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Article

Abundance and distribution of fecal indicator bacteria in recreational beach sand in the southern Baltic Sea

Abundancia y distribución de indicadores bacterianos fecales en playas arenosas recreacionales en el sur del Mar Báltico

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Resumen.- Se estimó la densidad y la distribución de indicadores bacterianos fecales en arena seca y húmeda y en el agua de mar adyacente de una playa marina recreacional de Polonia en el Mar Báltico. El número de coliformes totales, coliformes fecales y estreptococos fecales fueron entre 3 y 9 veces mayores en la arena seca que en el agua de mar y entre 2 y 6 veces mayores en arena seca que en arena húmeda. Dentro de un año, el número de bacterias fecales que habitaron la arena y el agua de mar mostraron considerables cambios mensuales. El mayor número de indicadores de bacterias fecales en el agua de mar y en la arena aparecieron en la estación de primavera-verano y el mejor estado sanitario se detectó en los meses de invierno. Hubo diferencias en el número de indicadores de bacterias fecales entre la capa de arena superficial y subsuperficial, con una clara tendencia decreciente en el número de las bacterias estudiadas al aumentar la profundidad.

Palabras clave: Mar Báltico, playa, bacterias fecales

Abstract.- Density and distribution of fecal indicator bacteria in dry and wet sand and the adjacent seawater of recreational marine beach in Poland, Baltic Sea, were estimated. Numbers of total coliforms, fecal coliforms and fecal streptococci were 3-9 times higher in dry sand than in the seawater and 2-6 times higher in dry sand than in wet sand. Within a year, number of fecal bacteria inhabiting the sand and seawater showed considerable monthly changes. The highest number of the studied fecal indicator bacteria in the seawater and sand occurred in spring-summer season and the best sanitary state was noted in the winter months. There were differences in the numbers of fecal indicator bacteria between the surface and subsurface sand layer with a clear decreasing trend in the number of the studied bacteria with increasing depth.

Key words: Baltic Sea, beach, fecal bacteria

Introduction

In recent years increased fecal contamination of the sand of many recreational marine beaches was observed, which consequently results in an increased risk of illness among beach users (Elmir *et al.* 2007, Stewart *et al.* 2008, Heaney *et al.* 2009). The sand of beaches acts as a passive element of cumulative pollution accumulating fecal bacteria from point sources such as municipal wastewater effluents, and non-point sources such as recreational users, fecal droppings from wild animals (mainly birds), agricultural run-off, storm drain and mats of green algae (Craig *et al.* 2002, Sato *et al.* 2005, Edge & Hill 2007). According to Shibata *et al.* (2004) and Bonilla *et al.* (2007) the accumulation of fecal bacteria in sand has two potential consequences for beach users. The washout of bacteria from the sand into nearshore waters might complicate the task of water quality managers' intent on monitoring the quality of bathing water. Moreover, if fecal indicators are being concentrated in beach sand, fecal-borne pathogens may also be accumulating raising the question of whether contact with sand poses additional health risks related to beach use. Numerous studies (Hartz *et al.* 2007, Heaney *et al.* 2009, Yamahara *et al.* 2009, Griffith *et al.* 2010) found that the conditions in foreshore, nearshore and backshore sand of marine beaches can favour the persistence, survival and regrowth of fecal indicator bacteria. The large surface area of sand grains and the unique microhabitats within the cracks and crevices provide microbes with variety of potentially suitable environments for enhanced survival and growth (Craig *et al.* 2004, Bonilla *et al.* 2007). Whitman & Nevers (2003), Byappanahalli *et al.* (2006) and Brownell *et al.* (2007) showed that bacterial fecal indicators can persist in sand throughout the year with little variation in counts. Therefore, sand of marine beaches may be an important reservoir of metabolically active fecal bacteria (Hartz *et al.* 2007).

Total coliforms, fecal coliforms and fecal streptococci are the main organisms indicating the possibility of fecal contamination of recreational water and sand of marine beaches (Shibata *et al.* 2004). Their presence indicates the potential presence of pathogenic bacteria and is a good predictor of health risks related to marine beach use (Colford *et al.* 2007, Griffith *et al*. 2010). Studies on abundance and distribution of fecal bacteria in marine beaches are necessary to understand their potential threat to human health and correctly target fecal pollution prevention actions (Edge & Hill 2007, Stewart *et al*. 2008). For that reason the aim of the present study was to determine number and distribution of total coliforms, fecal coliforms and fecal streptococci in sand and the adjacent seawater in recreational marine beach in Ustka located at the southern Baltic Sea.

Materials and Methods

Study area and sampling

The study was carried out on non-tidal sandy beach (54° 35'N and 16° 51'E) localized in Ustka town, Poland (southern Baltic Sea) (Fig. 1). It is located at the mouth of the River Słupia, which divides the studied beach into two parts: Eastern Beach and Western Beach. It represents a dissipative beach type with longshore bars and troughs and its width is about 75 m. In general, the beach is fine and medium-grained, and the sand grain-size is between 0.125 and 0.250 mm (Kramarska *et al.* 2003). The studied beach, particularly in autumn and winter, is exposed to strong winds that generate high waves, which cause strong erosion onshore. As a result the seashore along the Ustka beach is heavily destroyed and the coastline retreats on average 0.08 m every year (Zawadzka-Kahlau 1999). There are two main sources of contamination of this beach: the River Słupia and seabird/shorebird populations.

Dry and wet sand and seawater samples were collected bimonthly between November 2007 and October 2008 at Eastern Beach. Samples of sand were obtained from two sites along a profile perpendicular to the shoreline (Fig. 1). Wet sand was collected from a site situated at the waterline and dry sand was collected from a site located a halfway up the beach at a 30 m distance from the shore. Sand core samples were taken with a hand operated sampler (length 30 cm, inner diameter 15 cm). In the field, sand samples were divided into two sections: 0-5 cm and 10-15 cm and placed in sterile plastic jars. Seawater samples were collected in sterile bottles within a 1.5 m from the waterline at a depth of about 15 cm. Jars and bottles were put into containers with ice and transported to the laboratory. The time between sample collection and bacteriological analyses did not usually exceed 2-3 h.

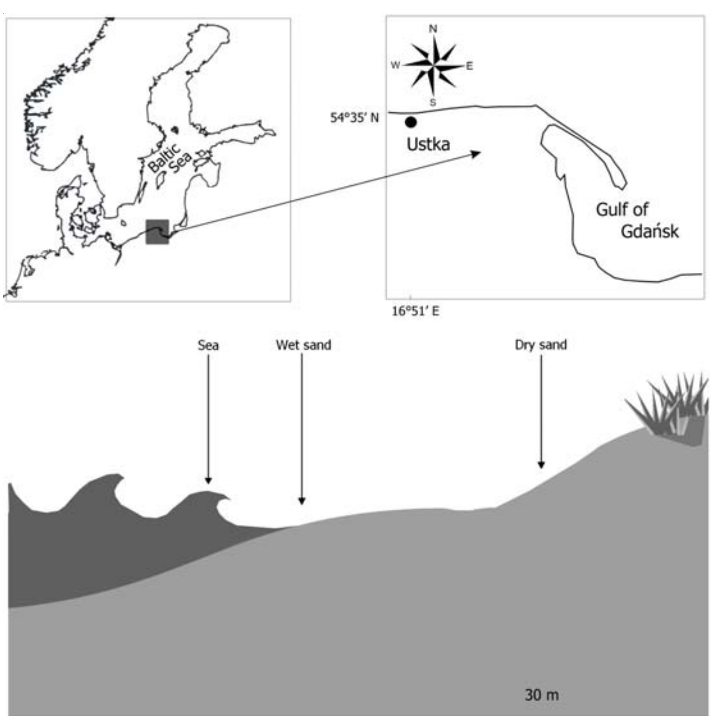

Figure 1. Location of sampling sites on the sandy beach in Ustka, Poland / Ubicación de los sitios de muestreo sobre la playa de arena en Ustka, Polonia

Bacteriological analyses

All collected sand and seawater samples were tested for the number of total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS). In order to determine the number of fecal indicator bacteria, 5.0 g of sand samples were weighed aseptically and transferred to 45 cm^3 of sterile phosphate-buffered saline (pH 7.2) and were shaken vigorously by hand for 1 min to suspend bacteria. Following 30 min sedimentation, the supernatant was serially diluted with sterile phosphate-buffered saline to reach final concentration ranging from 10^{-1} to 10^{-3}. Collected sample of the seawater was also diluted with sterile phosphate-buffered saline to reach final concentration ranging from 10^0 to 10^{-2}. Dilutions of sand and seawater samples were filtered through a 0.45 µm pore size, 47 mm diameter membrane filter (Whatman ME 25/31 ST). The filters with collected bacteria were then aseptically transferred to Petri dishes containing 10 cm^3 of selective media.

The number of total coliforms was determined using the Endo medium (Biocorp). TC cultures were incubated at 37˚C for 48 h and typical red colonies with metallic sheen were counted as the total of coliforms bacteria.

A count of fecal coliforms was determined by the ECD MUG Agar (Fluka). The incubation of inoculations was conducted at the temperature of 44˚C for 48 h. After incubation greenish fluorescent colonies indicated cleavage of 4-methylumbelliferone-β-D-glucuronide (MUG) by the β-D-glucuronidase and the released fluorescent MUG compound, which was detected under Wood's lamp (UV light 366 nm); the colonies were counted as FC.

In order to determine the number of fecal streptococci the medium Slanetz-Bartely (Biocorp) was used. After a 48 h incubation at 44˚C, red, maroon or pink colonies were counted as FS.

An additional sample of 10 g of sand was weighed (RADWAG WPS 30 S) and dried at 105˚C in order to determine the dry weight of sand. All counts were normalized to colony forming units (CFU) per 100 cm^3 of seawater or CFU per 100 g of the dry weight of sand.

Results

Data presented in Table 1 show that the mean number of total coliform bacteria in seawater samples (583 CFU per 100 cm^3) was about two times lower than in wet sand samples (966 CFU per 100 g dry wt of sand) and 3 times lower than in dry sand samples (1807 CFU per 100 g dry wt of sand). Within a year, the highest number of total coliforms bacteria in the seawater occurred in the period from May to July and the lowest in October. In wet sand the highest number of TC was noted in May, while in dry sand from May to August. The lowest number of TC in wet sand was observed in September and October, while in dry sand in September (Fig. 2).

Table 1. Abundance of total fecal coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) in seawater (CFU 100 cm^{-3}) and wet and dry sand (100 g dry weight of sand) (data derived from the pooled data of all months and depths) / Abundancia de coliformes fecales totales (TC), coliformes fecales (FC) y estreptococos fecales (FS) en agua de mar (CFU 100 cm^{-3}) y arena húmeda y seca (100 g peso seco de arena) (datos derivados de los datos agregados en todos los meses y profundidades)

Microbiological parameters	Sites	Mean		Min	Max	SD	CD	CV (%)
TC	seawater	583		100	1300	390.4	261.3	66.9
	wet sand	966	(1.7)*	0	7671	1542.8	2464.2	159.7
	dry sand	1807	(3.1)**	0	9028	2270.7	2852.9	125.6
FC	seawater	142		0	400	116.5	95.7	82.2
	wet sand	92	(0.6)*	0	511	123.9	166.9	134.6
	dry sand	394	(2.8)**	0	2883	666.5	1128.9	169.4
FS	seawater	150		0	500	138.2	127.3	92.1
	wet sand	204	(1.4)*	0	2060	414.7	844.9	203.8
	dry sand	1293	(8.6)**	0	10384	2400.7	4456.3	185.6

TC - total coliforms; FC - fecal coliforms; FS - fecal streptococci;
* wet sand/seawater
** dry sand/seawater

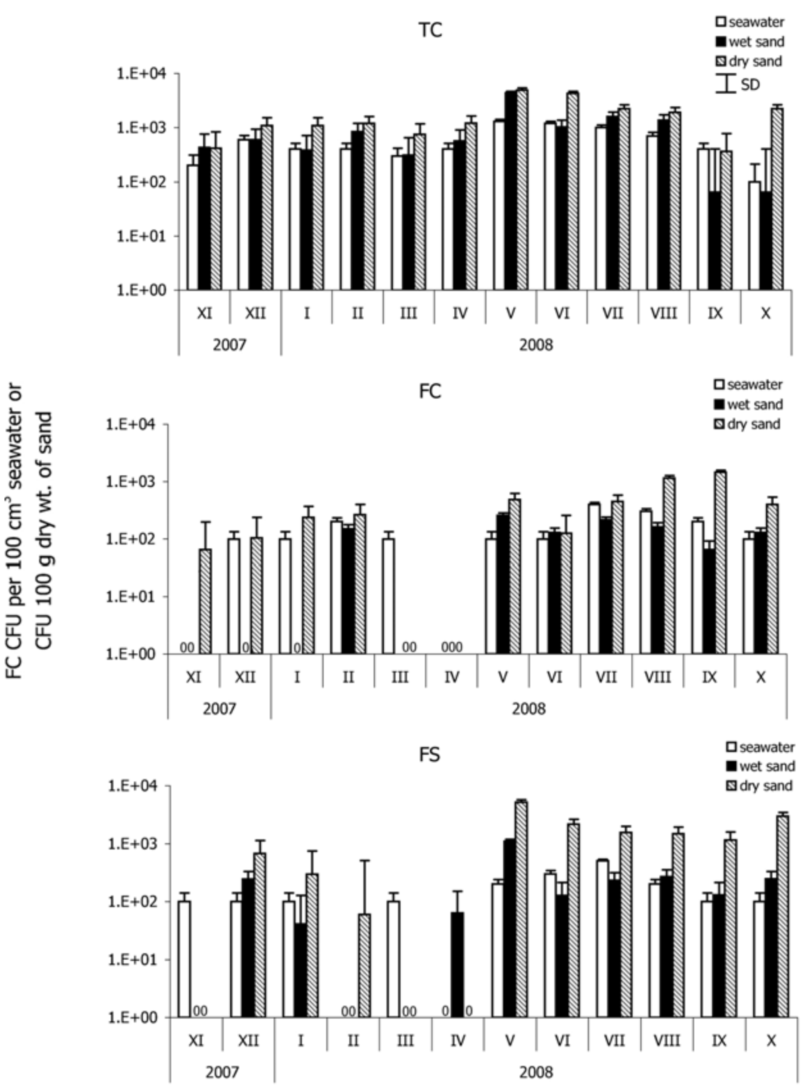

Figure 2. Numbers of total fecal coliforms, fecal coliforms and fecal streptococci in seawater, wet sand and dry sand during the year - long investigation (data derived from the pooled data of all depth) / Números de coliformes totales fecales, coliformes fecales y streptococci fecales en el agua de mar, arena húmeda y seca durante el año de investigación (datos derivados de los datos agregados de todas las profundidades)

The mean number of fecal coliforms in dry sand (394 CFU per 100 g dry wt of sand) was 4 times higher than in wet sand (92 CFU per 100 g dry wt of sand) and 3 times higher than in the seawater (142 CFU per 100 cm^3) (Table 1). The highest number of FC in the seawater was noted in July and August (Fig. 2), while in April no fecal coliforms were noted. Number of FC in wet sand increased in May and July, while in dry sand in August and September. No presence of fecal coliforms was noted in wet and dry sand in March and April.

The mean of bacterial counts of fecal streptococci in dry sand (1293 CFU per 100 g dry wt of sand) was 6 times higher compared to wet sand (204 CFU per 100 g dry wt of sand) and 9 times higher than in the seawater (150 CFU per 100 cm^3) (Table 1). Data presented in Figure 2 show that within a year, we observed the increase in the number of FS in the seawater that started in May and finished in August. In February and April no fecal streptococci were noted in the seawater. In wet and dry sand the highest numbers of FS were recorded in May. In February and March no fecal streptococci were noted in wet sand, while in dry sand in March and April.

Data on number of total coliforms, fecal coliforms and fecal streptococci isolated from the surface (0-5 cm) and subsurface (10-15 cm) sand layers are given in Figure 3. The results of this study showed that all studied fecal bacteria were more numerous in the surface sand layer. Number of these bacteria in the surface layer of wet sand was 2 to 4 times higher than in the subsurface layer. All 3 studied groups of fecal indicator bacteria were 3 to 10 times more abundant in the top layer than in the subsurface layer of dry sand.

To analyze the relationships among studied bacterial fecal indicators a statistical data evaluation of sand and seawater samples was undertaken and the results are given as the correlation matrix (Table 2). When analyzing wet and dry sand samples together, a very strong correlation (r = 0.86, $P < 0.01$) was found between total coliform bacteria (TC) in sand and water samples and also total coliforms (TC) and fecal streptococci (FS) in sand samples (r = 0.87, $P < 0.01$).

Linear regression analysis was also applied to compare relationships between fecal indicators bacteria inhabiting the seawater and sand of the studied beach (Fig. 4). In the seawater, we observed the relation of the number of fecal streptococci (FS) to total coliforms (TC) ($R^2 = 0.48$, $P < 0.01$), and also of fecal streptococci (FS) to fecal coliforms (FC) ($R^2 = 0.42$, $P < 0.01$). In sand samples (we analyzed wet and dry sand samples together) we found only statistically significant relation ($R^2 = 0.76$, $P < 0.01$) of the number of fecal streptococci (FS) to total coliforms (TC).

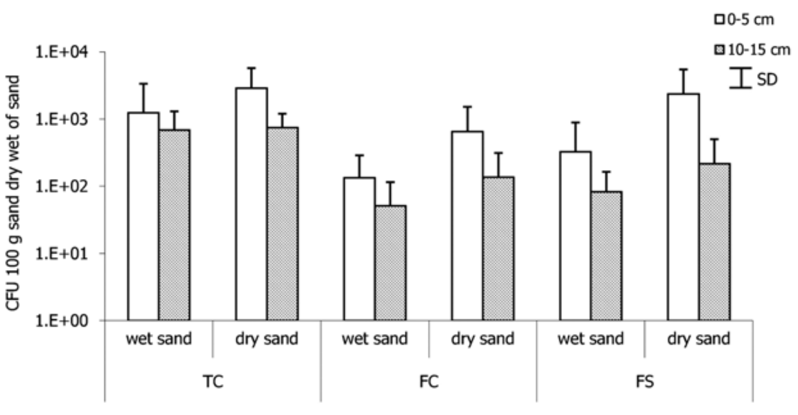

Figure 3. Fecal bacteria in surface (0-5 cm) and subsurface (10-15 cm) wet and dry sand layer (data derived from the pooled data of all months and sites) / Bacterias fecales en la capa superficial (0-5 cm) y subsuperficial (10-15 cm) de arena húmeda y seca (datos derivados de los datos agregados de todos los meses y sitios)

Table 2. Correlation coefficient fecal indicator bacteria in sand and seawater / Coeficiente de correlación de indicadores bacterianos fecales en arena y agua de mar

	TC-s	FC-s	FS-s	TC-w	FC-w	FS-w
TC-s	1	0.16	0.87**	0.86**	0.125	0.48
FC-s		1	0.38	0.21	0.63*	0.25
FS-s			1	0.63*	0.11	0.38
TC-w				1	0.34	0.69*
FC-w					1	0.65*
FS-w						1

TC - total coliforms; FC - fecal coliforms; FS - fecal streptococci; s-sand, w-seawater
*P-value <0.05 (significance at 0.05 level)
** P- value <0.01 (significance at 0.01 level)

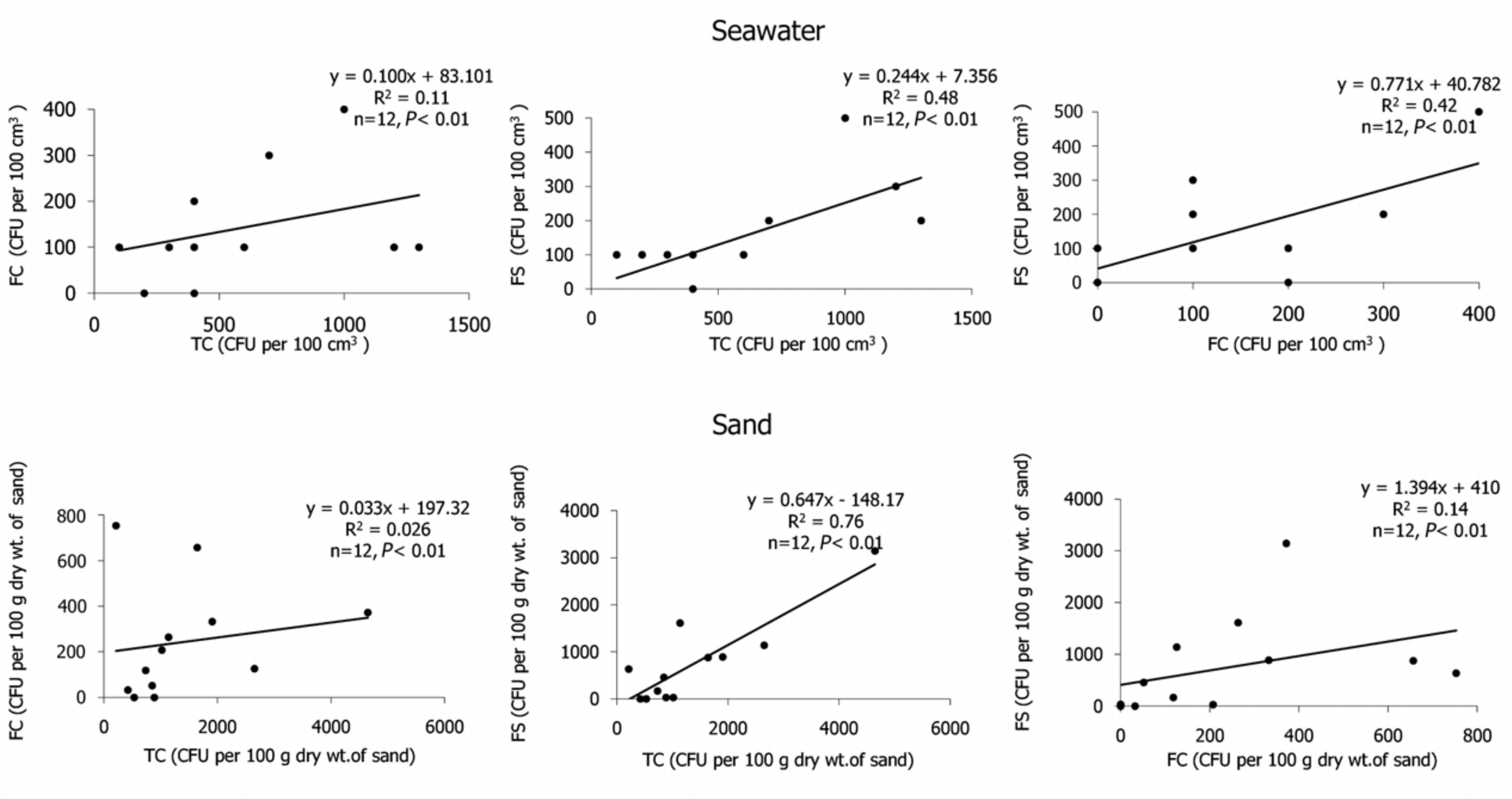

Figure 4. Relationship between mean fecal bacteria densities in seawater and sand of a Beach in Ustka, Poland / Relaciones entre la densidad fecal promedio en el agua de mar y arena en una playa de Ustka, Polonia

Discussion

Only recreational coastal waters along marine beaches are systematically sanitary monitored, while the concentration of fecal indicators in the beach sand is not routinely measured despite that the sand beach as a natural filter that may become contaminated with fecal indicator bacteria. These organisms can be transported from the sand to the sea where they may instigate beach advisories (Lee *et al*. 2006, Bonilla *et al*. 2007, Yamahara *et al*. 2009). Law and legislation has emphasised the beach visitors may not use seawater, but would use only the beach sand (Elmanama *et al*. 2005). They may risk their health due to microbiological contamination in the sand (Olanczuk-Neyman & Jankowska 2001, Vieira *et al*. 2001, Sato *et al*. 2005, Heaney *et al*. 2009).

The year-long study in the Ustka beach demonstrated that fecal bacteria were detected in all study sites (seawater, wet sand, dry sand). Moreover, the number of fecal bacteria was 3-9 times higher in dry sand than in the seawater and 2-6 times higher than in wet sand. Previous studies in marine beaches also showed that the number of fecal bacteria was higher in the sand than in the adjacent water. In 6 public freshwater beaches in St. Clair County, Michigan (USA) fecal bacteria counted in the sand were 3-48 times higher compared to water (Wheeler-Alm *et al*. 2003), while in a marine beach in Italy, the number of fecal bacteria in the sand was 1 to 30 times higher than in the adjacent seawater (Aulicino *et al*. 1985). The results of the study in 3 marine beaches of South Florida (USA) were even more striking: the levels of fecal indicator bacteria were on average 100-1000 times greater in the sand relative to seawater (Bonilla *et al*. 2007). This may be explained by the fact that allochthonous microorganisms inhabiting the beach can survive better in the sand than in the adjacent water (Craig *et al*. 2002).

According to Lee *et al*. (2006) and Yamahara *et al*. (2009) the sand of marine beach represents more stable conditions which are less subject to change than the adjacent seawater. The beach sand may be more conducive to fecal indicator bacteria survival relative to the seawater by reducing the sunlight radiation (Beversdorf *et al*. 2007, Brownwell *et al*. 2007), the capability of glycine-betaine accumulation that protects against osmotic stress and lower salinity variation (Heaney *et al*. 2009). Moreover, the sand is characterized by the significant thermal inertia that effectively reduces a temperature gradient from day to night. This phenomenon secures stable thermal conditions for microorganisms inhabiting the sand (Heaney *et al*. 2009). The sand also protects against predators (Wheeler-Alm *et al*. 2003, Lee *et al*. 2006), and provides colonizable surfaces (Craig *et al*. 2004, Elmanama *et al*. 2005). According to Vieira *et al*. (2001), Kischner *et al*. (2004) and Whitman *et al*. (2004) solar radiation, mainly UV light, in combination with salinity is arguably the most potent in the inactivation or killing fecal coliforms and fecal streptococci in seawater. The research of Fujioka *et al*. (1981) showed that in the absence of sunlight, fecal indicators survive for a few days in seawater samples, whereas in the presence of sunlight, 90% of fecal coliforms and fecal streptococci are inactivated within 30-90 and 60-180 min, respectively. According to Whitman *et al*. (2004) the process of inactivation or killing fecal bacteria by sunlight in natural waters is rather complex; however, the two major pathways involved in this process appear to be photobiological (DNA damage) and photooxidation (oxidation of cellular components).

Previous studies in marine beaches identified dry sand as the main reservoir of fecal bacteria (Shibata *et al*. 2004, Sato *et al*. 2005, Beversdorf *et al*. 2007, Yamahara *et al*. 2009). In the studied Ustka beach, the number of fecal bacteria was higher in dry than in wet sand. The results of our study are also consistent with those of Vieira *et al*. (2001) who found higher amounts of fecal bacteria in dry than in wet sand in 3 marine beaches in Brazil. Similar results were reported by Bonilla *et al*. (2007) from the Hobie Beach in South Florida, USA. The high number of fecal bacteria in dry sand that it is not under the influence of the tides may indicate that the seawater is not the main source of fecal contamination in this zone of the beach. The statistical analysis in 16 marine beaches of São Paulo State (Brazil) indicated a high correlation between fecal bacteria densities in wet sand and seawater, but not between dry sand and seawater (Sato *et al*. 2005). According to Haack *et al*. (2003), Whitman & Nevers (2003), Ishii *et al*. (2007) and Wright *et al*. (2009) humans and birds occupying the beach have been main non-point source of fecal bacteria in dry sand. Marine seabirds, for example gulls can excrete more fecal bacteria per day than humans (Jones & White 1984). Thus gulls' fecal droppings are the more prominent source of fecal bacteria in the sand beach (Fogarty *et al*. 2003, Edge & Hill 2007). Permanent residents of the studied beach are numerous gulls which population grows rapidly in the region of Ustka town every year (Zielinska *et al*. 2007). Gulls are the most familiar and social birds and quickly adapt to the presence of people (Levesque *et al*. 1993). They eat not only fish, but to a greater extend use waste and food remaining left by recreational users. Seagulls and other bird type species are attracted by the easy access to food. All of them contribute to the increase contamination of sand by excreting on the beach (Oshiro & Fujioka 1995). Gould & Flechter (1978) determined that the average wet weight of faeces excreted by different gull species ranged from 11.2 to 24.9 g day^{-1} and one gull could produce between 34 and 62 of fecal droppings in a day. Haack *et al*. (2003) found that gulls carried a burden of high fecal bacteria in their gastrointestinal tract, with numbers as 1.4 10^7 of fecal coliforms g^{-1} and 5.0 10^7 of fecal streptococci g^{-1} of faeces. Single gull dropping has been shown to increase the numbers of background streptococci by between 100 and 1000-fold in the 3 m^2 area around the dropping (Bonilla *et al*. 2006). Gull fecal material is considered a threat to human health. The presence of human pathogens in gull faeces such as *Salmonella* spp., *Aeromonas* spp., *Campylobacter* spp., *Listeria* monocytogenes and *Escherichia coli* serotype 0157 were documented by Hatch (1996), Levesque *et al*. (2000) and Fogarty *et al*. (2003). According to Haack *et al*. (2003) bird faeces are delivered to the beach via multiple pathways. The movement of people on the beach may contribute to the abundance of indicator bacteria and their distribution in dry sand. In high traffic areas, fecal bacteria can be translocated by people on average 1.6 m in just 4 h (Bonilla *et al*. 2007). In addition, a study by Alderisio & DeLuca (1999) indicated a fairly stable concentration of fecal bacteria in gull fecal material over 4 seasons during 2 sampling years. Even in non-bathing seasons many visitors of Ustka town, which is a health resort, walking along the beach can spread bird faeces delivered in the sand.

Apart from birds, particularly in summer season, a significant source of fecal bacteria in dry sand of the marine beach is recreational users (Haack *et al*. 2003, Whitman & Nevers 2003). The same applies to the studied recreational Ustka beach. In summer many people spend a lot of time on the beach dry sand. Bacteria in the skin of recreational users stick to sand or are washed into seawater during sea baths (Craig *et al*. 2004, Elmir *et al*. 2007).

In this area there are other serious faecal contamination sources of seawater. The polluted river Słupia with the surface of the river hydrological basin of about 1623 km^2 carries wastewater from urban and agriculture area as well as 200,000-300,000 m^3 year^{-1} of natural and anthropogenic sediments into the sea within the area of the studied beach (Zawadzka 1996).

In addition, according to Bonilla *et al*. (2007) the higher fecal bacteria densities observed in dry sand compared to wet sand may partially also be attributable to lower predation. Predation is a major biotic factor influencing fecal bacteria death rates; it accounted for 47-99% of mortality in water ecosystems (Chigbu *et al*. 2005). Dry sand contains approximately half of the water content of the intertidal wet sand leading to a reduced water film surrounding sand grains. Macroinvertebrate and larger protozoa, main consumers of bacteria, may not be active in this environment (Bonilla *et al*. 2007). The second factor contributing to the decrease of the number of faecal bacteria in wet sand is its salinity. The osmotic pressure of salt effectively stops the metabolic processes of bacteria causing the quicker death of cells (Podgórska *et al.* 2008).

In our study we observed seasonal variation in fecal coliform abundance at the Ustka beach. Generally, during the spring-summer season, the higher abundance of all studied fecal indicator bacteria both in seawater and sand was recorded. Olanczuk-Neyman & Jankowska (2001) in the earlier studies carried out at the Sopot beach (southern Baltic Sea) also reported an increasing trend in the number of fecal bacteria in spring-summer season. Similarly, in Wisconsin beach (Canada) (Zehms *et al*. 2008), beaches of South Coastal of São Paulo State (Brazil) (Sato *et al*. 2005) and Duluth Boat Club beach in Minnesota (USA) (Ishii *et al*. 2007) the number of fecal bacteria was highest from spring to summer months. The high number of fecal bacteria in summer is a potential health risk associated with the exposure of people to the contaminated sand and seawater; particularly children who stay there longer (Sato *et al.* 2005). This has been observed in the previous study (Whitman & Nevers 2003) and was attributed to the higher survival and perhaps growth rates of fecal bacteria in warmer temperatures. According to Sato *et al*. (2005) bathers and birds occupying the beach are main potential-point source of fecal indicator bacteria in seawater and the sand of marine beach in summer season.

The results of this study showed that on the Ustka beach all studied fecal bacteria were more numerous in the surface (0-5 cm) than subsurface (10-15 cm) sand layers. Olanczuk-Neyman & Jankowska (2001) in the earlier study carried out on the Sopot beach (southern Baltic Sea) also showed a clear decrease in the number of fecal bacteria with increasing depth. Similarly, in the sand of 6 beaches in St. Clair County, Michigan (USA) the number of fecal coliforms and fecal streptococci in the surface (0-10 cm) layer of the sand was much higher than at the depth of 15-20 cm (Wheeler-Alm *et al.* 2003). Such distribution results most probably from the fact that the concentrations of organic matter, oxygen and the primary production level of microphytobenthos, which are main stimulators of growth for heterotrophic bacteria, decrease with depth in sand (Mudryk & Podgórska 2007).

In conclusion, the results of this study showed that human pathogenic bacteria of intestinal origin were also present in the sand of marine beach. Therefore, sand of marine and freshwater beaches, which are used for recreational purposes, should be included in sanitary monitoring programs, and this may enhance their effectiveness in human health protection.

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