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Author-formatted, not peer-reviewed document posted on 27/01/2025

DOI: <https://doi.org/10.3897/arphapreprints.e147793>

Potentially Pathogenic Yeasts to Humans Isolated From Sandy Beaches Used For Recreational Purposes

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1 **Potentially Pathogenic Yeasts to Humans Isolated From Sandy Beaches Used For Recreational Purposes**

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12 **Running head:** Potentially pathogenic yeast isolated from beach sand

13

14 **Abstract**

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

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

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

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

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

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

38 1. Introduction

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

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

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

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

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

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

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

84 **2. Materials and Methods**

85 **2.1. Research area and material**

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

93 **2.2. Sampling**

94 Sampling sites were designated at the midpoint of the shoreline of each beach, 3 meters from the water’s edge.
 95 Fifty-gram sand samples were collected from two depths (10 cm and 50 cm) using a soil auger (Fiskars)
 96 sterilized with a 70% alcohol solution. For sample unification, a 1 m x 1 m square was marked at the designated
 97 location, and 5 soil cores were taken (one from each corner of the square and one from the intersection of the
 98 diagonals). From each core, 10 grams of soil were collected into a sterile ziplock bag, sealed, and mixed to
 99 obtain a unified 50-gram sample. Samples were collected during the first week of each month of the research
 100 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
 101 research seasons, a total of 448 50-gram sand samples were collected from 8 study sites. During sample
 102 collection, air temperature and humidity were measured, and the UV index was recorded (Tab. 1). Until yeast
 103 isolation, the collected sand samples were stored under refrigeration (4°C) (Wójcik et al. 2013).

104 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

105 **D** – average temperature of the month during the day

106 **N** – average temperature of the month at night

107

108

109 **2.3. Yeast isolation and cultivation**

110 The yeast isolation procedure was carried out within 48 hours of sand sample collection. Yeasts were isolated
111 from the sand samples using a modification of methods described in previous studies (Wójcik et al. 2013, 2016).

112 From the 50-gram sand samples, a 10-gram sand samples were placed in 90 ml of sterile tap water (1:10

113 dilution) and shaken for 15 minutes. Yeasts were isolated by surface inoculation of 0.1 ml of the shaken

114 suspension on Bengal Rose Agar with chloramphenicol (RBC) (Wójcik et al. 2013, 2016; Echevarría and Iqbal

115 2021). Each inoculation on the RBC medium was performed in triplicate. Given that 448 sand samples were

116 tested, the total number of inoculations was 1344. In pilot studies, the tested media included RBC agar,

117 Sabouraud agar with chloramphenicol, PDA (potato dextrose agar), and Czapek-Dox agar. The highest

118 efficiency in yeast isolation from the studied sites was achieved using RBC medium. This medium was selected

119 for further research due to the selective properties of Rose Bengal. Pilot studies demonstrated that RBC medium

120 effectively inhibits the growth of filamentous fungi, particularly rapidly growing species such as *Rhizopus* and

121 *Mucor*, thereby facilitating the growth and isolation of yeasts. Inoculated media were incubated at 25°C for 48-

122 72 hours. In pilot studies conducted to validate the method, no growth was obtained at 37°C, so a 25°C

123 incubation temperature was adopted for effective isolation in further studies. After incubation, macroscopic and

124 microscopic evaluations of the colonies were conducted to differentiate yeast colonies from bacterial and

125 filamentous fungal colonies. The yeast colonies were counted and passaged onto Sabouraud agar slants with

126 chloramphenicol and incubated at 25°C for 48 hours. After incubation the cultures were stored under

127 refrigeration (4°C) for further studies.

128 **2.4. Yeast count assessment**

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

132 **2.5. Yeast identification**

133 A macromorphological description of the verified yeast colonies was made, including features such as shape,
 134 color, consistency, surface prominence, edge structure, transparency, smell, and growth into the medium. The
 135 ability of the isolated yeasts to ferment a basic carbohydrate series (glucose, galactose, maltose, sucrose, lactose)
 136 was tested using a liquid medium for carbohydrate zymogram (tested sugar 20 g; peptone 0.5 g; distilled water
 137 1000 ml; bromothymol blue with NaOH to achieve a blue color). Suspensions of the colonies of the tested strains
 138 were prepared in sterile tap water with turbidity of 3° on the McFarland scale. A 40 µl drop of the suspension
 139 was added to the zymogram medium tubes and incubated at 25°C for 48 hours. Fermentation ability was
 140 indicated by a change in the color of the solution from blue to yellow (lowering of the medium's pH due to
 141 fermentation) and/or medium turbidity (increased yeast biomass).

142 To analyze micromorphological features, microcultures were prepared on Nickerson's agar (Dynowska and
 143 Ejdys 2011; Dynowska and Biedunkiewicz 2013; Kulesza et al. 2021; Bałabański and Biedunkiewicz 2023).
 144 Microcultures were set up in sterile moist chambers by passaging colony inoculum onto the surface of
 145 Nickerson's agar, followed by covering it with 20 µl of rabbit serum solution with bacteriological broth (1:1).
 146 The inoculum was then covered with a sterile coverslip. To maintain appropriate humidity, 1 ml of sterile
 147 distilled water was placed at the bottom of the chamber. The microcultures were incubated at 25°C for 144 hours
 148 to reveal diagnostically significant features, such as the size, shape, location, and formation of blastospores, the
 149 ability to produce chlamydospores, pseudohyphae, and true hyphae, as well as their size, shape, and arrangement
 150 (Dynowska and Ejdys 2011; Biedunkiewicz and Górska 2016; Biedunkiewicz et al. 2020; Kulesza et al. 2021;
 151 Bałabański and Biedunkiewicz 2023). Microscopic observations of the microcultures were made at 48, 72, and
 152 144 hours of incubation, with photographic documentation at each stage..

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

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

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

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

170 **2.6. Statistical analysis**

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

175 **3. Results**

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

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	<i>Aureobasidium pullulans</i> (de Bary & Löwenthal) G. Arnaud 1918	A	1	1	5	4	1	2	3	1	4	1	4	1	0	3	1
2	<i>Sporobolomyces xanthus</i> (Nakase, G. Okada & Sugiy.) Boekhout 1991	B	1	1	1	1	0	1	0	0	1	0	1	0	0	0	1
3	<i>Barnettozyma californica</i> (Lodder) Kurtzman, Robnett & Bas.-Powers 2008	A	1	1	12	7	5	11	1	8	4	8	4	2	0	1	9
4	** <i>Candida albicans</i> (C.P. Robin) Berkhout 1923	A	2	2	1	1	0	1	0	1	0	0	1	0	1	0	0
5	** <i>Nakaseomyces glabratus</i> (H.W. Anderson) Sugita & M. Takash 2022	A	2	2	12	6	6	8	4	7	5	3	9	0	5	1	6
6	<i>Pichia pseudolambica</i> (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	<i>Citeromyces matritensis</i> (Santa María) Santa María 1957	A	1	1	8	5	3	8	0	6	2	2	6	2	5	0	1
8	<i>Clavispora lusitaniae</i> Rodr. Mir. 1979 (anamorfa: <i>Candida lusitaniae</i>)	A	2	2	1	1	0	0	1	1	0	0	1	0	0	0	1
9	<i>Cryptococcus amyloletus</i> (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	<i>Cryptococcus uniguttulatus</i> (Wolfram & Zach) Phaff & Fell 1970	B	1	1	1	1	0	1	0	0	1	0	1	0	1	0	0
11	<i>Cutaneotrichosporon jirovecii</i> (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	<i>Cutaneotrichosporon moniliiforme</i> (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	<i>Cyniclomyces guttulatus</i> (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	** <i>Debaryomyces hansenii</i> (Zopf) Lodder & Kreger-van Rij 1984	A	1	1	24	7	17	10	14	13	11	5	19	4	7	2	11
15	** <i>Geotrichum albidum</i> (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	<i>Geotrichum galactomycetum</i> 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	<i>Dothiora sorbi</i> (Wahlenb.) Fuckel 1870	A	1	1	1	0	1	0	1	1	0	0	1	0	0	0	1
18	<i>Exophiala bergeri</i> Haase & de Hoog 1999	A	2	2	1	0	1	0	1	0	1	0	1	0	0	1	0
19	<i>Exophiala castellanii</i> Iwatsu, Nishim. & Miyaji 1984	A	2	2	2	1	1	2	0	0	2	0	2	0	0	2	0
20	<i>Exophiala jeanselmei</i> (Langeron) McGinnis & A.A. Padhye 1977	A	2	2	3	3	0	0	3	3	0	0	3	0	3	0	0
21	<i>Hanseniaspora osmophila</i> (Niehaus) Phaff, M.W. Mill. & Shifrine 1956	A	1	1	1	1	0	1	0	0	1	1	0	0	0	1	0
22	<i>Isabelozyma rhagii</i> (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	<i>Komagataella pastoris</i> (Guillerm.) 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	<i>Kondoa malvinella</i> (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	<i>Kregervanrija fluxuum</i> (Phaff & E.P. Knapp) Kurtzman 2006 (anamorfa: <i>Candida vini</i>)	A	1	1	1	1	0	1	0	1	0	0	1	0	1	0	0
26	<i>Leucosporidium scottii</i> Fell, Statzell, I.L. Hunter & Phaff 1970	B	1	1	1	0	1	1	0	0	1	0	1	0	0	0	1
27	<i>Cryptococcus lipoferus</i> (Den Dooren) C.E. Skinner 1970	A	1	1	2	2	0	2	0	2	0	0	2	0	0	0	2
28	<i>Lodderomyces elongisporus</i> (Recca & Mrak) Van der Walt 1971	A	1	1	1	0	1	1	0	1	0	0	1	0	0	0	1
29	<i>Metschnikowia pulcherrima</i> Pitt & M.W. Mill. 1968	A	1	1	1	1	0	1	0	0	1	1	0	0	1	0	0
30	<i>Moniliella spathulata</i> (de Hoog) C.A. Rosa & Lachance 2009	B	1	1	2	1	1	2	0	0	2	0	2	2	0	0	0
31	<i>Mycogloea nipponica</i> Bandoni 1998	B	1	1	6	2	4	4	2	4	2	0	6	1	3	0	2
32	** <i>Nadsonia commutata</i> Golubev 1973	A	1	1	3	3	0	1	2	1	2	2	1	0	0	0	3
33	<i>Nadsonia fulvescens</i> var. <i>elongata</i> (Konok.) Golubev & M.T. Sm. 1989	A	1	1	5	3	2	3	2	0	5	4	1	3	2	0	0
34	<i>Naganishia albida</i> (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	<i>Ogataea angusta</i> (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	<i>Ogataea minuta</i> (Wick.) Y. Yamada, K. Maeda & Mikata 1994	A	1	1	1	0	1	1	0	0	1	0	1	0	1	0	0

37	<i>Oosporidium margaritiferum</i> Stautz 1931	A	1	1	6	5	1	3	3	1	5	0	6	2	3	0	1
38	<i>Papiliotrema laurentii</i> (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	<i>Papiliotrema pseudoalba</i> (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	** <i>Pichia fermentans</i> Lodder 1932	A	1	1	1	0	1	1	0	1	0	1	0	0	0	0	1
41	<i>Pichia membranifaciens</i> (E.C. Hansen) E.C. Hansen 1904	A	1	1	6	4	2	5	1	2	4	4	2	0	5	0	1
42	<i>Pichia terricola</i> Van der Walt 1957	A	1	1	1	0	1	1	0	1	0	0	1	0	0	0	1
43	<i>Rhodotorula diobovata</i> (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	<i>Saccharomyces bayanus</i> Sacc. 1895	A	1	1	3	1	2	0	3	2	1	0	3	1	2	0	0
45	<i>Saccharomyces cerevisiae</i> (Desm.) Meyen 1838	A	1	1	5	1	4	4	1	1	4	1	4	0	3	1	1
46	<i>Saccharomyces mikatae</i> 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	** <i>Saccharomycodes ludwigii</i> (E.C. Hansen) E.C. Hansen 1904	A	1	1	3	2	1	3	0	1	2	1	2	1	1	1	0
48	<i>Saitozyma podzolica</i> (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	<i>Octosporomyces octosporus</i> (Beij.) Kudryavtsev 1960	A	1	1	1	0	1	1	0	0	1	1	0	1	0	0	0
50	<i>Schizosaccharomyces pombe</i> Lindner 1893	A	1	1	1	1	0	1	0	0	1	1	0	0	0	1	0
51	<i>Schwanniomyces capriotti</i> M. Suzuki & Kurtzman 2010	A	1	1	5	4	1	3	2	2	3	5	0	2	0	1	2
52	<i>Schwanniomyces occidentalis</i> Klöcker 1909	A	1	1	2	0	2	2	0	0	2	2	0	2	0	0	0
53	** <i>Schwanniomyces polymorphus</i> (Klöcker) M. Suzuki & Kurtzman 2010	A	1	1	8	4	4	5	3	2	6	3	5	0	4	2	2
54	<i>Schwanniomyces vanrijae</i> (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	<i>Solicoccozyma aeria</i> (Saito) Yurkov 2015	B	1	1	11	7	4	10	1	3	8	3	8	7	1	0	3
56	<i>Sydowia polyspora</i> (Bref. & Tavel) E. Müll. 1953	A	1	1	1	0	1	1	0	1	0	0	1	0	1	0	0
57	<i>Tausonia pullulans</i> (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	<i>Thelebolus globosus</i> Brumm. & de Hoog 2005	A	1	1	2	2	0	0	2	0	2	0	2	2	0	0	0
59	<i>Torulaspora globosa</i> (Klöcker) Van der Walt & Johannsen 1975	A	1	1	4	2	2	4	0	1	3	2	2	0	3	0	1
60	<i>Vanderwaltozyma polyspora</i> (Van der Walt) Kurtzman 2003	A	1	1	5	3	2	3	2	0	5	3	2	1	3	1	0
61	<i>Vanrija humicola</i> (Dasz.) R.T. Moore 1980	B	1	1	1	0	1	0	1	0	1	1	0	1	0	0	0
62	<i>Wickerhamomyces anomalus</i> (E.C. Hansen) Kurtzman, Robnett & Bas.-Powers 2008 (anamorfa: <i>Candida peliculosa</i>)	A	1	1	1	0	1	1	0	1	0	0	1	0	0	0	1
63	<i>Bullera</i> sp.	B	1	1	2	1	1	1	1	2	0	0	2	0	0	0	2
64	* <i>Cryptococcus</i> sp.	B	1/2	1/2	5	3	2	3	2	2	3	3	2	2	1	0	2
65	<i>Dipodascus</i> sp.	A	1	1	1	1	0	1	0	0	1	0	1	0	0	0	1
66	<i>Kluyveromyces</i> 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

186 *Candida albicans* – species classified to biosafety level 2 (BSL-2)

187 ***Saccharomycodes ludwigii* – species included in two-species isolates

188 **Cryptococcus* sp. – the BSL index value varies depending on the species

189 **SUM** – total number of isolates of a given species

190 **Sum*** – total number of isolates (species of two-species isolates were counted separately and included in the total sum of isolates)

191 **DV** – division (A – *Ascomycota*; B - *Basidiomycota*)

192 **M** – number of isolates obtained in the morning

193 **E** – number of isolates obtained in the evening
194 **d10** – number of isolates obtained from sand collected from a depth of 10 cm
195 **d50** – number of isolates obtained from sand collected from a depth of 50 cm
196 **S** – number of isolates obtained from the sand of supervised beaches
197 **US** – number of isolates obtained from the sand of unsupervised beaches
198 **s19** – number of isolates obtained in the 2019 research season
199 **s20** – number of isolates obtained in the 2020 research season
200 **LK** - number of isolates obtained from the beach of lake Kortowskie
201 **LS** - number of isolates obtained from the beaches of lake Skanda
202 **LT** - number of isolates obtained from the beach of lake Tyrsko
203 **LU** - number of isolates obtained from the beach of lake Ukiel
204
205
206
207

208

209 Table 2. List of two-species isolates

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

210
211

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

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

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

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

228 Among the yeasts isolated during the study, the most frequently represented species were: *Barnettozyma*
229 *californica*, *Nakasemomyces glabratus*, *Cutaneotrichosporon moniliiforme*, *C. jirovecii*, *Debaryomyces*
230 *hansenii*, and *Solicoccozyma aeria*. Other species occurred in smaller numbers (8 isolates or less) (Tab. 2, Fig.
231 3).

232 In the 2019 season, the most frequently isolated species were: *B. californica*, *Cutaneotrichosporon jirovecii*, *C.*
233 *moniliiforme*, *Schwanniomyces capriottii*, and *Tausonia pullulans* (Tab. 2) Among the species isolated in the
234 2020 season, *D. hansenii*, *Mycogloea nipponica*, *N. glabratus*, and *S. aeria* dominated (Tab. 2). Twenty-one
235 species and five strains identified only to genus (1 genus) were isolated in both research seasons, representing
236 33.3% of the species and genera pool obtained during the study. Twelve species and one strain identified only to
237 genus (1 genus) were noted exclusively in the 2019 season (19.7% of the total species and genera pool). Twenty-
238 nine species and four strains identified only to genus (2 genera) were found only in the 2020 season (46.9% of
239 the total species and genera pool) (Tab. 2).

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

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

255 In the sand from supervised beaches, the most frequently isolated species were: *B. californica*, *N. glabratus*, *C.*
 256 *moniliiforme* and *D. hansenii* (Tab. 2). From the sand of unsupervised beaches, the most common species were:
 257 *Cutaneotrichosporon jirovecii*, *C. moniliiforme*, *D. hansenii* and *S. aeria* (Tab. 2). Twenty-six species and one
 258 genus were isolated from both types of beaches (supervised and unsupervised), representing 40.9% of the total
 259 species and genera pool detected during the study (Tab. 2). Twelve species and two genera were noted only in
 260 samples collected from supervised public beaches (21.2% of the total species and genera pool). Twenty-four
 261 species and one genus (strain identified only to genus) were found only in the sand from unsupervised beaches
 262 (37.8% of the total species and genera pool).

263 During the two-year study, the most isolates were obtained from the beaches of Lake Skanda (87 isolates;
 264 32.95% of all isolates) and Lake Ukiel (87 isolates; 32.95% of all isolates). From Lake Kortowskie, 63 isolates
 265 were recorded (23.9% of all isolates). The fewest isolates were from Lake Tyrsko (27 isolates; 10.2% of all
 266 isolates) (Tab. 2).

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

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

286 The number of viable cells of individual strains was counted, and the obtained data were compiled in tabular
 287 form (Appen. A). On average, nearly twice as many viable yeast cells were isolated in the 2019 season (9.91×10^2
 288 CFU/g) compared to the 2020 season (5.56×10^2 CFU/g) (Appen. A; Fig. 5). The fluctuations in the average
 289 number of yeasts in 1 g of sand in the 2019 season ranged from 1.40×10^2 CFU/g in October to 5.48×10^3
 290 CFU/g in July, while in the 2020 season, they ranged from 1.40×10^2 CFU/g in October to 1.94×10^3 CFU/g in
 291 September. The highest average number of viable yeast cells was found in sand samples collected in July 2019
 292 (5.56×10^2 CFU/g), and in August and September 2020 (1.03×10^3 CFU/g and 1.94×10^3 CFU/g, respectively).
 293 The lowest concentrations were in samples collected in April, September, and October 2019, and in October
 294 2020 (1.48×10^2 CFU/g, 1.47×10^2 CFU/g, 1.40×10^2 CFU/g, and 1.40×10^2 CFU/g, respectively) (Appen. A;
 295 Fig. 5). In 2019, compared to 2020, more viable yeast cells were isolated in May, June, and July. In July 2019,
 296 the average number of viable yeast cells was an order of magnitude higher than in the 2020 season. The opposite
 297 trend was observed in April, August, and September. In August and September 2020, the average number of
 298 viable yeast cells was an order of magnitude higher than in the corresponding months of 2019. In October of
 299 both seasons, the same average number of viable yeast cells was recorded (Appen. A; Fig. 5). The differences in
 300 the average concentration of strains between individual months were statistically significant ($p = 0.0300$). Post
 301 hoc analysis showed that this difference occurred between April and July (the test considered numerical data
 302 from both research seasons and all studied beaches).

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

307 Table 4. Statistical dependence of the number of isolates and strain concentration on
 308 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 ($R=0,3396$; $p=0,0770$)

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

310

311 4. Discussion

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

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

328 Quantitative analysis and species pool of microfungi in beach sand depends on numerous atmospheric and
 329 environmental factors (Brandão et al. 2021, 2022; Frenkel et al. 2022; Novak Babič et al. 2022). The own
 330 research found a statistically significant effect of air temperature on the average concentration of strains in the
 331 sand (higher temperature resulted in a higher average strain concentration). Other authors highlight the direct
 332 impact of temperature on the survival of microorganisms in water and sand (Brandão et al. 2022; Cuartero et al.
 333 2024). Moreover, they suggest a possible connection between global warming and the microorganisms growth
 334 kinetics, pointing out that, despite the general belief that increased temperatures favor microbial growth, in the
 335 case of mesophilic microorganisms, it will act as a limiting factor, while favoring the growth of pathogens that
 336 prefer conditions closer to the human body temperature (Brandão et al. 2022). An example could be yeasts of the
 337 genus *Candida*, which exhibit xerotolerant properties (Shah et al. 2011). Furthermore, it is noted that increased
 338 temperatures affect other environmental parameters that shape the microbiological structure of beaches (Brandão
 339 et al. 2022). For instance, an indirect negative correlation between the abundance of fungi in sand and the length
 340 of exposure to sunlight has been demonstrated, with the latter causing an increase in sand temperature (Brandão
 341 et al. 2021). Similar to the own research, no statistically significant effect of air humidity on the number of
 342 yeasts per gram of soil was observed (Brandão et al. 2021).

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

351 number of users is confirmed by other studies (Lindahl et al. 2015; Kauppinen et al. 2017). This issue, however,
 352 requires further analysis, especially in the context of the number of users, due to incomplete data on population
 353 flow provided by supervisors of the studied municipal beaches (Appen. B.). Additionally, an analysis of the
 354 average concentration of yeasts in the sand of beaches across studied lakes was conducted. In both research
 355 seasons, the lowest average concentration of yeasts in the sand was recorded for the lake with the smallest
 356 surface area. This observation is likely due to the varying number of beach users, which correlates with the size
 357 and availability of recreational areas at the studied water bodies. Lake Tyrsko has the shortest shoreline,
 358 significantly limiting the bathing area and the number of users. Other researchers have pointed out the possible
 359 influence of the water body's structure on the taxonomic and numerical composition of yeast populations in the
 360 associated ecological niches (Biedunkiewicz and Górska 2016; Nowacka et al. 2018; Biedunkiewicz et al.
 361 2020).

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

366 Moreover, no significant changes in the concentration and number of yeast isolates were observed between the
 367 times of day studied (morning; evening). The own research correlates with the results of other studies, which
 368 demonstrated the weakest seasonal correlation between air and soil temperature in the spring and summer. The
 369 authors noted that soil temperature is mainly influenced by factors unrelated to the diurnal cycle (Kossowski
 370 2005; Skowera and Wojkowski 2017). Furthermore, the authors found that the diurnal amplitude of soil
 371 temperatures and the dependence of soil temperature on air temperature decrease with soil profile depth
 372 (Kossowski 2005; Skowera and Wojkowski 2017). This suggests that sand is less susceptible to temperature
 373 changes with depth. Samples for own research were taken from depths of 10 and 50 cm, which significantly
 374 limited the influence of atmospheric factors on the numerical structure of yeast populations in the sand over a
 375 diurnal cycle.

376 Analyzing the yeast abundance at different depths, it was found that the obtained results are consistent with those
 377 reported by Grishkan and Kidron (2016), who also observed higher average concentrations of yeast strains at a
 378 depth of 10 cm. This trend persisted across different research seasons. However, in the case of both parameters,
 379 the observed differences were not statistically significant. This observation may be linked to the higher content
 380 of organic carbon, water, and oxygen in the surface soil layers, where sand layers mix.. Fang and Moncrieff's
 381 (2005) studies indicate decreased organic carbon content and oxygen availability with soil depth. The same study
 382 showed that this trend does not significantly change depending on the climate and that respiratory processes and
 383 biomass development of oxygen-affiliated organisms occur most intensively in the surface soil layers. Grishkan
 384 and Kidron's (2016) research suggests that the distribution of fungal communities in soil is related to the
 385 availability of organic matter, water, aeration, salinity, and UV radiation, emphasizing its complex and non-
 386 linear nature. Another factor influencing the distribution and abundance of microfungus consortia is soil and sand
 387 porosity (Grishkan and Kidron 2016; Brandão et al. 2021). Microbial biomass density reaches its maximum
 388 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
 389 decline down to 50 cm (Grishkan and Kidron 2016). This observation aligns with the present study.

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

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

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

430 noted, along with 13 genera (81.3%) that were isolated in their research from the phyllosphere. These
 431 observations indicate the continuous circulation of microfungi between the various components of the lake
 432 ecosystem, including during the washing of sand and vegetation by lake water, as well as through surface and
 433 subsurface runoff caused by precipitation. Beach users and animals living there, e.g. waterfowl, may also be an
 434 important vector of such transmission (Biedunkiewicz et al. 2020; Brandão et al. 2021, 2022; Novak Babič et al.
 435 2022). Numerous scientific papers indicate that species such as *B. californica*, *N. glabratus*, *C. moniliiforme*, *C.*
 436 *jirovecii*, *D. hansenii* and *S. aeria* are soil microorganisms or are frequently isolated from soil, including beach
 437 sand (Yurkov 2018; Brandão et al. 2021, 2022; Kaewkrajay et al. 2021; Samarasinghe et al. 2021). The frequent
 438 notation of these species in the present study should therefore be considered a conventional observation.

439 A comparison of the taxonomic structure between the designated research seasons showed that 21 species and 1
 440 genus (strain identified only to the genus level) were isolated in both seasons. Among this group, *B. californica*,
 441 *Nakaseomyces glabratus*, *C. moniliiforme*, *D. hansenii*, *Schwanniomyces polymorphus* and *S. aeria* were noted
 442 with 3 or more isolates in each season, suggesting that they are indigenous to the studied environments. This is
 443 supported by studies that classify these species as soil yeasts (Kurtzman et al. 2011; Góralaska and Biedunkiewicz
 444 2016; Yurkov 2018; Brandão et al. 2021; Salah et al. 2021). For the species: *C. moniliiforme* and *D. hansenii* the
 445 discrepancy in the number of isolates between seasons reached an order of magnitude. It can be hypothesized
 446 that these species may be more sensitive to seasonal changes in environmental parameters, such as weather
 447 conditions or beach users traffic and activity. This is corroborated by studies from other authors (Biedunkiewicz
 448 et al. 2013, 2020; Biedunkiewicz and Góralaska 2016; Yurkov 2018; Brandão et al. 2021, 2022; Kaewkrajay et al.
 449 2021). In the 2019 season, 29 species and 2 genera (strains identified only to the genus level) were recorded,
 450 while in 2020, 12 species and 1 genus were found. The probable cause of these observed differences is the
 451 limited traffic of beach users, a potential vector of microfungal transmission at bathing sites, due to the sanitary
 452 regulations related to the COVID-19 pandemic in 2020.

453 A total of 26 species and 1 genus (strains identified only to the genus level) were found on both supervised and
 454 unsupervised beaches. Among these, the species *B. californica*, *N. glabratus*, *Cutaneotrichosporon jirovecii*, *C.*
 455 *moniliiforme*, *D. hansenii*, and *S. aeria* were noted with 3 or more isolates on each type of beach. These species
 456 have previously been identified as characteristic or frequently isolated from soil and lake environments (Yurkov
 457 2018; Biedunkiewicz et al. 2020; Brandão et al. 2021, 2022; Kaewkrajay et al. 2021; Samarasinghe et al. 2021).
 458 For the species, *B. californica*, *N. glabratus* and *D. hansenii* more isolates were found on supervised beaches.
 459 *N. glabratus* often forms part of the physiological mycobiota of humans and, in immunocompromised
 460 individuals, can become pathogenic (Biedunkiewicz and Góralaska 2016; Biedunkiewicz et al. 2020; Brandão et
 461 al. 2021). *B. californica* is classified as a soil yeast (Kurtzman et al. 2011; Yurkov 2018) and has also been
 462 isolated from water, plant debris, and animal droppings (Mullen et al. 2018). *B. californica* and *D. hansenii* are
 463 also found in food products (Desnos-Ollivier et al. 2008; Drumonde-Neves et al. 2016). The presence of these
 464 species suggests a link to human activity, as supervised beaches generally attract more visitors during the
 465 summer season. However, overall, half as many species were found exclusively on supervised beaches compared
 466 to those isolated solely from unsupervised beaches. This discrepancy is likely due to the better sanitary condition
 467 of supervised beaches, which are cleaned and have their sand replenished by supervisors.

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

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

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

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

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

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

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

509 During the study, numerous strains classified in the genus *Candida* were isolated. Species belonging to this
 510 genus are the most commonly isolated from the blood of hospitalized patients. Additionally, they are frequently
 511 found in the oral cavity, urinary tract, and female genital tract (Silva et al. 2012). There is an increase in
 512 infections caused by *Candida* fungi in immunocompromised individuals, including cancer patients and
 513 postoperative patients (Silva et al. 2012). Factors predisposing *Candida* species to infect humans include their
 514 ability to adhere to host epithelial cells and form biofilms (Silva et al. 2012; Kulesza et al. 2021; Martin et al.
 515 2021; Zarnowski et al. 2021; Bałabański and Biedunkiewicz 2023). In the present study, the dominant species
 516 was *Nakaseomyces glabratus* (formely *C. glabrata*) with a single isolate of *C. albicans* also recorded. *N.*
 517 *glabratus* and *C. albicans* are among the four species responsible for approximately 95% of diagnosed
 518 candidiasis (Silva et al. 2012). Both species are classified as BSL-2 and RG-2. They mainly cause infections of
 519 the skin and mucous membranes in immunocompromised individuals (Silva et al. 2012; Biedunkiewicz and
 520 Góralaska 2016; Góralaska and Biedunkiewicz 2016; Biedunkiewicz et al. 2020; Frenkel et al. 2022). *N. glabratus*
 521 primarily causes infections of the oral cavity, urogenital system, and bloodstream, and it exhibits resistance to
 522 many antifungal agents (Silva et al. 2014). *C. albicans* is considered an indicator of fecal contamination in the
 523 environment (Brandão et al. 2021). Among the frequently isolated species was also *Citeromyces matritensis*, the
 524 teleomorphic stage of *Candida globosa*. Research by Kheireddine and co-authors (2019) indicated weak growth
 525 of this species at 37°C and emphasized the lack of clinical infection cases caused by this species.

526 Numerous strains of *B. californica* were isolated during the study This species is not pathogenic but rather
 527 saprotrophic (Ni et al. 2011). Similarly, *Solicoccozyma aerea* was frequently noted. Research indicates that it can
 528 be isolated from animals, but there are no reports of human infections caused by this species (Bertout et al.
 529 2022). *S. aerea* may be a part of the physiological mycobiota of the colon, as it has also been isolated from
 530 human feces (Guo et al. 2021). Additionally, it has been linked to appetite suppression and the activation of
 531 interleukin 17 in sensitive individuals, which may be associated with disturbances in gut microbiota and
 532 mycobiota. Studies also show an increase in *S. aerea* expression in cancer patients, with the potential for
 533 considering this species as a marker of gastric cancer, while excluding it as an etiological factor in the disease
 534 (Zhang et al. 2022). In summary, the literature suggests that this species may have a positive impact on human
 535 health as a cancer marker. There are no reports of its potential pathogenicity to humans; however, its isolation
 536 from animals suggests it could be considered as a potential human pathogen.

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

546 Six isolates from the genus *Exophiala* were recorded during the study. Of the identified species, *E. jeanselmei*
 547 was the most frequently isolated. This species can cause subcutaneous ulcers and nodules in transplant recipients
 548 (Lief et al. 2011). In advanced cases, it leads to chronic, purulent infections of subcutaneous tissue (Badali et al.
 549 2009). The less frequently isolated species, *E. bergeri*, has been reported as an etiological agent of nail infections
 550 (Woo et al. 2013), while *E. castellanii* has been noted in subcutaneous infections (Biedunkiewicz and Schulz
 551 2012). All species from the genus *Exophiala* isolated in this study are potential pathogens for
 552 immunocompromised individuals, as they are classified as BSL-2 and RG-2 (Biedunkiewicz and Schulz 2012;
 553 Biedunkiewicz and Górska 2016; Biedunkiewicz et al. 2020; Kulesza et al. 2021; Bałabański and
 554 Biedunkiewicz 2023).

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

558 **5. Conclusions**

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

574 **Statements and declarations**

575 ***Funding***

576 This work was supported by the Department of Microbiology and Mycology, Faculty of Biology and
 577 Biotechnology, University of Warmia and Mazury in Olsztyn (12.610.009-300), and European Social Fund
 578 (POWR.03.05.00-00-Z310/17)

579 ***Competing interests***

580 Authors declare that they have no conflict of interest

581 ***Availability of data and material***

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

584 ***Code availability***

585 Not applicable

586 ***Authors' contributions***

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

592 ***Ethics approval***

593 Not applicable

594 **Acknowledgments**

595 We would like to extend our heartfelt gratitude to the staff of the Department of Microbiology and Mycology,
596 Professor Dorota Górnica, and Professor Aleksander Świątecki, for their support in conducting molecular
597 research, which enriched the substantive value of the study.

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800 **Figure legends**

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

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

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

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

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

812 **Table legends**

813 **Tab. 1.** Values of meteorological parameters during sand sampling

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

816 **Tab. 3.** List of two-species isolates

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

819 **Appendage legends**

820 **Appen. A.** The number of isolated yeasts

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

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