# Quantitative estimation and sampling optimisation of fungi in beach sand



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## with the use of GIS and geostatistical analysis

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### **INTRODUCTION**

Sandy beaches attract many people for recreation during the summer months. Beach sand has been recognised as a potentially large reservoir of pathogenic microorganisms (WHO, 2003) and their presence may imply increased health risks for bathers and non-bather visitors. The presence of faecal indicator bacteria in beach sand has been documented (Bonilla et al., 2007). Fungi can pose potentially serious health hazards as they are easily transmitted by contact. Yet, little has been published on the occurrence of fungi in the sand of recreational beaches (Velegraki et al., 1987).

#### **AIM OF STUDY**

The objectives of the present study are:

1. Estimation of the numbers of fungi in the sand of a recreational beach, during the bathing season.

**Table 1.** Fungal concentrations in sand categorized by beach use.

Recreational use/service (no of sampling stations)	Min (cfu/g of sand)	Max (cfu/g of sand)	Range (cfu/g of sand)	Mean (cfu/g of sand)
Showers (6)	$3.6 \times 10^2$	$18.0 \times 10^3$	17. $6 \times 10^3$	$5.3 \times 10^3$
Playground (8)	$3.0 \times 10^2$	$8.6 \times 10^3$	$8.3 \times 10^3$	$1.9 \times 10^3$
Sun bathing (22)	0	$15.9 \times 10^3$	$15.9 \times 10^3$	$2.9 \times 10^3$
Shoreline (8)	1.0 x 10	$1.4 \ge 10^2$	$1.3 \times 10^2$	5.9 x 10

- 2. Evaluation of the spatial distribution of fungi in the sand with respect to diverse activities that take place: sunbathing, beach-volley, showers, refreshment bar, children playground.
- 3. Optimisation of the sampling design by using geostatistical and spatial analysis methods within a geographical information system (GIS) environment.
- 4. Development of a thematic map illustrating the spatial distribution of the fungal concentrations on the recreational beach.

#### **STUDY SITE**

Sand samples were collected in the summer of 2010 from a recreational beach located on the island of Lesvos, Greece (Fig. 1). Forty nine sampling stations (49) were selected to be representative of the various activities that take place on that beach. Forty two (42) samples were collected on the 21<sup>st</sup> of July and 7 samples on the 4<sup>th</sup> of August 2010. There were no rain incidences during the 2 weeks preceding sampling. The 7 extra samples were deemed necessary after the geostatistical analysis; therefore, optimisation of the number and the location of the sampling stations was achieved. Sand was collected aseptically from a depth of 1-2 cm, was carried to the laboratory in a cold box and processed within a maximum of four hours after collection.

5.2 x  $10^2$  4.3 x  $10^3$  3.8 x  $10^3$  2.4 x  $10^3$ Refreshment Bar (2)  $9.1 \times 10^2$  $8.4 \times 10^2$  $4.0 \times 10^2$ Beach volley (3) 6.6 x 10

The application of geostatistical analysis methods (Fig. 4) showed lower spatial heterogeneity of fungal concentrations along the shoreline (major axis of the ellipse of the surface variogram) and higher heterogeneity vertical to the shoreline (minor axis of the ellipse of the surface variogram). The developed thematic map based on the optimised sampling network is shown in Fig. 5. The lowest concentrations were observed ca 5m from the water's edge, while the highest concentrations on the spot of several of the recreational uses/services provided – namely showers, refreshment bar and the children playground.







Fig. 1. Satellite view of Lesvos island.

#### **MATERIALS AND METHODS**

Fungi were isolated from each sand specimen using standard culture techniques, after an initial stage of extraction from sand-grains in quarter strength Ringer's solution. Appropriate concentrations or dilutions were prepared and analysed. The enumeration of fungi was carried out using the spread plate technique on Sabouraud Dextrose agar with chloramphenicol. All yeast and mould colonies were counted (Fig. 2).

Geostatistical analysis methods (Battilani et al., 2006) were applied in order to examine the spatial heterogeneity of fungal concentrations. Omni-directional and surface variograms were developed (Fig. 4) and the directions of maximum spatial anisotropy of fungal concentrations were detected. This led to optimisation of the sampling design. The analysis of the spatial anisotropy using the surface variograms is carried out based on the study of the major and the minor axis of the produced ellipsis. The major axis represents the direction of the maximum homogeneity of the parameter and the minor axis the direction of the maximum anisotropy. The  $\gamma$  represents the semi-variance. The spatial distribution of fungal concentrations was estimated using the IDW (Inverse Distance Weighted) interpolation method based on the optimised sampling data points and is illustrated on the thematic map in Fig. 5.

#### RESULTS

Fungal concentrations were between 0-18,000 cfu/g of sand. In 63% of the samples, the numbers of fungi ranged between 0-1,000 cfu/g. In 8% of the samples the fungal counts were above 10,000 cfu/g. (Table 1). In Fig. 3 minimum and maximum concentrations of the fungi is given per recreational use.





Fig. 5. Thematic map illustrating the spatial distribution of fungal concentration on the beach.

#### **DISCUSSION**

Sandy beaches attract many people for recreation, and attendance on this type of beach is maximized during the summer. Application of geostatistical methods of analysis and surface variograms led to the optimisation of the selection of sampling stations. The results indicate that the application of geostatistical and spatial analysis methods are a useful tool in the process of quantitative estimation of fungi in beach sand.

In this study we observed the highest concentrations of fungi in the shower area. High concentrations were detected in the spot where children are offered various activities, as well as by the refreshment bar. The lowest concentrations were observed in sand washed by the sea (shoreline). Increased levels of fungal concentration on some spots could also be due to the existence of organic matter and bird droppings.

Fig. 2. Petri dishes with characteristic fungal colonies.



Fig. 3. Fungal minimum and maximum concentrations per recreational use.

Overall, the relative abundance and distribution of fungi could be attributed to the degree each of these spots is used by the beach visitors, many of which walk on the sand in their bare feet. The underway qualitative determinations will ascertain the role of these fungal isolates in public health.

#### REFERENCES

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