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Microfungal diversity in the swash zone interstitial water (SZIW) of three Ligurian urban beaches (NW, Italy)

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Abstract

Microfungal species abundance and specific composition of three urban beaches near Genoa (Liguria, NW Italy) in the swash zone interstitial water (SZIW) were evaluated. These beaches are characterized by a high presence of tourists. The investigation was carried out in order to: i) characterize the beach SZIWs microfungal diversity; ii) evaluate the presence of potential pathogenic and/or allergenic fungi for human; iii) investigate the influence of biotic and abiotic parameters on fungal species distribution. Qualitative and quantitative data were collected during April, July and September 2013.
On the whole, 1,157 Morphological Taxonomic Units (MTUs), belonging to 52 *taxa*, were identified. Among these 52 *taxa*, 45% were common to all three beaches. The majority of them were saprotrophic species. Several opportunistic (mainly belonging to genus *Aspergillus*) and allergenic (such as *Aspergillus*, *Cladosporium*, *Mucor*, etc) fungi were found. Moreover several colonies of pathogenic species *Aspergillus niger* were found. Concerning the quantitative results, only the month (sampling period) seems to affect the amount of MTUs.

Keywords: filamentous microfungi; fungal community; environmental stress; seashore; anthropic impact.

Riassunto

È stata studiata l'abbondanza e la composizione specifica della comunità microfungina presente nell'acqua interstiziale di battigia in tre spiagge urbane ubicate in prossimità di Genova caratterizzate da una forte pressione antropica. In particolare si è voluto: 1) caratterizzare la diversità microfungina nell'acqua interstiziale di battigia; 2) verificare la presenza di funghi potenzialmente patogeni e/o allergenici per l'uomo; 3) studiare l'influenza dei parametri biotici e abiotici sulla distribuzione delle specie fungine. A tal fine sono state effettuate tre campagne di campionamento (aprile, luglio e settembre 2013) nelle quali sono stati raccolti dati qualitativi e quantitativi.
Complessivamente sono stati isolati e identificati 1157 morfotipi (MTU) appartenenti a 52 *taxa*. Tra i 52 *taxa* identificati, il 45% era comune a tutte e tre le spiagge. Sono state isolate diverse specie opportuniste (principalmente appartenenti al genere *Aspergillus*) e allergeniche (come *Aspergillus*, *Cladosporium*, *Mucor*,

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ecc). Inoltre, sono state individuate diverse colonie della specie patogena *Aspergillus niger*. Per quanto riguarda i dati quantitativi solo il mese di campionamento sembra influenzare il numero di MTU presenti.

Parole chiave: funghi filamentosi; comunità fungina; stress ambientale; costa; impatto antropico.

Introduction

Marine coastal environments are complex and extreme ecosystems characterized by a high level of biodiversity (Hyde 1989; Gonzalez et al. 1998; Migahed 2003; Babu *et al.* 2010; Gonçalves de Oliveira et al. 2011). The human exploitation of beaches often alters ecological balance, and causes the deterioration of the environment itself (Lucrezi et al. 2009; Montefalcone et al. 2009). In this scenario, urban beach represents an interesting and multidimensional environment subjected to the marine, terrestrial and human influence (Salvo et al. 2005; Roca and Villares 2008). Several studies have shown that urban beach soils host a great number of organisms, such as small invertebrates, bacteria, fungi, virus, algae, and diatoms which can adapt in constantly changing environments (Mancini et al., 2004; Goncalves de Oliveira et al., 2011; Zakaria et al., 2011). Specifically, Moore-Landecker (1996) has demonstrated that microfungi are important components of beach ecosystems, in both soil and water. Indeed, they play a key role in nutrient cycle decomposing complex organic substrates such as cellulose, and animal products, biodegrading hydrocarbons, or living as opportunistic parasite on marine organisms (Gonzales et al., 1998; Kohlmeyer and Volkmann-Kohlmeyer, 2003; Vogel et al., 2007; Gomes et al., 2008; Babu et al., 2010; Matallah-Boutiba et al., 2011; Zakaria et al., 2011).

However, the microfungal communities of urban beach swash zones are poorly investigated and only few studies are available till now (Vezzulli et al., 2009; Onofri et al., 2011; Vassallo et al., 2012). These zones represent a dynamic environment, characterized by large sediment transport rates, rapid morphological changes, and high turbulence levels (Masselink and Puleo, 2006). Some authors compared several beach habitats (e.g. backshore, foreshore) and found a microbial reservoir and a high level of species richness in swash zones (Vassallo and Fabiano, 2005; Vezzulli et al., 2009; Onofri et al., 2011; Vassallo et al., 2012). Owing to their wide ecological range (plasticity), several urban beach microfungi may also behave as opportunistic or pathogenic (Hyde, 1989; de Hoog et al., 2000; Heaney et al., 2009; Lee et al., 2011), becoming a human health potential risk. Nevertheless, urban beach microorganism communities have been often neglected and real health risks have not been assessed (Hyde, 1989; Salvo and Fabiano, 2007; Vezzulli et al., 2009; Onofri et al., 2011). Recently, the study of such communities has received a greater attention in view of the safety of beach environments since they are very attractive and intensely visited recreational areas (Guidelines for recreational water environments – Volume 1 Coastal and Fresh Waters – World Health Organization, 2003). Therefore, the investigation of urban beaches swash zone interstitial water (SZIW) mycobiota results essential to know the related mycological biodiversity, to prevent potential sources of pollution, and possible contaminations of adjacent waters. In addition, the study of SZIW mycobiota permits to estimate the quality and health of urban beaches, and to assess the risks of potential contaminations by opportunistic, pathogenic or allergenic fungi.

The paper deals with the microfungal species abundance and diversity of three urban beaches SZIWs in Liguria (NW Italy) and presents the results of some statistical tests on the potential influence of biotic and abiotic parameters on microfungal communities.

The main goals are: i) characterize the beaches SZIWs microfungal diversity; ii) evaluate the presence of potential pathogenic or allergenic fungal species to humans; and iii) investigate the influence of biotic (in

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particular human frequentation, organic matter content) and abiotic (seasonality, pH, temperature of air and water, relative humidity) parameters on fungal species temporal distribution.

Materials and methods

Study area

The study focuses on three intensely visited urban beaches located within the boundaries of the Municipality of Genoa (Italy, Liguria, NW Mediterranean Sea), from west to east: Voltri, Multedo and Quinto, hereinafter referred as VB, MB and QB respectively (Fig. 1).

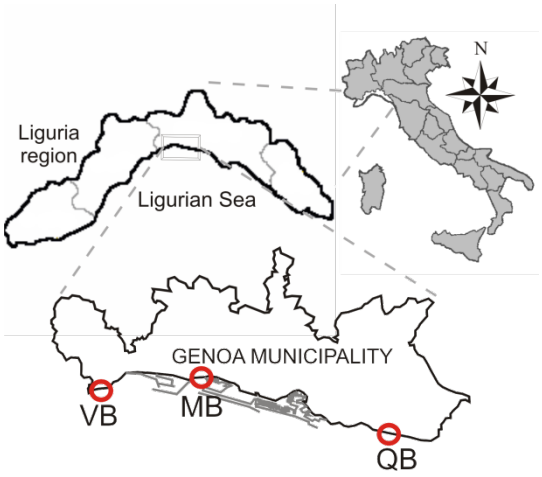

Fig. 1. Localization of studied beaches (red circles) within Genoa municipality, Voltri (VB), Multedo (MB) and Quinto (QB).
Fig. 1. Localizzazione delle spiagge oggetto di studio (cerchi rossi) all'interno del comune di Genova, Voltri (VB), Multedo (MB) and Quinto (QB).

VB is located on the western boundary of Genoa, and limited westward by the presence of a freshwater outlet. It is characterized by a great confluence of urban activities located in the immediate nearby of the beach: residential and municipal buildings, commercial activities, sports facilities, railway, and beach resorts (Fig. 2). MB is located within the Genoa harbor, between the mouth of a stream (west) and the Genoa oil terminal (east). For this reason bathing activities are forbidden all-year-round. It undergoes several pressures by sports facilities, residential buildings, petrol stations, and some sailing and fishing associations (Fig. 2). QB is a typical gravely cobble pocket beach, which is nested within one of the most valuable stretches of the urban coast. The residential context is characterized by an alternation of ancient seafaring centers, historical mansion houses with wide gardens, and more recent buildings. QB hosts a freshwater discharge that may cause an increase of nutrients due to the organic matter and fecal pollution contamination in the adjacent coastal waters (Fig. 2).

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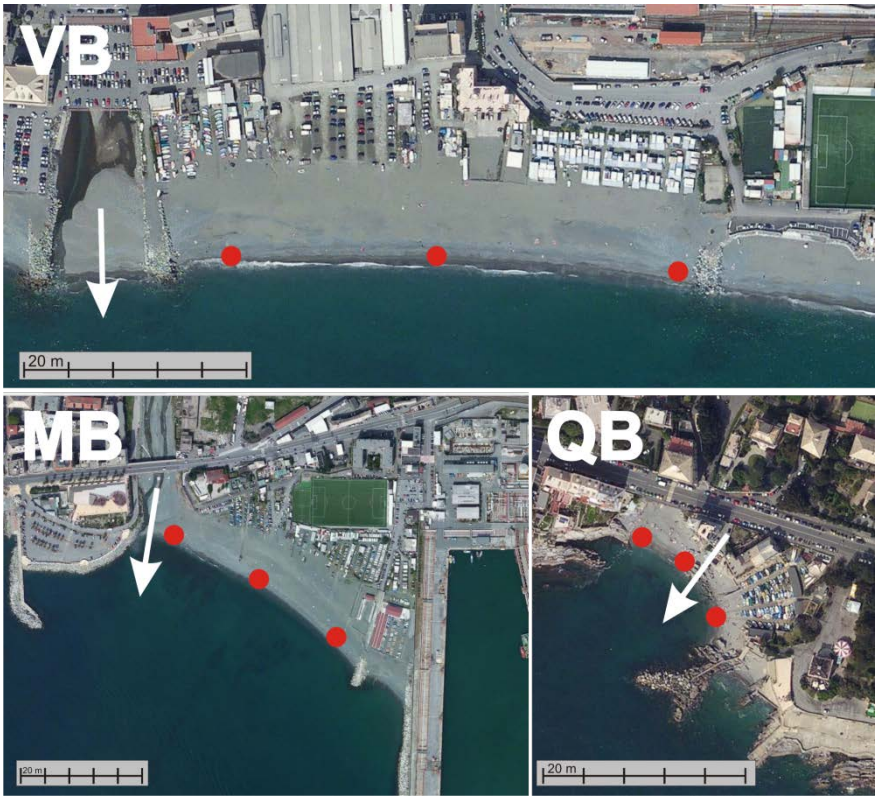

Fig. 2. Stellite view of the analyzed beaches. Red dots identify sampling stations distribution. White arrows show main freshwater outlet (images from www.regione.liguria.it)
Fig. 2. Immagini satellitari delle spiagge analizzate. I punti rossi indicano la distribuzione delle stazioni di campionamento. Le frecce bianche indicano le pricipali foci di torrenti (immagini prese da www.regione.liguria.it)

Sampling and samples processing

The three beaches were sampled during April, July, and September 2013. For each beach, three sampling areas were chosen along the swash zone equally spaced along the beach extent. In each sampling area three samples were collected. As a consequence, 27 samples of water and sediment were collected overall in each month. On the whole 81 water and sediment samples were collected during this study.
Sampling activities were always carried out with dry weather conditions (from at least three days before sampling) and calm sea (Beaufort scale 2 or lower). Sample areas were determined by first locating the landward edge of the swash zone, which is the zone alternatively covered and exposed by waves, and then chosen 1 m inland from the outer edge of the swash zone. For the interstitial water, the depth of dry sand was measured and the samples were harvested with a shovel. Each hole was dug deep 20 cm enough to allow free interstitial water to accumulate (Fig. 3).
Interstitial water was collected by means of sterile Falcons (45 ml) and conserved at 4°C until the arrival at the laboratory. Additional samples of interstitial water were also collected for the pH measurement, and sediment samples were collected for the quantification of the total organic matter present (% Total Organic Matter; TOM) in the sediments of the swash zone.
In addition, data about air temperature (°C), humidity (%) and seawater temperature (°C) were acquired from www.dicat.unige.it and www.ambienteinliguria.it for every sampling month.
Microfungi were isolated by a dilution soil-plate method (Gams 1987) that permits to isolate culturable fungal strains. The final dilution (1:100000) was obtained mixing 1 ml of interstitial water with sterile water.

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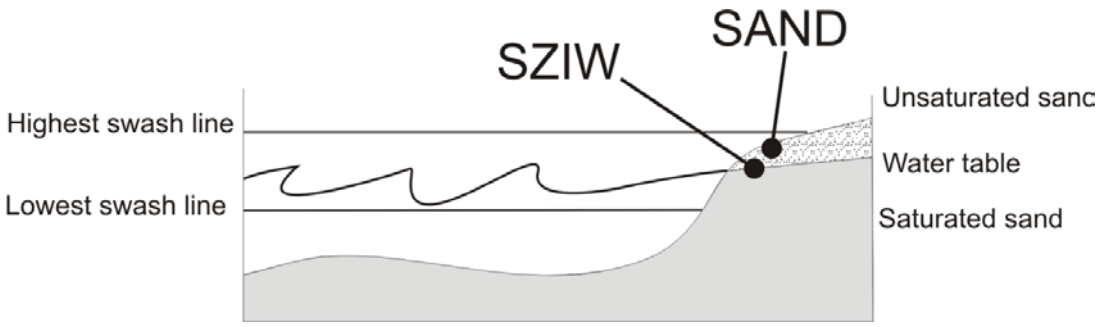

Fig. 3. Plan for the SZIW collection
Fig. 3. Schema di campionamento della SZIW.

Each sample was inoculated in Petri dishes (12 cm diameter) on modified Sabouraud (15% agar, 10% glucose, 5% neopepton) and Malt Extract Agar at 24 °C in the dark (Hyde and Pointing, 2000). The Petri dishes were inspected daily for 20 days. Single colonies of fungi and yeasts were counted and isolated. The number of morphological taxonomic units (MTU) per 100 ml was calculated in order to quantify the total amount of colonies for each station.
The colonies were identified by polyphasic approach. In order to obtain a clear identification, in particular for the most critical strains, the results of morphological and physiological identification (Rifai 1969; Ellis 1971; Raper and Fennel 1977; Schipper 1978; Kohlmeyer and Kohlmeyer 1979; Bisset 1984, 1991a, 1991b, 1991c; Kiffer et al. 1999; Klich 2002) were compared with the results of sequence of the b-tubulin and ITS gene (Glass N.L., Donaldson 1995). The sequence data were searched by means of sequence databases (NCBI/BLASTN algorithm) to confirm the identification.
Interstitial water pH was measured with a digital pH-Meter, and the TOM was determined through mass loss-on-ignition (LOI) method that involves the heated destruction of all organic matter in sediments. A known quantity of sample oven-dried at 60 °C for 24 h, was washed at 450 °C for 4 hours. The sample was then cooled and weighed. The TOM is confuted as the difference between the initial and final sample weights divided by the initial sample weight.

Statistical data analyses

The species accumulation curves were plotted (number of samples vs number of identified species) and the estimation of species richness was computed to interpret the effect of sampling effort on survey efficiency.
A Principal Component Analysis (PCA) was performed in order to analyze the effect of environmental variables (organic matter, pH, humidity, water and air temperature) on the mycobiota. Since the environmental variables are expressed in different measurement scales, we computed a PCA on the correlation matrix. The Euclidian distances were used to jointly display sites and variables in the PCA plot. Vector fitting was executed to interpret environmental variables onto ordination. A two-way ANOVA test was performed to analyze the fungal abundance dependence on beach and season, respectively (Beach: three fixed levels; Month: three fixed levels). All the statistical analyses were computed by using Vegan package in R system (version 3.1.0; 2014).

Results

From the SZIW samples were isolated 1,157 MTUs belonging to 52 taxa (Table 1). 37 species of filamentous fungi were identified and 15 taxa were unidentified. Among the latter taxa, seven fungi were

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unable to complete their lifecycle and grew as sterile mycelia. In spite of the filamentous fungi that were the main target of our survey, 8 different morphotypes of yeasts were distinguished on the basis of the macro-morphological features of the colony. The number of MTUs per 1 ml is summarized in Table 2 and the data collected in each sampling month about pH, TOM, air and water temperature and humidity values are displayed in Table 3.

In table 4 the results of a TWO WAY ANOVA revealed significant differences among sampling month (0.1 confidence level) whereas differences among beaches did not result statistically significant. Figure 4 shows the accumulation curve approach asymptote for all the three areas, both plotted as total and separated per sites. The PCA plots are shown in Figure 5 and Table 5. The cumulative percentage variance explained along the first two axes amounts to 84% (50% on the first axis; 34% on the second axis). The eigenvalues associated to the first and second axes are 2.50 and 1.72, respectively (Tab. 5). The gradient along the first axis appears to be correlated with a seasonality trend; whereas, air and water temperatures are significantly correlated with the different sites (= beaches) composition. This trend is further confirmed by the results of vector fitting test (Tab. 6). The most significant variables (see r^2 and Pr(>r)) in fact, were the water and air temperatures which depend on the season. Relevant for the communities species composition it was also the value of pH.

Tab. 1. List of 52 *taxa* discerned (37 species of filamentous fungi and 15 unidentified *taxa*) and their abundance of MTU measured during the isolation procedure.
Tab. 1. Lista dei 52 *taxa* individuati (37 species di fungi filamentosi e 15 *taxa* non identificati) e la loro abbondanza di MTU misurata durante le procedure di isolamento.

	April			**July**			**September**		
Fungal species	**QB**	**MB**	**VB**	**QB**	**MB**	**VB**	**QB**	**MB**	**VB**
Aspergillus flavus var. oryzae (Ahlb.) Kurtzman	5	4	3	-	22	3	-	-	16
Aspergillus flavus Link	-	1	-	-	-	-	6	2	-
Aspergillus niger Tiegh.	1	-	-	2	2	-	20	1	-
Aspergillus ochraceus Wilh.	-	-	2	-	2	2	18	-	-
Aspergillus parasiticus Speare	-	-	-	-	-	-	-	-	4
Aspergillus pulverulentus (McAlpine) Thom	-	-	2	-	-	-	-	-	-
Aspergillus tamarii Kita	-	-	-	2	1	1	-	-	-
Aspergillus terreus Thom	-	12	18	-	-	-	9	1	-
Aspergillus tubingensis Mosseray	-	-	-	-	3	-	-	-	-
Boeremia exigua var. *exigua* (Desm.) Aveskamp, Gruyter & Verkley	3	-	-	-	3	-	-	-	-
Circinella minor Lendn.	-	4	1	4	-	-	-	3	1
Cladosporium cladosporioides (Fresen.) G.A. de Vries	-	-	-	-	-	-	1	-	1
Cladosporium herbarum (Pers.) Link	-	-	-	-	-	2	-	-	-
Cladosporium sphaerospermum Penz.	-	-	-	-	-	-	-	-	1
Cladosporium tenuissimum Cooke	5	-	-	-	-	-	-	-	-
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	2	2	-	-	-	2	-	-	-
Mucor hiemalis Wehmer	-	-	-	-	-	3	2	3	2
Mucor mucedo Fresen.	1	-	3	1	2	10	5	-	-
Mucor racemosus Bull.	-	-	-	-	-	1	1	-	1
Penicillium brevicompactum Dierckx	26	14	7	7	-	3	7	1	8
Penicillium chrysogenum var. *chrysogenum* Thom	1	-	-	-	6	3	-	-	1
Penicillium citrinum Thom	-	-	1	-	11	-	-	-	-
Penicillium decumbens Thom	7	-	-	-	4	-	-	-	-
Penicillium digitatum (Pers.) Sacc.	-	2	-	-	-	-	-	-	-
Penicillium griseofulvum Dierckx	-	-	-	-	5	4	-	-	-
Penicillium italicum Wehmer	-	-	2	-	-	-	-	-	-
Penicillium simplicissimum Thom	-	-	-	-	-	7	-	-	-

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Penicillium spinulosum Thom	3	-	-	-	39	-	-	-	1
Phoma pomorum Thüm.	3	-	-	1	-	-	-	-	-
Rhizopus arrhizus A. Fisch.	1	3	-	1	1	2	-	-	1
Rhodotorula Type 1 F.C. Harrison	40	2	6	-	1	7	19	-	-
Rhodotorula Type 2 F.C. Harrison	-	-	-	-	1	-	-	-	-
Sterile mycelia Type 1	23	3	9	2	-	10	7	6	-
Sterile mycelia Type 2	4	-	-	-	4	1	-	-	-
Sterile mycelia Type 3	1	-	-	1	6	1	10	3	19
Sterile mycelia Type 4	-	1	7	1	5	3	11	-	5
Sterile mycelia Type 5	-	-	-	-	-	4	-	-	-
Sterile mycelia Type 6	-	-	-	1	-	-	-	-	-
Sterile mycelia Type 7	-	-	-	2	1	-	-	-	-
Talaromyces purpureogenus Samson, Yilmaz, Houbraken, Spierenburg, Seifert, Peterson, Varga & Frisvad	6	7	3	-	-	-	-	-	-
Talaromyces ruber (Stoll) Yilmaz, Houbraken, Frisvad & Samson	3	6	6	-	38	5	11	21	6
Trichoderma asperellum Samuels, Lieckf. & Nirenberg	-	-	-	4	-	-	4	-	4
Trichoderma atroviride P. Karst.	-	-	-	-	1	-	-	-	-
Trichoderma harzianum Rifai	5	7	15	-	-	-	7	-	1
Trichoderma koningii Oudem	-	-	-	-	1	-	-	-	-
Trichoderma viride Pers.	26	3	-	1	1	-	15	2	1
Yeast Type 1	19	-	12	6	1	-	2	14	3
Yeast Type 2	50	28	93	27	3	-	4	-	4
Yeast Type 3	-	-	-	-	1	3	-	-	-
Yeast Type 4	6	-	-	2	3	-	-	-	1
Yeast Type 5	-	17	1	-	1	-	-	-	-
Yeast Type 6	-	-	-	-	1	-	-	-	-

Tab. 2. Mean amount of MTUs in 1 ml for each beach and month of sampling: QB = Quinto, MB = Multedo and VB = Voltri.
Tab. 2. Media delle MTUs in 1 ml calcolate in ogni campionamento effettuato su ogni spiaggia: QB = Quinto, MB = Multedo e VB = Voltri.

Site	**April**	**July**	**September**	**Total**
QB	8.0 E+03	2.2 E+03	5.3 E+03	5.2 E+03
MB	3.9 E+03	5.7 E+03	1.9 E+03	3.8 E+03
VB	6.4 E+03	2.6 E+03	2.7 E+03	3.9 E+03
Total	6.1 E+03	3.5 E+03	3.3 E+03	4.3 E+03

Tab. 3. Environmental parameters measured during each sampling month for each station: QB = Quinto, MB = Multedo and VB = Voltri.
Tab. 3. Parametri ambientali misurati durante ogni mese di campionamento nelle rispettive stazioni: QB = Quinto, MB = Multedo e VB = Voltri

Sampled beaches	**Air temperature (°C)**	**Water temperature (°C)**	**Humidity (%)**	**Organic matter (%)**	**pH interstitial water**
QB April	14.6	15	75.2	0.55	8.2
MB April	14.6	15	75.2	0.49	8.3
VB April	14.6	15	75.2	0.34	8.3
QB July	24.3	26	83.7	0.45	8.2
MB July	24.3	26	83.7	0.42	8.2
VB July	24.3	26	83.7	0.51	8.2
QB September	20.5	24	68.8	0.35	7.7
MB September	20.5	24	68.8	0.38	8.2
VB September	20.5	24	68.8	0.28	8

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Tab. 4. Two-way ANOVA results.
Tab. 4. Risultati dell'analisi ANOVA a due vie.

Source	**Sum Sq**	**d.f.**	**Mean Sq**	**F**	**Prob > F**
Beach	60.6	2	30.32	0.6	0.5475
Month	254.1	2	127.05	2.53	0.0809
Error	23271.9	463	50.26		
Total	23586.6	467			

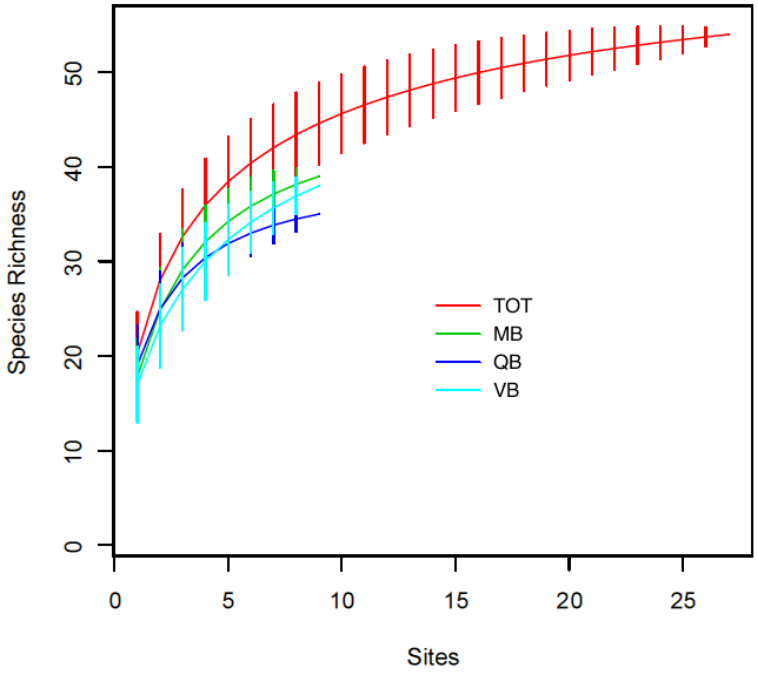

Fig. 4. Accumulation curves of species richness in the three areas (MB = Multedo, QB = Quinto and VB = Voltri) plotted both separately, and as total.
Fig. 4. Curve di accumulo della ricchezza specifica nelle tre aree analizzate (MB = Multedo, QB = Quinto e VB = Voltri) e del loro totale.

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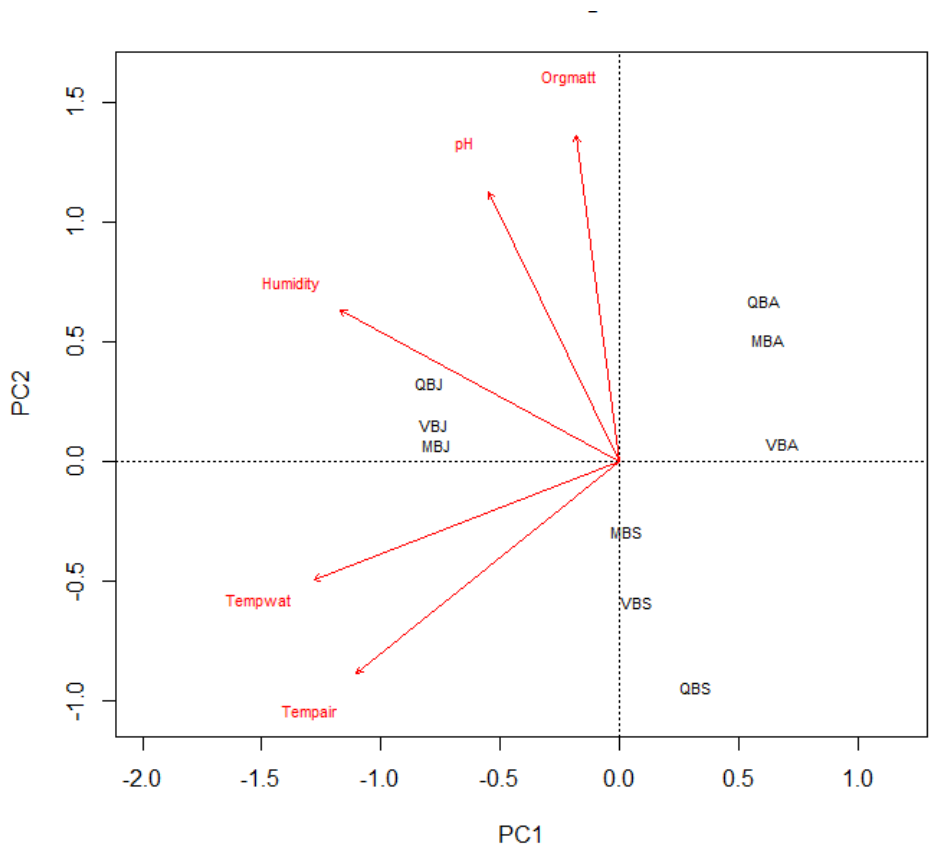

Fig. 5. Ordination diagrams obtained through Principal Component Analysis (PCA). Sites abbreviations: QBA=site QB in April, QBJ= site QB in July; QBS= site QB in September etc. Variable abbreviations: Orgmatt=Organic Matter, pH=pH, Humidity= Humidity, Tempwat=water temperature, Tempair=air temperature.
Fig. 5. Diagramma relative all'analisi delle component principali (PCA). Abbreviazione dei siti: QBA=sito QB ad aprile, QBJ= sito QB a luglio; QBS= sito QB a settembre ecc. Abbreviazione delle variabili: Orgmatt=Materia Organica, pH=pH, Humidity= Umidità, Tempwat=Temperatura dell'Acqua, Tempair=Temperatura dell'Aria.

Tab. 5. Summary of PCA: eigenvalues, their contribution to the correlations and variables (Organic Matter, pH, Humidity, temperature water, temperature air) scores.
Tab. 5. Risultato della PCA e valori delle singole variabili (Materia Organica, pH, Umidità, Temperatura dell'Acqua, Temperatura dell'Aria) rispetto agli assi (PCA1, PCA2, PCA3, PCA4).

	PC1	PC2	PC3	PC4
Eigenvalue	2.5083	1.7226	0.6018	0.16731
Proportion Explained	0.5017	0.3445	0.1204	0.03346
Cumulative Proportion	0.5017	0.8462	0.9665	1.00000
Temperature water	-1.5081	-0.5811	-0.2116	-0.2579
Temperature air	-1.2995	-1.0420	-0.1578	-1.1032
Organic matter	-0.2150	1.6067	-1.6221	-1.0323
pH	-0.6458	1.3280	1.8870	-0.7637
Humidity	-1.3778	0.7456	-0.2509	1.

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Tab. 6. Results of vector fitting test of significant variables. The first column shows the environmental variables; the second and third column show eigenvalues for Axis 1 (PC1) and Axis2 (PC2). r^2 corresponds to correlation values. P values, in the last column, are based on 1000 permutations. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tab. 6. Risultati del test vector fitting per le variabili significative. La prima colonna mostra le variabili ambientali; la seconda e la terza colonna mostrano l'eigenvalues per l'Asse 1 (PC1) e Axis2 (PC2). r^2 corrisponde al valore di correlazione. Il P values, nell'ultima colonna è basato su 1000 permutazioni. Significatività: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	PCA 1	**PCA 2**	**r^2**	**Pr(>r)**
Temperature water	-0.93313	-0.35953	0.9940	0.002997 **
Temperature air	-0.78014	-0.62560	0.9654	0.002997 **
Organic matter	-0.13264	0.99116	0.7214	0.021978 *
pH	-0.43732	0.89931	0.6458	0.003996 **
Humidity	-0.87947	0.47596	0.9043	0.025974 *

Discussion and Conclusion

Some researches (Salvo and Fabiano, 2007; Vezzulli et al., 2009) indicated that SZIW is characterized by good mycological diversity, and represent a reservoir of fungal micro-organisms due to the optimal combination of environmental factors (e.g. high levels of humidity and organic matter, etc…). For this reason SZIW environment can be fruitfully taken into account to characterize urban beach fungal diversity and composition, and to evaluate the quality of the beach by means of the presence of fungal pathogenic or allergenic species.

Our quantitative results according to the data reported in Vezzulli et al. (2009) reveal a high presence of fungi in the SZIW of all beaches. The total number of MTUs from each beach is almost the same probably due to the high human frequentation that acts as a vector of fungal propagules. A significant variation of MTU amount was detected among months, and the total abundance appear to be higher in April than in July and September. In general, organic matter on the beach represents the essential substrate and nutrient source for microbial organisms, and surely it can affect the fungal abundance. However, on urban beaches organic matter has different origin, such as decayed wood and algae, washed-up leaves, animal products, (e.g. chitin and keratin), and sewage discharges from publicly owned treatment works (Hyde, 1989; Babu et al., 2010; Gonçalves de Oliveira et al., 2011; Lee et al., 2011, Zakaria et al., 2011).

PCA results show that environmental factors, mainly water and air temperature, seem to have influenced on site fungal composition in April and July, and not in September. This is likely due to the high human frequentation, which during the summer increase the number of fungal propagules transported; and as suggested by Pereira et al. (2013) the other recreational seashore activities contribute to increase the organic matter amount.

In spite of the high MTUs abundance, there are few differences in fungal species composition among the beaches themselves. In fact, we identify 52 taxa: 45% of which are common to all three beaches, and 70% are common to at least two beaches This similarity probably is due to the anthropic pressure that often tends to support a limited group of widespread species manly related to the human activity.

As concerns filamentous fungi, the most recurrent genera are: *Aspergillus*, *Mucor, Penicillium, Talaromyces, Trichoderma*, *Circinella* and *Clonostachys.* Concerning the yeasts, four of seven morphotypes discerned were isolated from all beaches.

The most fungal species are really ubiquitous, saprotrophic potential allergenic species (e.g. *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Mucor mucedo* Fresen., *Rhizopus arrhizus* A. Fisch., *Trichoderma*

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harzianum Rifai), and only few species are opportunistic or true pathogens to human (e.g. *Aspergillus flavus* Link, *A. ochraceus* Wilh., *A. niger* Tiegh., *A. terreus* Thom, *A. tubingensis* Mosseray, *Mucor racemosus* Bull., and *Rhodotorula* sp.) in accord with previous researches on urban beaches (Salvo and Fabiano, 2007). Opportunistic species could be threatening for human immunodepressed individuals infecting by conidia/spores inhalation, or by contact with sand causing irritations and/or sensitivity (Matavulj et al., 2005). In particular, *Aspergillus tubingensis* rarely producted osteomyelitis (Bathoorn et al., 2013), while *Rhodotorula* spp. recently caused allergenic reactions on skin (Wirth and Goldani, 2012).

Our results show that no dermatophitic and keratinophilic fungi were found in urban beach SZIW zones. Furthermore, visitors could be possible vectors of opportunistic fungi that can find a suitable habitat in the SZIW itself (Sabino et al., 2011). This suggests that human impact in general contribute to affect seasonality distribution of fungal species on beaches. In our case, however, these beaches are undergone to human stress throughout the year and, as a consequence, the effect caused by summer beach frequentation is hidden by background noise.

Our results show how urban SZIW represents a reservoir of microfungi. Most of the fungal species were saprotrophic, some of which were opportunistic. As concerning fungal pathogens for human only *A. niger* were found. Furthermore, a statistical analysis has revealed that the fungal composition in April and July is affected by water and air temperature. The human impact, in particular beach recreational frequentation and the related activity, seems to affect the fungal communities in September. Further studies are needed in order to better characterize the urban beaches mycobiota and to well understand the effect of environmental factors on organisms distribution.

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