1	1	**1**	0	1	1	0	1	0	1	0	0	0	0	1
41	*Pichia membranifaciens* (E.C. Hansen) E.C. Hansen 1904	A	1	1	**6**	4	2	5	1	2	4	4	2	0	5	0	1
42	*Pichia terricola* Van der Walt 1957	A	1	1	**1**	0	1	1	0	1	0	0	1	0	0	0	1
43	*Rhodotorula diobovata* (S.Y. Newell & I.L. Hunter) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout 2015	B	1	1	**1**	1	0	1	0	0	1	0	1	1	0	0	0
44	*Saccharomyces bayanus* Sacc. 1895	A	1	1	**3**	1	2	0	3	2	1	0	3	1	2	0	0
45	*Saccharomyces cerevisiae* (Desm.) Meyen 1838	A	1	1	**5**	1	4	4	1	1	4	1	4	0	3	1	1
46	*Saccharomyces mikatae* G.I. Naumov, S.A. James, E.S. Naumova, E.J. Louis & I.N. Roberts 2000	A	1	1	**1**	1	0	0	1	0	1	0	1	0	0	1	0
47	***Saccharomycodes ludwigii* (E.C. Hansen) E.C. Hansen 1904	A	1	1	**3**	2	1	3	0	1	2	1	2	1	1	1	0
48	*Saitozyma podzolica* (Babeva & Reshetova) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout 2015	B	1	1	**3**	2	1	1	2	2	1	1	2	0	1	0	2
49	*Octosporomyces octosporus* (Beij.) Kudryavtsev 1960	A	1	1	**1**	0	1	1	0	0	1	1	0	1	0	0	0
50	*Schizosaccharomyces pombe* Lindner 1893	A	1	1	**1**	1	0	1	0	0	1	1	0	0	0	1	0
51	*Schwanniomyces capriottii* M. Suzuki & Kurtzman 2010	A	1	1	**5**	4	1	3	2	2	3	5	0	2	0	1	2
52	*Schwanniomyces occidentalis* Klöcker 1909	A	1	1	**2**	0	2	2	0	0	2	2	0	2	0	0	0
53	***Schwanniomyces polymorphus (Klöcker)* M. Suzuki & Kurtzman 2010	A	1	1	**8**	4	4	5	3	2	6	3	5	0	4	2	2
54	*Schwanniomyces vanrijiae* (Van der Walt & Tscheuschner) M. Suzuki & Kurtzman 2010	A	1	1	**1**	0	1	1	0	0	1	1	0	1	0	0	0
55	*Solicoccozyma aeria* (Saito) Yurkov 2015	B	1	1	**11**	7	4	10	1	3	8	3	8	7	1	0	3
56	*Sydowia polyspora* (Bref. & Tavel) E. Müll. 1953	A	1	1	**1**	0	1	1	0	1	0	0	1	0	1	0	0
57	*Tausonia pullulans* (Lindner) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout 2015	B	1	1	**6**	2	4	5	1	1	5	6	0	2	2	0	2
58	*Thelebolus globosus* Brumm. & de Hoog 2005	A	1	1	**2**	2	0	0	2	0	2	0	2	2	0	0	0
59	*Torulaspora globosa* (Klöcker) Van der Walt & Johannsen 1975	A	1	1	**4**	2	2	4	0	1	3	2	2	0	3	0	1
60	*Vanderwaltozyma polyspora* (Van der Walt) Kurtzman 2003	A	1	1	**5**	3	2	3	2	0	5	3	2	1	3	1	0
61	*Vanrija humicola* (Dasz.) R.T. Moore 1980	B	1	1	**1**	0	1	0	1	0	1	1	0	1	0	0	0
62	*Wickerhamomyces anomalus* (E.C. Hansen) Kurtzman, Robnett & Bas.-Powers 2008 (anamorfa: *Candida peliculosa*)	A	1	1	**1**	0	1	1	0	1	0	0	1	0	0	0	1
63	*Bullera* sp.	B	1	1	**2**	1	1	1	1	2	0	0	2	0	0	0	2
64	**Cryptococcus* sp.	B	1/2	1/2	**5**	3	2	3	2	2	3	3	2	2	1	0	2
65	*Dipodascus* sp.	A	1	1	**1**	1	0	1	0	0	1	0	1	0	0	0	1
66	*Kluyveromyces* sp.	A	1	1	**1**	0	1	0	1	1	0	1	0	0	0	0	1
	Sum*	**-**	**-**	**-**	**264**	147	117	175	89	100	164	115	149	63	87	27	87

Candida albicans – species classified to biosafety level 2 (BSL-2)
****Saccharomycodes ludwigii* –** species included in two-species isolates
Cryptococcus* sp. **– the BSL index value varies depending on the species
SUM – total number of isolates of a given species
Sum* – total number of isolates (species of two-species isolates were counted separately and included in the total sum of isolates)
DV – division (A – *Ascomycota;* B - *Basidiomycota)*
M – number of isolates obtained in the morning

E – number of isolates obtained in the evening
d10 – number of isolates obtained from sand collected from a depth of 10 cm
d50 – number of isolates obtained from sand collected from a depth of 50 cm
S – number of isolates obtained from the sand of supervised beaches
US – number of isolates obtained from the sand of unsupervised beaches
s19 – number of isolates obtained in the 2019 research season
s20 – number of isolates obtained in the 2020 research season
LK - number of isolates obtained from the beach of lake Kortowskie
LS - number of isolates obtained from the beaches of lake Skanda
LT - number of isolates obtained from the beach of lake Tyrsko
LU - number of isolates obtained from the beach of lake Ukiel

Table 2. List of two-species isolates

ON.	SPECIES	RESEARCH SEASON	LAKE
1	*Nakaseomyces glabratus* (H.W. Anderson) Sugita & M. Takash 2022 *Pichia fermentans* Lodder 1932	2019	Ukiel
2	*Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij 1984 *Nadsonia commutata* Golubev 1973	2019	Ukiel
3	*Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij 1984 *Nadsonia commutata* Golubev 1973	2019	Ukiel
4	*Candida albicans* (C.P. Robin) Berkhout 1923 *Geotrichum albidum* (Lagerh.) H.Y. Zhu, X.Z. Liu & F.Y. Bai 2024	2020	Skanda
5	*Saccharomycodes ludwigii* (E.C. Hansen) E.C. Hansen 1904 *Schwanniomyces polymorphus* (Klöcker) M. Suzuki & Kurtzman 2010	2020	Tyrsko

Analysis of individual research seasons showed differences in the number of isolates obtained from the beaches. Overall, more isolates were obtained in the second research season (2020; 149 isolates, including two two-species isolates), while 86 fewer isolates were obtained in the first research season (2019; 63 isolates, including three two-species isolates) (Tab. 2, Tab. 3). The difference in the number of isolates was not statistically significant (p=0.0927).

In the total number of isolates, those obtained from sand in the morning hours predominated (147 isolates, 55.7% of all isolates). Fewer yeasts were isolated from sand collected in the evening (117 isolates, 44.3% of all isolates) (Tab. 2). No statistically significant differences were found in the number of isolates obtained in the morning and evening (p=0.7955).

Larger differences were observed in the case of sand samples collected from different depths (Tab. 2). In the total pool of isolates, yeasts collected from a depth of 10 cm clearly dominated (175 isolates, 66.3% of all isolates). Fewer yeasts were isolated from soil at a depth of 50 cm, below the mixing layer of the sand (89 isolates, 33.7% of all isolates) (Tab. 2). These differences, however, were not statistically significant (p=0.0927).

From the beaches supervised by authorized municipal institutions, 100 isolates were obtained (37.9% of all isolates), while 164 isolates were obtained from unsupervised beaches (62.1% of all isolates) (Tab. 2). These differences were not statistically significant (p=0.2131).

Among the yeasts isolated during the study, the most frequently represented species were: *Barnettozyma californica*, *Nakasemomyces glabratatus, Cutaneotrichosporon moniliiforme, C. jirovecii*, *Debaryomyces hansenii*, and *Solicoccozyma aeria*. Other species occurred in smaller numbers (8 isolates or less) (Tab. 2, Fig. 3).

In the 2019 season, the most frequently isolated species were: *B. californica*, *Cutaneotrochosporon jirovecii*, *C. moniliiforme*, *Schwanniomyces capriottii*, and *Tausonia pullulans* (Tab. 2) Among the species isolated in the 2020 season, *D. hansenii*, *Mycogloea nipponica*, *N. glabratatus*, and *S. aeria* dominated (Tab. 2). Twenty-one species and five strains identified only to genus (1 genus) were isolated in both research seasons, representing 33.3% of the species and genera pool obtained during the study. Twelve species and one strain identified only to genus (1 genus) were noted exclusively in the 2019 season (19.7% of the total species and genera pool). Twenty-nine species and four strains identified only to genus (2 genera) were found only in the 2020 season (46.9% of the total species and genera pool) (Tab. 2).

From morning-collected sand samples, the most frequently isolated species were: *B. californica*, *Cutaneotrichosporon jirovecii*, *C. moniliiforme*, *D. hansenii* and *S. aeria* (Tab. 2). In the evening-collected sand, the most common species were: *C. glabrata*, *Cutaneotrichosporon jirovecii*, *C. moniliiforme* and *D. hansenii* (Tab. 2). Thirty-two species and nine strains identified to genus (2 genera) were isolated both in the morning and evening, representing 51.5% of the total species and genera pool detected during the study (Tab. 2). Seventeen species and one strain identified to genus (1 genus) were found only in the morning samples (27.3% of the total species and genera pool). Thirteen species and one genus were found only in evening samples (21.2% of the total species and genera pool) (Tab. 2).

From a depth of 10 cm, the most frequently isolated species were: *B. californica*, *Citeromyces matritensis*, *Cutaneotrichosporon jirovecii*, *C. moniliiforme*, *D. hansenii*, *N. glabratus* and *S. aeria* (Tab. 2). Among species isolated from a depth of 50 cm, *N. glabratus*, *C. moniliiforme*, and *D. hansenii* dominated (Tab. 2). Twenty-four species and seven strains identified only to genus (2 genera) were isolated from both depths (10 cm and 50 cm), representing 39.4% of the total species and genera pool (Tab. 2). Twenty-eight species and one genus were found only in the 10 cm deep samples (43.9% of the total species and genera pool). Ten species and one genus were isolated only from the 50 cm deep samples (16.6% of the total species and genera pool) (Tab. 2).

In the sand from supervised beaches, the most frequently isolated species were: *B. californica*, *N. glabratus*, *C. moniliiforme* and *D. hansenii* (Tab. 2). From the sand of unsupervised beaches, the most common species were: *Cutaneotrichosporon jirovecii*, *C. moniliiforme*, *D. hansenii* and *S. aeria* (Tab. 2). Twenty-six species and one genus were isolated from both types of beaches (supervised and unsupervised), representing 40.9% of the total species and genera pool detected during the study (Tab. 2). Twelve species and two genera were noted only in samples collected from supervised public beaches (21.2% of the total species and genera pool). Twenty-four species and one genus (strain identified only to genus) were found only in the sand from unsupervised beaches (37.8% of the total species and genera pool).

During the two-year study, the most isolates were obtained from the beaches of Lake Skanda (87 isolates; 32.95% of all isolates) and Lake Ukiel (87 isolates; 32.95% of all isolates). From Lake Kortowskie, 63 isolates were recorded (23.9% of all isolates). The fewest isolates were from Lake Tyrsko (27 isolates; 10.2% of all isolates) (Tab. 2).

Taking into account the strains identified to the species level, the greatest taxonomic diversity was observed in the case of lakes Ukiel and Skanda (33 and 32 isolated species, respectively), and the least in the case of Lake Tyrsko (19 isolated species). From the sand of the beaches of Lake Kortowskie, 27 species were isolated (Tab. 2, Fig. 4). The obtained results allowed for a detailed characterization of the species structure of the sand on the beaches of individual lakes (Tab. 2). Nine species were found exclusively in the sand of the beaches of Lake Kortowskie, the most frequently isolated was *Papiliotrema pseudoalba*. Seven species were isolated exclusively from the sand of the beaches of Lake Skanda, with *Exophiala jeanselmei*, classified as BSL-2 and RG-2, being the most frequent. Four species were found only in the sand of the beach of Lake Tyrsko, with *Exophiala castellanii*, also classified as BSL-2 and RG-2, being the most frequent. In the case of Lake Ukiel, nine species and five strains identified only to the genus (3 genera) were noted exclusively in the sand of the beaches of this lake, with *Nadsonia commutata* being the dominant species.

Three species were recorded in the sand of the beaches of all the lakes (*Cutaneotrichosporon jirovecii*, C. *moniliiforme*, and *D. hansenii*). Two of them, *C. jirovecii* and *C. moniliiforme*, were classified as BSL-2 and RG-2 (Tab. 2). Fourteen species were found in the sand of the beaches of three out of the four studied lakes, including one (*N. glabratus*, formely *C. glabrata*) classified as BSL-2 and RG-2 (Tab. 2). Fourteen species were isolated from the sand of the beaches of two lakes, while 35 species were found in the sand of beaches located near only one lake (Tab. 2). Five of the species present in the sand of the beaches of only one lake (*Candida albicans*, *Clavispora lusitaniae*, *Exophiala bergeri*, *E. castellanii*, and *E. jeanselmei*) were classified as BSL-2 and RG-2 (Tab. 2).

The number of viable cells of individual strains was counted, and the obtained data were compiled in tabular form (Appen. A). On average, nearly twice as many viable yeast cells were isolated in the 2019 season (9.91 x 10^2 CFU/g) compared to the 2020 season (5.56 x 10^2 CFU/g) (Appen. A; Fig. 5). The fluctuations in the average number of yeasts in 1 g of sand in the 2019 season ranged from 1.40 x 10^2 CFU/g in October to 5.48 x 10^3 CFU/g in July, while in the 2020 season, they ranged from 1.40 x 10^2 CFU/g in October to 1.94 x 10^3 CFU/g in September. The highest average number of viable yeast cells was found in sand samples collected in July 2019 (5.56 x 10^2 CFU/g), and in August and September 2020 (1.03 x 10^3 CFU/g and 1.94 x 10^3 CFU/g, respectively). The lowest concentrations were in samples collected in April, September, and October 2019, and in October 2020 (1.48 x 10^2 CFU/g, 1.47 x 10^2 CFU/g, 1.40 x 10^2 CFU/g, and 1.40 x 10^2 CFU/g, respectively) (Appen. A; Fig. 5). In 2019, compared to 2020, more viable yeast cells were isolated in May, June, and July. In July 2019, the average number of viable yeast cells was an order of magnitude higher than in the 2020 season. The opposite trend was observed in April, August, and September. In August and September 2020, the average number of viable yeast cells was an order of magnitude higher than in the corresponding months of 2019. In October of both seasons, the same average number of viable yeast cells was recorded (Appen. A; Fig. 5). The differences in the average concentration of strains between individual months were statistically significant ($p = 0.0300$). Post hoc analysis showed that this difference occurred between April and July (the test considered numerical data from both research seasons and all studied beaches).

Statistical analysis was conducted to assess the impact of meteorological parameters (temperature, humidity, UV index) on the number of isolates and strain concentrations in the sand in individual months of the research seasons, finding that temperature affected the average strain concentration in the sand ($p = 0.0210$). The test results for individual parameters are presented in the table (Tab. 4).

Table 4. Statistical dependence of the number of isolates and strain concentration on meteorological parameters (Spearman rank correlation coefficient)

PARAMETER	ISOLATES NUMBER	AVERAGE STRAINS CONCENTRATION
Temperature	Independent of the parameter (R=0,2991; p=0,1355)	***Depends on temperature (R=0,4339; p=0,0210)**
Air humidity	Independent of the parameter (R=-0,0837; p=0,6716)	Independent of the parameter (R=-0,0766; p=0,6984)
UV index	Independent of the parameter (R=0,1056; p=0,5926)	Independent of the parameter (R=0,3396; p=0,0770)

***Depends on temperature** - statistically significant results (p<0,05)

4. Discussion

Research shows high ecological and taxonomic diversity of yeasts found in soil ecosystems (Botha 2011; Yurkov 2018; Abdullabekova et al. 2021). The taxonomic structure of yeasts in sand is very uneven and diverse. It includes many species, classified both in the phylum *Ascomycota* and *Basidiomycota*. There is a relationship between the taxonomic and numerical structure of soil yeast populations and the cross-section of the soil profile (Yurkov 2018; Abdullabekova et al. 2021). The results of own research confirm this observation. According to them, the largest number of yeasts occurs at a depth of about 10 cm, although their number is quite variable and may range from 10 to over 100 CFU (colony-forming units) per gram of dry soil mass. A clear correlation was found between the size of yeast populations and the content of carbon and nitrogen in the soil (Botha 2011; Yurkov 2018). Thanks to their adaptive abilities, soil and sand provide good environments for yeast development due to the favorable microclimate, richness in available carbon and nitrogen sources, as well as other macro- and microelements (Kurtzman et al. 2011; Yurkov 2018; Brandão et al. 2021; Novak Babič et al. 2022).

Sanitary-epidemiological monitoring of recreational beaches currently includes only various parameters of bathing waters. This legal issue has been pointed out in other studies (Jang et al. 2017; Frenkel et al. 2022; Novak Babič et al. 2022). Other authors emphasize the unique and complex structure of sand, which requires going beyond standard beach monitoring parameters and developing independent quality control standards and microbiological norms to ensure the safety of beach users (Brandão et al. 2022).

Quantitative analysis and species pool of microfungi in beach sand depends on numerous atmospheric and environmental factors (Brandão et al. 2021, 2022; Frenkel et al. 2022; Novak Babič et al. 2022). The own research found a statistically significant effect of air temperature on the average concentration of strains in the sand (higher temperature resulted in a higher average strain concentration). Other authors highlight the direct impact of temperature on the survival of microorganisms in water and sand (Brandão et al. 2022; Cuartero et al. 2024). Moreover, they suggest a possible connection between global warming and the microorganisms growth kinetics, pointing out that, despite the general belief that increased temperatures favor microbial growth, in the case of mesophilic microorganisms, it will act as a limiting factor, while favoring the growth of pathogens that prefer conditions closer to the human body temperature (Brandão et al. 2022). An example could be yeasts of the genus *Candida*, which exhibit xerotolerant properties (Shah et al. 2011). Furthermore, it is noted that increased temperatures affect other environmental parameters that shape the microbiological structure of beaches (Brandão et al. 2022). For instance, an indirect negative correlation between the abundance of fungi in sand and the length of exposure to sunlight has been demonstrated, with the latter causing an increase in sand temperature (Brandão et al. 2021). Similar to the own research, no statistically significant effect of air humidity on the number of yeasts per gram of soil was observed (Brandão et al. 2021).

There were no statistically significant differences in the total number of isolates and the total concentration of strains between the 2019 and 2020 seasons. A broader analysis was conducted, comparing data across different months of the season (April-October). It was found that the differences in the number of isolates were not statistically significant; however, in the case of strain concentration, differences were noted between April and July. Similar observations were made by other researchers, who explained the results by the increased number of beach and bathing area users in the summer season, as well as the frequent organization of mass events during this period, which, according to the authors, may contribute to a higher microbial load in beach environments (Brandão et al. 2022). The positive correlation between the concentration of microfungi in beach sand and the

number of users is confirmed by other studies (Lindahl et al. 2015; Kauppinen et al. 2017). This issue, however, requires further analysis, especially in the context of the number of users, due to incomplete data on population flow provided by supervisors of the studied municipal beaches (Appen. B.). Additionally, an analysis of the average concentration of yeasts in the sand of beaches across studied lakes was conducted. In both research seasons, the lowest average concentration of yeasts in the sand was recorded for the lake with the smallest surface area. This observation is likely due to the varying number of beach users, which correlates with the size and availability of recreational areas at the studied water bodies. Lake Tyrsko has the shortest shoreline, significantly limiting the bathing area and the number of users. Other researchers have pointed out the possible influence of the water body's structure on the taxonomic and numerical composition of yeast populations in the associated ecological niches (Biedunkiewicz and Góralska 2016; Nowacka et al. 2018; Biedunkiewicz et al. 2020).

There is no statistical relationship between the season and the number of isolates or the concentration of yeasts in the soil. Similar results were presented in the other study (Brandão et al. 2021). This suggests that the numerical structure of yeasts in beach sand is determined not by the season, but rather by long-term atmospheric conditions.

Moreover, no significant changes in the concentration and number of yeast isolates were observed between the times of day studied (morning; evening). The own research correlates with the results of other studies, which demonstrated the weakest seasonal correlation between air and soil temperature in the spring and summer. The authors noted that soil temperature is mainly influenced by factors unrelated to the diurnal cycle (Kossowski 2005; Skowera and Wojkowski 2017). Furthermore, the authors found that the diurnal amplitude of soil temperatures and the dependence of soil temperature on air temperature decrease with soil profile depth (Kossowski 2005; Skowera and Wojkowski 2017). This suggests that sand is less susceptible to temperature changes with depth. Samples for own research were taken from depths of 10 and 50 cm, which significantly limited the influence of atmospheric factors on the numerical structure of yeast populations in the sand over a diurnal cycle.

Analyzing the yeast abundance at different depths, it was found that the obtained results are consistent with those reported by Grishkan and Kidron (2016), who also observed higher average concentrations of yeast strains at a depth of 10 cm. This trend persisted across different research seasons. However, in the case of both parameters, the observed differences were not statistically significant. This observation may be linked to the higher content of organic carbon, water, and oxygen in the surface soil layers, where sand layers mix.. Fang and Moncrieff's (2005) studies indicate decreased organic carbon content and oxygen availability with soil depth. The same study showed that this trend does not significantly change depending on the climate and that respiratory processes and biomass development of oxygen-affiliated organisms occur most intensively in the surface soil layers. Grishkan and Kidron's (2016) research suggests that the distribution of fungal communities in soil is related to the availability of organic matter, water, aeration, salinity, and UV radiation, emphasizing its complex and non-linear nature. Another factor influencing the distribution and abundance of microfungal consortia is soil and sand porosity (Grishkan and Kidron 2016; Brandão et al. 2021). Microbial biomass density reaches its maximum values in the surface soil layers (0.2-2 cm), with a second peak at a depth of 5-10 cm, followed by a sharp decline down to 50 cm (Grishkan and Kidron 2016). This observation aligns with the present study.

During the research, differences in the taxonomic structure of soil yeast consortia depending on the soil profile depth were noted. The dominant part of the isolates obtained included species occurring at both depths (24 species; 2 genera). Species recorded in the number of 3 isolates or more were distinguished from this group. These were: *N. glabratus, C. jirovecii, C. moniliiforme, D. hansenii, Oosporidium margaritiferum* and *Schwanniomyces polymorphus*. Based on these findings, it can be hypothesized that the mentioned species are insensitive to limited oxygen availability and are less susceptible to changes in oxygen content in the sand compared to other microorganisms. In the current study, 28 species and 1 genus were found exclusively at a depth of 10 cm, which may indicate their high oxygen affinity. At a depth of 50 cm, only 10 species and 1 genus were found, indicating their ability to grow in low-oxygen conditions. The taxonomic structure of the mycobiota at a depth of 50 cm was significantly poorer, showing a clear trend that yeasts can develop at various depths but are more commonly found in the surface soil layers. This is likely because most yeasts require oxygen for growth (Mooiman et al. 2021). Nonetheless, other researchers suggest that yeasts may acquire the ability to develop in anaerobic conditions through horizontal gene transfer It should be noted that other factors, such as soil structure (e.g., porosity), organic compound content (mainly carbon), water availability, UV radiation, salinity, and temperature, may also influence the species richness of yeasts in various soil layers (Grishkan and Kidron 2016; Brandão et al. 2021, 2022; Novak Babič et al. 2022).

Overall, more yeast strains were isolated from unsupervised beaches; however, a comparison of the number of isolates and yeast concentration in the sand did not reveal statistically significant differences. Similar results were obtained by other researchers (Brandão et al. 2021; Frenkel et al. 2022). The likely cause of this difference was the similar purpose of the beaches studied, as well as the cleaning and replenishment of sand on supervised beaches by municipal institutions, which may improve the sanitary condition of the sand. It should be noted, however, that the presence of municipal supervision at swimming beaches may encourage more people to use them, and the presence of users increases the quantitative and taxonomic structure of the mycobiota in the sand and water (Biedunkiewicz et al. 2020; Brandão et al. 2022; Frenkel et al. 2022).

Among the most frequently reported yeasts on beaches are representatives of the genera: *Aureobasidium*, *Candida*, *Geotrichum*, *Exophiala*, *Metschnikowia*, *Rhodothorula* and *Yarrowia* (Libkind et al. 2017; Frenkel et al. 2022; Novak Babič et al. 2022). Yeasts from the genera *Candida, Cryptococcus* and *Rhodotorula* are associated with sandy beaches (Brandão et al. 2021). In the present study, nearly 260 yeast strains were isolated from the sand of lake swimming beaches, among which 47 genera were recorded, including *Aureobasidium, Candida, Cryptococcus, Exophiala, Metschnikowia* and *Rhodotorula*, confirming that beach sand is their natural ecological niche. Additionally, the literature indicates that the most commonly noted genera in lake waters are: *Candida, Cryptococcus, Geotrichum, Hansenula, Pichia, Rhodotorula, Saccharomyces, Sporobolomyces* and *Trichosporon* (Dynowska and Biedunkiewicz 2013; Biedunkiewicz et al. 2020; Brandão et al. 2021), and on the coastal phyllosphere, the genera: *Candida*, *Cryptococcus*, *Debaryomyces*, *Metschnikowia*, *Pichia*, *Saccharomyces* and *Rhodotorula* (Biedunkiewicz et al. 2020; Brandão et al. 2021; Gouka et al. 2022). Five of the genera considered typical for bathing water and all those considered characteristic for coastal aquatic vegetation were isolated from the sand in this study. A summary of long-term studies conducted by Biedunkiewicz demonstrated the constant presence of over 40 yeast genera in lake waters in the Olsztyn region, and 16 associated with the aquatic vegetation found in their vicinity (Biedunkiewicz et al. 2020). In the present study, 21 genera (48.8%) from those recorded by Biedunkiewicz and colleagues (2020) in bathing water were

noted, along with 13 genera (81.3%) that were isolated in their research from the phyllosphere. These observations indicate the continuous circulation of microfungi between the various components of the lake ecosystem, including during the washing of sand and vegetation by lake water, as well as through surface and subsurface runoff caused by precipitation. Beach users and animals living there, e.g. waterfowl, may also be an important vector of such transmission (Biedunkiewicz et al. 2020; Brandão et al. 2021, 2022; Novak Babič et al. 2022). Numerous scientific papers indicate that species such as *B. californica*, *N. glabratus*, *C. moniliiforme*, *C. jirovecii*, *D. hansenii* and *S. aeria* are soil microorganisms or are frequently isolated from soil, including beach sand (Yurkov 2018; Brandão et al. 2021, 2022; Kaewkrajay et al. 2021; Samarasinghe et al. 2021). The frequent notation of these species in the present study should therefore be considered a conventional observation.

A comparison of the taxonomic structure between the designated research seasons showed that 21 species and 1 genus (strain identified only to the genus level) were isolated in both seasons. Among this group, *B. californica*, *Nakaseomyces glabratus*, *C. moniliiforme*, *D. hansenii*, *Schwanniomyces polymorphus* and *S aeria* were noted with 3 or more isolates in each season, suggesting that they are indigenous to the studied environments. This is supported by studies that classify these species as soil yeasts (Kurtzman et al. 2011; Góralska and Biedunkiewicz 2016; Yurkov 2018; Brandão et al. 2021; Salah et al. 2021). For the species: *C. moniliiforme* and *D. hansenii* the discrepancy in the number of isolates between seasons reached an order of magnitude. It can be hypothesized that these species may be more sensitive to seasonal changes in environmental parameters, such as weather conditions or beach users traffic and activity. This is corroborated by studies from other authors (Biedunkiewicz et al. 2013, 2020; Biedunkiewicz and Góralska 2016; Yurkov 2018; Brandão et al. 2021, 2022; Kaewkrajay et al. 2021). In the 2019 season, 29 species and 2 genera (strains identified only to the genus level) were recorded, while in 2020, 12 species and 1 genus were found. The probable cause of these observed differences is the limited traffic of beach users, a potential vector of microfungal transmission at bathing sites, due to the sanitary regulations related to the COVID-19 pandemic in 2020.

A total of 26 species and 1 genus (strains identified only to the genus level) were found on both supervised and unsupervised beaches. Among these, the species *B. californica*, *N. glabratus*, *Cutaneotrichosporon jirovecii*, *C. moniliiforme*, *D. hansenii*, and *S. aeria* were noted with 3 or more isolates on each type of beach. These species have previously been identified as characteristic or frequently isolated from soil and lake environments (Yurkov 2018; Biedunkiewicz et al. 2020; Brandão et al. 2021, 2022; Kaewkrajay et al. 2021; Samarasinghe et al. 2021). For the species, *B. californica*, *N. glabratatus* and *D. hansenii* more isolates were found on supervised beaches. *N. glabratatus* often forms part of the physiological mycobiota of humans and, in immunocompromised individuals, can become pathogenic (Biedunkiewicz and Góralska 2016; Biedunkiewicz et al. 2020; Brandão et al. 2021). *B. californica* is classified as a soil yeast (Kurtzman et al. 2011; Yurkov 2018) and has also been isolated from water, plant debris, and animal droppings (Mullen et al. 2018). *B. californica* and *D. hansenii* are also found in food products (Desnos-Ollivier et al. 2008; Drumonde-Neves et al. 2016). The presence of these species suggests a link to human activity, as supervised beaches generally attract more visitors during the summer season. However, overall, half as many species were found exclusively on supervised beaches compared to those isolated solely from unsupervised beaches. This discrepancy is likely due to the better sanitary condition of supervised beaches, which are cleaned and have their sand replenished by supervisors.

The diurnal cycle was the parameter for which the greatest number of species were recorded in the sand, regardless of this parameter, compared to the other parameters adopted in the study (depth, season, type of supervision). Other researchers indicate that the taxonomic structure of yeasts in sand and soil generally changes due to seasonal dynamics rather than the diurnal cycle (Kossowski 2005; Skowera and Wojkowski 2017; Brandão et al. 2021).

The taxonomic structure of yeast assemblages in sand was also compared between the beaches of different lakes. The most species were isolated from Ukiel and Skanda lakes, and the fewest from Tyrsko Lake. The beaches of Ukiel and Skanda have the longest shorelines, the largest usable area, and are among the most frequently used for recreational purposes. The length of the shoreline increases the area where sand is washed by water, and beachgoers act as natural vectors of fungal transmission, promoting yeast circulation between the different components of the lake ecosystem (Lossow et al. 2005; Biedunkiewicz et al. 2020). The beach at Tyrsko Lake is managed by a private owner and is only accessible to guests of the nearby hotel, which significantly limits the number of users. Additionally, Ukiel, Kortowskie, and Skanda lakes are eutrophic and exhibit high primary production, which is associated with high levels of nitrogen, oxygen, and phosphorus, and generally correlates with increased taxonomic diversity. In contrast, Tyrsko Lake, despite showing signs of aging, is characterized by low trophic status and poor water mineralization, which may limit the number of species transmitted to the sand (Lossow et al. 2005).

Sixteen species and one genus were isolated from the beaches of more than two of the studied lakes. Among them, *C. moniliiforme, C. jirovecii* and *D. hansenii* were isolated from the sand of all four studied lakes. Other studies confirm the presence of species belonging to these genera in lake water and beach sand (Biedunkiewicz and Góralska 2016; Libkind et al. 2017; Biedunkiewicz et al. 2020; Kaewkrajay et al. 2021). These species can therefore be considered characteristic of the beach sand of the lakes studied and likely continuously associated with this type of ecological niche, which has sanitary and epidemiological significance.

All obtained yeast isolates were assigned BSL and RG index values in the context of their potential pathogenicity. Additionally, for the most frequently isolated species, a literature review was conducted regarding the infections they can cause.

Among the most frequently isolated species were *Cutaneotrichosporon jirovecii* and *C. moniliiforme.* These are potential human pathogens in immunocompromised individuals (Kurtzmann et al. 2011, Biedunkiewicz and Góralska, 2016; Biedunkiewicz et al., 2020; Kulesza et al., 2021; Bałabański and Biedunkiewicz, 2023). Studies indicate that species from the genus *Cutaneotrichosporon* can grow at human body temperature and produce hydrolytic enzymes that act as virulence factors promoting infections. Furthermore, the scientific literature indicates an increase in infections and resistance of *Cutaneotrichosporon* species to antifungal agents used by clinicians (do Espírito Santo et al. 2020). Researchers have noted that *C. jirovecii* can cause liver and skin damage, while *C. moniliiforme* tends to cause less invasive pathological changes (Ma et al. 2019). The high risk these species pose to beachgoers is demonstrated by the fact that they were isolated from the sand of beaches at all the lakes studied.

The second most frequently noted species, *D. hansenii*, found on all the studied beaches, is associated with food products. It is generally considered saprotrophic, but there are reports in the literature of isolates from clinical material. Studies by Breuer and Harms (2006) and Desnos-Ollivier *et al.*, (2008) showed that this species rarely

causes infections such as bone infections, subcutaneous abscesses, keratitis, and allergic alveolitis in both immunocompetent and immunocompromised individuals.

During the study, numerous strains classified in the genus *Candida* were isolated. Species belonging to this genus are the most commonly isolated from the blood of hospitalized patients. Additionally, they are frequently found in the oral cavity, urinary tract, and female genital tract (Silva et al. 2012). There is an increase in infections caused by *Candida* fungi in immunocompromised individuals, including cancer patients and postoperative patients (Silva et al. 2012). Factors predisposing Candida species to infect humans include their ability to adhere to host epithelial cells and form biofilms (Silva et al. 2012; Kulesza et al. 2021; Martin et al. 2021; Zarnowski et al. 2021; Bałabański and Biedunkiewicz 2023). In the present study, the dominant species was *Nakaseomyces glabratus* (formely *C. glabrata*) with a single isolate of *C. albicans* also recorded. *N. glabratus* and *C. albicans* are among the four species responsible for approximately 95% of diagnosed candidiasis (Silva et al. 2012). Both species are classified as BSL-2 and RG-2. They mainly cause infections of the skin and mucous membranes in immunocompromised individuals (Silva et al. 2012; Biedunkiewicz and Góralska 2016; Góralska and Biedunkiewicz 2016; Biedunkiewicz et al. 2020; Frenkel et al. 2022). *N. glabratus* primarily causes infections of the oral cavity, urogenital system, and bloodstream, and it exhibits resistance to many antifungal agents (Silva et al. 2014). *C. albicans* is considered an indicator of fecal contamination in the environment (Brandão et al. 2021). Among the frequently isolated species was also *Citeromyces matritensis*, the teleomorphic stage of *Candida globosa.* Research by Kheireddine and co-authors (2019) indicated weak growth of this species at 37°C and emphasized the lack of clinical infection cases caused by this species.

Numerous strains of *B. californica* were isolated during the study This species is not pathogenic but rather saprotrophic (Ni et al. 2011). Similarly, *Solicoccozyma aeria* was frequently noted. Research indicates that it can be isolated from animals, but there are no reports of human infections caused by this species (Bertout et al. 2022). *S. aeria* may be a part of the physiological mycobiota of the colon, as it has also been isolated from human feces (Guo et al. 2021). Additionally, it has been linked to appetite suppression and the activation of interleukin 17 in sensitive individuals, which may be associated with disturbances in gut microbiota and mycobiota. Studies also show an increase in *S. aeria* expression in cancer patients, with the potential for considering this species as a marker of gastric cancer, while excluding it as an etiological factor in the disease (Zhang et al. 2022). In summary, the literature suggests that this species may have a positive impact on human health as a cancer marker. There are no reports of its potential pathogenicity to humans; however, its isolation from animals suggests it could be considered as a potential human pathogen.

Yeasts from the genus *Cryptococcus* were also noted in the beach sand. Many species from this genus exhibit pathogenic properties in both immunocompromised and immunocompetent individuals (Passer et al. 2019; Gupta et al. 2020). Fungi of the genus *Cryptococcus* cause cryptococcosis, which can affect the skin, lungs, and bloodstream, and in advanced stages, lead to meningitis and brain edema (Passer et al. 2019; Gupta et al. 2020). The virulence factors of *Cryptococcus* include the production of polysaccharide capsules and high enzymatic activity (protease, urease, phospholipase) (Gupta et al. 2020). The only identified species, *C. amylolentus*, belongs to the non-pathogenic cryptococci, closely related to pathogenic species. This species has been shown to have infectious potential under induced conditions, but due to its sensitivity to thermal stress, it does not grow at human body temperature (Passer et al. 2019; Gupta et al. 2020).

Six isolates from the genus *Exophiala* were recorded during the study. Of the identified species, *E. jeanselmei* was the most frequently isolated. This species can cause subcutaneous ulcers and nodules in transplant recipients (Lief et al. 2011). In advanced cases, it leads to chronic, purulent infections of subcutaneous tissue (Badali et al. 2009). The less frequently isolated species, *E. bergeri*, has been reported as an etiological agent of nail infections (Woo et al. 2013), while *E. castellanii* has been noted in subcutaneous infections (Biedunkiewicz and Schulz 2012). All species from the genus *Exophiala* isolated in this study are potential pathogens for immunocompromised individuals, as they are classified as BSL-2 and RG-2 (Biedunkiewicz and Schulz 2012; Biedunkiewicz and Góralska 2016; Biedunkiewicz et al. 2020; Kulesza et al. 2021; Bałabański and Biedunkiewicz 2023).

All remaining species were isolated in numbers of six or fewer strains. Due to their rare (episodic) occurrence in the studied environments and simultaneous classification as BSL-1 and RG-1, it was assumed that they do not pose a potential threat to beach users.

5. Conclusions

The examination of the sanitary condition of beach sand at supervised and unsupervised swimming areas near Lakes Kortowskie, Skanda, Tyrsko, and Ukiel revealed the presence of species classified as potential human pathogens, Particular attention should be given to *N. glabratus*, *Cutaneotrichosporon jirovecii*, and *C. moniliiforme*, which were among the five most frequently isolated species, exhibited a cosmopolitan character in relation to the ecological niches studied, and were classified as Biosafety Level 2 (BSL-2) and Risk Group 2 (RG-2). The research demonstrated that beach sand at swimming areas may pose a potential health risk for individuals with temporary or chronic immunocompromised conditions. In this context, it is essential to conduct continuous sanitary-epidemiological monitoring of beach sand and implement preventive measures to mitigate the risks associated with the presence of potentially pathogenic yeasts in the sand, such as regular sand cleaning and replenishment. The present study, combined with literature data, revealed that knowledge regarding the ecological and taxonomic characteristics of yeast populations in beach sand at swimming areas is significantly limited. Furthermore, there is a lack of legal regulations defining reference standards for the mycological cleanliness of such sand. In light of this, the introduction of such standards and the supervision of beach sand at swimming areas by institutions authorized to conduct sanitary inspections is recommended, along with further research in this field.

Statements and declarations

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Competing interests

Authors declare that they have no conflict of interest

Availability of data and material

Sequences of ITS regions have been deposited in the GenBank database. Accession number range: PQ882533 - PQ882625.

Code availability

Not applicable

Authors' contributions

Literature review [Tomasz Bałabański]. Research designing [Anna Biedunkiewicz]. Conducting experiments [Tomasz Bałabański, Anna Biedunkiewicz]. Writing the manuscript [Tomasz Bałabański, Anna Biedunkiewicz]. Proofreading the manuscript and approving the final version of the manuscript and agreeing on the order of presentation by the authors [Anna Biedunkiewicz, Tomasz Bałabański, Jan P. Jastrzębski] . Preparation and placement of sequences in GenBank [Jan P. Jastrzębski].

Ethics approval

Not applicable

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References

Abdullabekova DA, Magomedova ES, Magomedov GG, Kachalkin A V. (2021) Yeasts as an Element of Ampelocenosis Soil Biodiversity in an Arid Climate. Arid Ecosyst 11:299–303. https://doi.org/10.1134/S2079096121030021

Badali H, Najafzadeh MJ, Van Esbroeck M, et al (2009) The clinical spectrum of Exophiala jeanselmei, with a case report and in vitro antifungal susceptibility of the species. Med Mycol 48:318–327. https://doi.org/10.1080/13693780903148353

Bałabański T, Biedunkiewicz A (2023) Threats Resulting from the Presence of Potentially Pathogenic Fungi in the Sport Changing Rooms – a Preliminary Study. Polish J Environ Stud 32:3033–3042. https://doi.org/10.15244/pjoes/162052

Bertout S, Gouveia T, Krasteva D, et al (2022) Search for Cryptococcus neoformans/gattii Complexes and Related Genera (Filobasidium, Holtermanniella, Naganishia, Papiliotrema, Solicoccozyma, Vishniacozyma) spp. Biotope: Two Years Surveillance of Wild Avian Fauna in Southern France. J Fungi 8:1–20. https://doi.org/10.3390/jof8030227

Biedunkiewicz A, Dynowska M, Ejdys E, Sucharzewska E (2013) Species diversity of yeast-like fungi in some eutrophic lakes in Olsztyn. Acta Mycol 48:61–71. https://doi.org/10.5586/am.2013.008

Biedunkiewicz A, Góralska K (2016) Microfungi Potentially Pathogenic for Humans Reported in Surface Waters Utilized for Recreation. Clean - Soil, Air, Water 44:599–609. https://doi.org/10.1002/clen.201500696

Biedunkiewicz A, Schulz Ł (2012) Fungi of the genus exophiala in tap water - Potential etiological factors of phaeohyphomycoses. Mikol Lek 19:23–26

Biedunkiewicz A, Sucharzewska E, Kulesza K, et al (2020) Phyllosphere of Submerged Plants in Bathing Lakes as a Reservoir of Fungi—Potential Human Pathogens. Microb Ecol 79:552–561. https://doi.org/10.1007/s00248-019-01447-y

Botha A (2011) The importance and ecology of yeasts in soil. Soil Biol. Biochem. 43:1–8

Brandão J, Albergaria I, Albuquerque J, et al (2020) Untreated sewage contamination of beach sand from a leaking underground sewage system. Sci Total Environ 740:. https://doi.org/10.1016/j.scitotenv.2020.140237

Brandão J, Gangneux JP, Arikan-Akdagli S, et al (2021) Mycosands: Fungal diversity and abundance in beach sand and recreational waters — Relevance to human health. Sci Total Environ 781:1–15. https://doi.org/10.1016/j.scitotenv.2021.146598

Brandão J, Weiskerger C, Valério E, et al (2022) Climate Change Impacts on Microbiota in Beach Sand and Water: Looking Ahead. Int J Environ Res Public Health 19:1444–1459. https://doi.org/10.3390/ijerph19031444

Breuer U, Harms H (2006) Debaryomyces hansenii - An extremophilic yeast with biotechnological potential. Yeast 23:415–437. https://doi.org/10.1002/yea.1374

Buczyńska A, Cyprowski M, Piotrowska M, Szadkowska-Stańczyk I (2007) Grzyby pleśniowe w powietrzu pomieszczeń biurowych-wyniki interwencji środowiskowej. Indoor moulds: results of the environmental study in office rooms. Med Pr 58:521–525

Cuartero J, Querejeta JI, Prieto I, et al (2024) Warming and rainfall reduction alter soil microbial diversity and co-occurrence networks and enhance pathogenic fungi in dryland soils. Sci Total Environ 949:175006. https://doi.org/10.1016/j.scitotenv.2024.175006

de Hoog G., Guarro J, Gené J, Figueras MJ (2000) Atlas of Clinical Fungi, 2nd edn. Centraalbureau voor Schimmelcultures/Universitat Rovira and Virgili, Reus

de Hoog S, Guarro J, Gené J, et al (2019a) Atlas of Clinical Fungi: The Ultimate Benchtool for Diagnostics: Part α: Introductions, lower fungi, basidiomycetes, yeasts, filamentous ascomycetes A-B., 4th edn. Westerdijk Institute / Universitat Rovira i Virgili, Reus

de Hoog S, Guarro J, Gené J, et al (2019b) Atlas of Clinical Fungi: The Ultimate Benchtool for Diagnostics: Part β: Filamentous ascomycetes C-Z, 4th edn. Westerdijk Institute / Universitat Rovira i Virgili, Reus

Desnos-Ollivier M, Ragon M, Robert V, et al (2008) Debaryomyces hansenii (Candida famata), a rare human fungal pathogen often misidentified as Pichia guilliermondii (Candida guilliermondii). J Clin Microbiol 46:3237–3242. https://doi.org/10.1128/JCM.01451-08

do Espírito Santo EPT, Monteiro RC, da Costa ARF, Marques-da-Silva SH (2020) Molecular Identification, Genotyping, Phenotyping, and Antifungal Susceptibilities of Medically Important Trichosporon, Apiotrichum, and Cutaneotrichosporon Species. Mycopathologia 185:307–317.

https://doi.org/10.1007/s11046-019-00407-x

Drumonde-Neves J, Franco-Duarte R, Lima T, et al (2016) Yeast biodiversity in vineyard environments is increased by human intervention. PLoS One 11:1–13. https://doi.org/10.1371/journal.pone.0160579

Dynowska M (2018) The variability of fungi and its significance. In: Kurnatowska A, Kurnatowski P (eds) Mykologia Medyczna. Edra Urban & Partner, Wrocław, pp 17–25

Dynowska M, Biedunkiewicz A (2013) Mikrogrzyby wód o potencjalnych właściwościach bioindykacyjnych [Water microfungi with potential bioindicative properties]. In: Ciecierska H, Dynowska M (eds) Biologiczne metody oceny stanu środowiska. Tom 2. Ekosystemy wodne [Biological methods of assessing the state of the environment. Volume 2. Water ecosystems]. mantis, Olsztyn, pp 284–310

Dynowska M, Ejdys E (2011) Mikologia laboratoryjna – przygotowanie materiału badawczego i diagnostyka [Laboratory mycology – preparation of research material and diagnostics]. Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego, Olsztyn

Dynowska M, Sucharzewska E, Biedunkiewicz A (2001) Enzymatic activity of fungi of the genus Candida isolated from the skin and the digestive tract in people and from municipal sewage. Acta Mycol 36:293–302. https://doi.org/10.5586/am.2001.021

Echevarría L, Iqbal MN (2021) Identification of Fungi and Yeasts from the Sands of the Pyramids of Giza , in Cairo , Egypt. PSM Biol Res 6:13–18

Ejdys E, Dynowska M, Biedunkiewicz A, Sucharzewska E (2013) An overview of the species of fungi occurring in school rooms - A four-year study. Polish J Environ Stud 22:1691–1700

Ergül C, Çalişkan E (2018) Endospore formed bacteria and staining techniques. In: Christov I, Strauss E, Gad A-A, Curebal I (eds) Science, Ecology and Engineering Research in the Globalizing World. World. St. Kliment Ohridski University Press, Sofia, pp 362–374

Fedorova MD, Kurakov A V. (2023) Microbiota of Bottom Sediments in the Coastal Zone of Lake Baikal. Contemp Probl Ecol 16:492–508. https://doi.org/10.1134/S1995425523040030

Frenkel M, Serhan H, Blum SE, et al (2022) What Is Hiding in the Israeli Mediterranean Seawater and Beach Sand. J Fungi 8:950–961. https://doi.org/10.3390/jof8090950

Góralska K, Biedunkiewicz A (2016) Human Mycobiota-Selected Ontocenoses of Students of Natural Science and Medicine. J Bacteriol Parasitol 7:3–8. https://doi.org/10.4172/2155-9597.1000279

Gouka L, Raaijmakers JM, Cordovez V (2022) Ecology and functional potential of phyllosphere yeasts. Trends Plant Sci 27:1109–1123. https://doi.org/10.1016/j.tplants.2022.06.007

Gregorczyk-Maga I, Kania M, Sulik-Tyszka B, et al (2023) Oral Myco- and Bacteriobiota and Yeast Infections in Mechanically Ventilated COVID-19 Patients. Microorganisms 11:1442–1457. https://doi.org/10.3390/microorganisms11061442

Grishkan I, Kidron GJ (2016) Vertical Divergence of Cultured Microfungal Communities Through the Depth in Different Soil Formations at Nahal Nizzana, Western Negev Desert, Israel. Geomicrobiol J 33:564–577. https://doi.org/10.1080/01490451.2015.1062063

689 Guo Y, Guo H, Qiu L, et al (2021) Appetite Suppression and Interleukin 17 Receptor Signaling Activation of
690 Colonic Mycobiota Dysbiosis Induced by High Temperature and High Humidity Conditions. Front Cell
691 Infect Microbiol 11:1–15. https://doi.org/10.3389/fcimb.2021.657807

692 Gupta S, Paul K, Kaur S (2020) Diverse species in the genus Cryptococcus: Pathogens and their non-pathogenic
693 ancestors. IUBMB Life 72:2303–2312. https://doi.org/10.1002/iub.2377

694 Jang J, Hur HG, Sadowsky MJ, et al (2017) Environmental Escherichia coli: ecology and public health
695 implications—a review. J Appl Microbiol 123:570–581. https://doi.org/10.1111/jam.13468

696 Kaewkrajay C, Putchakarn S, Limtong S (2021) Cultivable yeasts associated with marine sponges in the Gulf of
697 Thailand, South China Sea. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 114:253–274.
698 https://doi.org/10.1007/s10482-021-01518-6

699 Kauppinen A, Al-Hello H, Zacheus O, et al (2017) Increase in outbreaks of gastroenteritis linked to bathing
700 water in Finland in summer 2014. Eurosurveillance 22:1–8. https://doi.org/10.2807/1560-
701 7917.ES.2017.22.8.30470

702 Kossowski J (2005) Relationship of daily amplitudes of soil temperature in the near-surface layer with air
703 temperature amplitudes and other meteorological elements [Związek amplitud dobowych temperatury
704 gleby w warstwie przypowierzchniowej z amplitudami temperatury powietrz. Acta Agrophysica 5:657–
705 667

706 Kowalski M, Pastuszka JS (2018) Effect of ambient air temperature and solar radiation on changes in bacterial
707 and fungal aerosols concentrationin the urban environment. Ann Agric Environ Med 25:259–261.
708 https://doi.org/10.26444/aaem/75877

709 Krajewska-Kułak E, Łukaszuk C, Gniadek A, et al (2010) Zanieczyszczenie powietrza w pomieszczeniach
710 mieszkalnych, ze szczególnym uwzglednieniem roli grzybów [Air pollution in aviation air, with detailed
711 consideration of the role of fungi]. Mikol Lek 17:177–181

712 Kulesza K, Biedunkiewicz A, Nowacka K, et al (2021) Dishwashers as an extreme environment of potentially
713 pathogenic yeast species. Pathogens 10:1–17. https://doi.org/10.3390/pathogens10040446

714 Kurnatowska A, Kurnatowski P (2018) Mykologia Medyczna [Medical Mycology]. Edra Urban & Partner,
715 Wrocław

716 Kurtzman CP, Fell JW, Boekhout T (2011) The Yeasts: A Taxonomic Study, 5th edn. Elsevier, Amsterdam

717 Libkind D, Buzzini P, Turchetti B (2017) Yeasts in continental and seawater. In: Buzzini P, Lachance M-A,
718 Yurkov AM (eds) Yeasts in Natural Ecosystems: Diversity. Springer, pp 1–61

719 Lief MH, Caplivski D, Bottone EJ, et al (2011) Exophiala jeanselmeiinfection in solid organ transplant
720 recipients: Report of two cases and review of the literature. Transpl Infect Dis 13:73–79.
721 https://doi.org/10.1111/j.1399-3062.2010.00552.x

722 Lindahl JF, Grace D, Strand T (2015) The consequences of human actions on risks for infectious diseases: a
723 review. Infect Ecol Epidemiol 5:1–11. https://doi.org/10.3402/IEE.V5.30048

724 Lossow K, Gawrońska H, Mientki C, et al (2005) Olsztyn lakes, trophic status, threats [Jeziora Olsztyna, stan

troficzny, zagrożenia]. Studio Przygotowawcze Wydawnictw “Edycja” s.c., Olsztyn

Ma X, Jiang Y, Wang C, et al (2019) Identification, genotyping, and pathogenicity of Trichosporon spp. Isolated from Giant pandas (Ailuropoda melanoleuca). BMC Microbiol 19:1–18. https://doi.org/10.1186/s12866-019-1486-7

Maciel NOP, Johann S, Brandão LR, et al (2019) Occurrence, antifungal susceptibility, and virulence factors of opportunistic yeasts isolated from brazilian beaches. Mem Inst Oswaldo Cruz 114:1–15. https://doi.org/10.1590/0074-02760180566

Martin H, Kavanagh K, Velasco-Torrijos T (2021) Targeting adhesion in fungal pathogen Candida albicans. Future Med Chem 13:313–334. https://doi.org/10.4155/fmc-2020-0052

Menu E, Filori Q, Dufour JC, et al (2023) A Repertoire of the Less Common Clinical Yeasts. J Fungi 9:1099–1132. https://doi.org/10.3390/jof9111099

Monapathi ME, Bezuidenhout CC, James Rhode OH (2020) Aquatic yeasts: Diversity, characteristics and potential health implications. J Water Health 18:91–105. https://doi.org/10.2166/wh.2020.270

Mooiman C, Bouwknegt J, Dekker WJC, et al (2021) Critical parameters and procedures for anaerobic cultivation of yeasts in bioreactors and anaerobic chambers. FEMS Yeast Res 21:1–13. https://doi.org/10.1093/femsyr/foab035

Mullen AA, Lynch CD, Hill SM, et al (2018) crossm Draft Genome Sequences of Two Natural Isolates of the Yeast. Genome Announc 6:1–2

Nagahama T (2006) Yeast Biodiversity in Freshwater, Marine and Deep-Sea Environments. In: Péter G, Rosa C (eds) Biodiversity and Ecophysiology of Yeasts. Springer Berlin Heidelberg, Berlin, pp 241–262

Ni H, Li L, Xiao A, et al (2011) Identification and characterization of a new naringinase-producing strain, Williopsis californica Jmudeb007. World J Microbiol Biotechnol 27:2857–2862. https://doi.org/10.1007/s11274-011-0766-7

Novak Babič M, Gunde-Cimerman N, Breskvar M, et al (2022) Occurrence, Diversity and Anti-Fungal Resistance of Fungi in Sand of an Urban Beach in Slovenia—Environmental Monitoring with Possible Health Risk Implications. J Fungi 8:860–885. https://doi.org/10.3390/jof8080860

Nowacka K, Kulesza, K, Glinka, P, et al (2018) The phyllosphere as a little-known reservoir of the Fusarium genus, a fungi of importance to medical mycology. Ann Parasitol 64:225–228. https://doi.org/10.17420/ap6403.156

Passer AR, Coelho MA, Billmyre RB, et al (2019) Genetic and genomic analyses reveal boundaries between species closely related to Cryptococcus pathogens. MBio 10:e00764-19. https://doi.org/10.1128/mBio.00764-19

Salah HA, Temerk HA, Salah NA, et al (2021) Production and optimization of xylanase and α-amylase from non-saccharomyces yeasts (Pichia membranifaciens). J Pure Appl Microbiol 15:452–461. https://doi.org/10.22207/JPAM.15.1.43

Samarasinghe H, Lu Y, Aljohani R, et al (2021) Global patterns in culturable soil yeast diversity. iScience

24:103098. https://doi.org/10.1016/j.isci.2021.103098

Schoeman H, Wolfaardt GM, Botha A, et al (2009) Establishing a risk-assessment process for release of genetically modified wine yeast into the environment. Can J Microbiol 55:990–1002. https://doi.org/10.1139/W09-039

Shah AH, Abdelzaher AM, Phillips M, et al (2011) Indicator microbes correlate with pathogenic bacteria, yeasts and helminthes in sand at a subtropical recreational beach site. J Appl Microbiol 110:1571–1583. https://doi.org/10.1111/j.1365-2672.2011.05013.x

Silva L, Ramirez M, Osorio E (2014) Yeast diversity associated to sediments and water from two Colombian artificial lakes. Brazilian J Microbiol 45:135–142

Silva S, Negri M, Henriques M, et al (2012) Candida glabrata, Candida parapsilosis and Candida tropicalis: Biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev 36:288–305. https://doi.org/10.1111/j.1574-6976.2011.00278.x

Skowera B, Wojkowski J (2017) Relation of Soil Temperature With Air Temperature At the Jurassic River Valley. Ecol Eng 18:18–26. https://doi.org/10.12912/23920629/65855

Sultana S, Biró J, Kucska B, Hancz C (2024) Factors Affecting Yeast Digestibility and Immunostimulation in Aquatic Animals. Animals 14:1–20. https://doi.org/10.3390/ani14192851

Tedersoo L, Bahram M, Põlme S, et al (2014) Global diversity and geography of soil fungi. Science (80-) 346:1078–1089. https://doi.org/10.1126/science.1256688

Walker JKM, Ward V, Jones MD (2016) Ectomycorrhizal fungal exoenzyme activity differs on spruce seedlings planted in forests versus clearcuts. Trees - Struct Funct 30:497–508. https://doi.org/10.1007/s00468-015-1239-7

Wójcik A, Błaszkowska J, Kurnatowski P, Góralska K (2016) Sandpits as a reservoir of potentially pathogenic fungi for children. Ann Agric Environ Med 23:542–548. https://doi.org/10.5604/12321966.1226843

Wójcik A, Kurnatowski P, Błaszkowska J (2013) Potentially pathogenic yeasts from soil of children's recreational areas in the city of Łódź (Poland). Int J Occup Med Environ Health 26:477–487. https://doi.org/10.2478/s13382-013-0118-y

Woo PCY, Ngan AHY, Tsang CCC, et al (2013) Clinical spectrum of Exophiala infections and a novel Exophiala species, Exophiala hongkongensis. J Clin Microbiol 51:260–267. https://doi.org/10.1128/JCM.02336-12

Wrzosek M, Ruszkiewicz-Michalska M, Sikora K, et al (2017) The plasticity of fungal interactions. Mycol Prog 16:101–108. https://doi.org/10.1007/s11557-016-1257-x

Yurkov AM (2018) Yeasts of the soil – obscure but precious. Yeast 35:369–378. https://doi.org/10.1002/yea.3310

Zarnowski R, Sanchez H, Andreu C, et al (2021) Formation and characterization of biofilms formed by salt-tolerant yeast strains in seawater-based growth medium. Appl Microbiol Biotechnol 105:2411–2426. https://doi.org/10.1007/s00253-021-11132-1

Zhang Z, Feng H, Qiu Y, et al (2022) Dysbiosis of Gastric Mucosal Fungal Microbiota in the Gastric Cancer Microenvironment. J Immunol Res 2022:1–14. https://doi.org/10.1155/2022/6011632

Figure legends

Fig. 1. Seasonal circulation of yeasts in lake ecosystem (based on BIEDUNKIEWICZ i in. 2020)

Fig. 2. Location of research sites against the background of the administrative map of Olsztyn (north-east Poland) **1** – Unsupervised beach on lake Kortowskie; **2** - Unsupervised beach on lake Skanda; **3** - Supervised beach on lake Skanda; **4** - Unsupervised beach on lake Tyrsko; **5** - Unsupervised beach on lake Ukiel; **6** - Supervised beach on lake Ukiel („ Miejska"); **7** - Supervised beach on lake Ukiel („Omega"); **8** - Supervised beach on lake Ukiel („Słoneczna Polana").

Fig. 3. Most often isolated species **A** – *Barnettozyma californica* 1000x; **B** – *Nakasemomyces glabratatus* 1000x; **C** – *Cutaneotrichosporon jrovecii* 1000x; **D** – *Cutaneotrichosporon moniliiforme* 1000x; **E** – *Debaryomyces hansenii* 1000x; **F** – *Solicoccozyma aeria* 1000x.

Fig. 4. Number of isolated species divided into beaches of individual lakes

Fig. 5. Average yeast concentration in sand over both research seasons

Table legends

Tab. 1. Values of meteorological parameters during sand sampling

Tab. 2. List of species isolated in the study, including division, BSL and RG indicator values, and the number of isolates obtained, categorized by research parameters

Tab. 3. List of two-species isolates

Tab. 4. Statistical dependence of the number of isolates and strain concentration on meteorological parameters (Spearman rank correlation coefficient)

Appendage legends

Appen. A. The number of isolated yeasts

Appen. B. Estimated number of users of the „Ukiel" recreational complex and supervised beach on Lake Skanda during the year - data of the Sports and Recreation Center in Olsztyn