Bacteriological investigations of the sandy beach ecosystem in Sopot*

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Abstract

The paper presents the results of bacteriological investigations relating to the coastal seawater and sandy sediments along the beach in Sopot in an area between the mouths of two streams, Grodowy Potok and Kamienny Potok. The sandy sediments investigated at four sites along a transect perpendicular to the shore contained variable numbers of saprophytic bacteria. In areas close to the littoral zone large numbers of allochthonous bacteria were found. With increasing distance from the waterline their domination declines to the advantage of autochthonous bacteria, an indication of the decreasing influence of anthropogenic pollution.

1. Introduction

The ecosystem of the Baltic sandy beaches, *i.e.* the shallow littoral between the high and low watermark has been little studied. However, preliminary investigations have indicated that it is an area exceptionally rich in biological life. The hypothesis has been put forward that the beach can function as a biological filter. Nevertheless, the effectiveness with which it removes pollutants and prevents their mineralisation depends to a large extent on the number and variety of organisms living in this environment.

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The prime objective of these investigations was to assess the composition of the beach fauna and its regenerative capacity, as well as the efficacy with which it removes the organic pollution occurring on different types of shore. The aim of this particular work was to determine the numbers of saprophytic bacteria occurring in the sandy substrate of the Sopot beach, and to assess the sanitary state of this environment.

2. Materials and methods

2.1. The study area and sampling procedures

The investigations refer to the sandy sediments of the beaches and coastal seawater of the Łazienki Północne (Northern Baths) region in Sopot. Samples for testing were taken from four measurement sites, the positions of which are given in Fig. 1. Site I was located approximately 1–1.5 m from the waterline in a zone where the water depth was about 0.5 m.

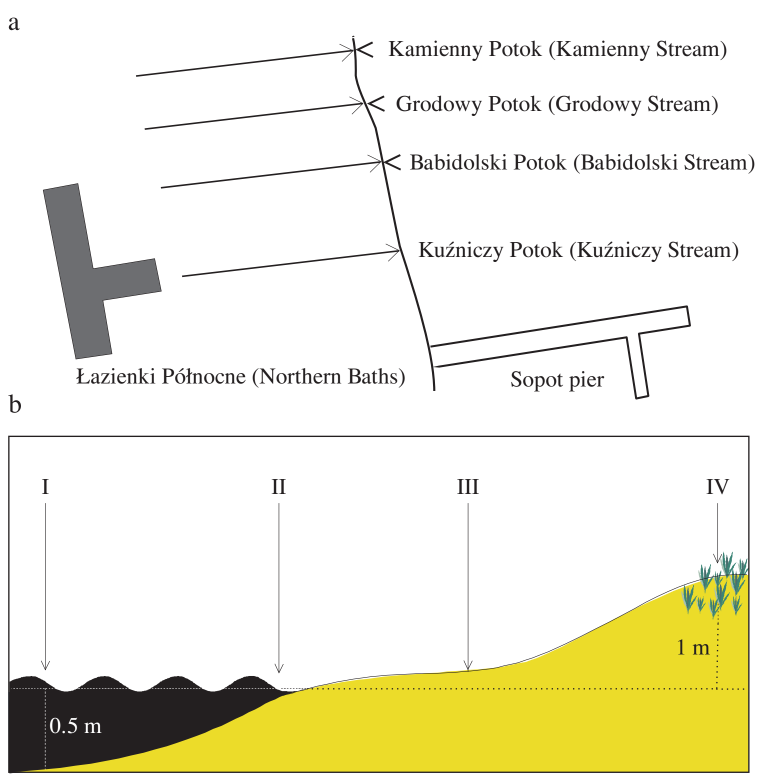

Fig. 1. Location of the study area (a) and the test sites (b)

The water samples were taken from a depth of 30 cm below the surface, while samples of sandy sediments were taken from the surface layer (0–5 cm). Site II was situated at the waterline. Site III lay at a distance of 32 m from the waterline, halfway up the beach. Site IV was situated in a sheltered region of the dunes 60 m from the shore.

Sedimentary core samples were taken at sites II, III and IV from a depth of 0–15 cm with a 10 cm-diameter Morduchaj-Boltowski core scoop. To obtain representative sedimentary samples at each site, three parallel cores were taken about 50 m from each other. Each of these cores was divided into three sections: 0–5, 5–10, 10–15 cm. An average sample was obtained by mixing corresponding layers. Further samples of sediments were taken at site III, at a depth of 50–70 cm (the water-table level), and at site IV at a depth 50–55 cm. All the water and sandy sediment samples were taken using sterile samplers under sterile conditions. The bacteriological tests were carried out 2 hours after sampling at the latest. At the same time, the water and air temperatures, and the wind speed and direction were measured. The cloud cover was also assessed. All the measurements were carried out between 24 April and 24 November 1997. A total of six measurement series were performed at one-month intervals.

2.2. Bacterial counts

Averaged samples of sandy sediments were thoroughly mixed, after which 100 g samples were placed in sterile flasks containing 100 ml Ringer's solution, diluted four times (Korzeniewski, 1978) with 0.28% sodium pyrophosphate (Chapelle, 1993). This solution was shaken for 20 min and then decanted for 2 min prior to further dilution. The saprophytic bacteria count was determined using two different media: nutrient agar (NA) prepared with fresh water, and ZoBell 2216E (ZO) medium (Rheinheimer, 1977) prepared with old brackish water, the salinity of which (7 PSU) corresponded to the sampling site salinity (Cyberska, 1990). The numbers of spore-forming bacteria were determined on NA following pasteurisation for 20 min at the requisite dilution at 80°C. The plates were incubated for 7 days at 20°C. The numbers of faecal coliform bacteria were determined using LMX medium following a 24 to 48 h incubation at 37°C, while mesophilic bacteria were cultivated on NA at 37°C for 72 h. The sanitary state of the water and sand samples was determined on the basis of the quantitative faecal coliform bacteria test. The permissible number of the above-mentioned indicator bacteria in the various surface water quality classes was taken into account in the assessment of the results (Sobol and Szumilas, 1989). To simplify the problem, similar assessment criteria for determining the sanitary condition of the sand were applied as in the case of surface waters.

The results of this bacteriological investigation are given as the number of colony forming units (c.f.u.) per 1 g of wet sediment, or per 1 ml of water. Comparison of the loss of mass due to the drying of samples showed that the differences in the bacterial counts between the wet and dry sediment samples were no greater than 15%. The faecal coliform bacteria count was determined as MPN per 100 g of sediment or 100 ml of water. The values given in the diagrams (Figs. 2 and 3) refer to 1 g of sand or 1 ml of water.

3. Results

3.1. Saprophytic and spore-forming bacteria in water and sandy sediments

The total number of saprophytic bacteria cultivated on NA and ZO, together with the number of spore-forming bacteria was determined in samples of coastal seawater and beach sand.

In general, the number of bacteria under investigation varied widely: in the case of NA the number of bacteria was usually larger than in the case of ZO.

The results are given in the form of a semi-logarithmic scale (Fig. 2). To simplify the description of the results in the later part of the paper the abbreviation ZO/NA denotes the percentage of saprophytic bacteria cultivated on the seawater medium (ZO) in the total number of bacteria incubated on the fresh water medium (NA).

Numbers of bacteria in the coastal water (site I)

In the water over the sediment (site I) the saprophytic bacteria count on NA ranged from < 10 c.f.u. g^{-1} to 1.1×10^5 c.f.u. g^{-1}; on ZO the number varied from < 10 c.f.u. g^{-1} to 1.2×10^4 c.f.u. g^{-1}. The minimum value of both bacteria groups occurred in April, maxima were recorded in June. Between May and August there were more saprophytic bacteria on NA than on ZO. The ZO/NA ratio ranged from 33% (in May) to 0.4% (in August). However, in April and October the numbers in both bacteria groups were similar.

The number of spore-forming bacteria in the water from April to October remained at a low level (< 10 c.f.u. g^{-1}), approaching the lowest values noted in the surface sediments at sites I, II and III. A distinct increase in the number of these bacteria in water occurred in November (2×10^3 c.f.u. g^{-1}). Throughout the investigation period the proportion of spore-forming bacteria in the total number of saprophytic bacteria (on NA) did not exceed 1%; only in April and November was it as high as 20%.

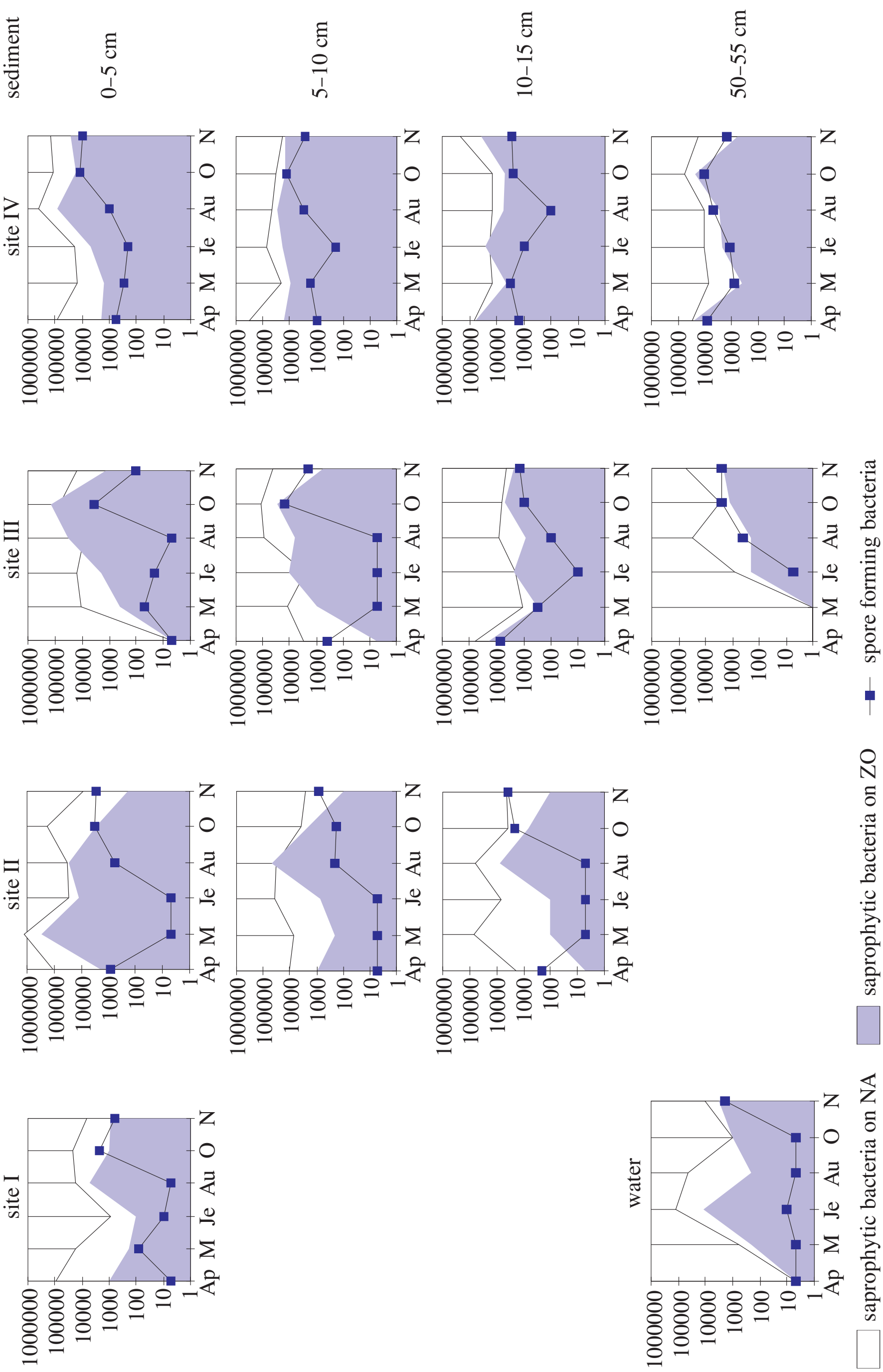

Fig. 2. Seasonal fluctuations in the counts of saprophytic and spore-forming bacteria

Numbers of bacteria in the surface layer of sandy sediments (sites II, III, IV)

With regard to the surface layer of sediments, the lowest number of saprophytic bacteria was found at site I. In sediment covered with water the number of bacteria on NA ranged between *ca* 10^3 c.f.u. g^{-1} and *ca* 10^5 c.f.u. g^{-1}, on ZO the respective figures were from 10^2 c.f.u. g^{-1} to 5.4×10^3 c.f.u. g^{-1}. Minimum counts of bacteria on both media were noted in June, maxima in April and August. The ZO/NA ratio varied from *ca* 1% in April and May to 32% in August. At this location, the mean value of ZO/NA with respect to the whole six-month period was 5.3%, the lowest at any of the sites under investigation.

The largest number of saprophytic bacteria in the surface layer of sediments was recorded at site II, on the waterline, and at site IV on a dune. The numbers of saprophytic bacteria on NA at site II ranged from 8×10^3 c.f.u. g^{-1} in November to 1.3×10^6 c.f.u. g^{-1} in May, and at site IV from 1.5×10^3 c.f.u. g^{-1} in May to 3.9×10^5 c.f.u. g^{-1} in August. At both sites, average ZO/NA values were similar: 19.2% at site II and 17.4% at site IV.

At site III, situated half-way up the beach, the numbers of saprophytic bacteria on NA in the surface layer of the sediment (0–5 cm) during consecutive months (with the exception of April) were in the range from 1.1×10^4 c.f.u. g^{-1} in May to 7×10^4 c.f.u. g^{-1} in October. However, the numbers of saprophytic bacteria on ZO indicated a greater variability, fluctuating from 4×10^2 c.f.u. g^{-1} in May to 1.5×10^5 c.f.u. g^{-1} in October. From April to June it was the saprophytic bacteria on NA (ZO/NA = *ca* 10%) that were highest in number; exceptionally, however, from August to October saprophytic bacteria on ZO dominated.

In general, the numbers of spore-forming bacteria in the surface layer of sediment at site I were higher than in the water, in particular in October. At the other sites (II, III, and IV) the numbers of spore-forming bacteria varied. The smallest quantities (< 10 c.f.u. g^{-1}) occurred in August at sites I and III and in May and June at site II. The largest numbers of such bacteria and the lowest numerical fluctuations were found to occur at site IV.

Numbers of bacteria in the core samples (site II, III and IV)

In general, the numbers of saprophytic bacteria in the core sediment samples tended to decrease, depending on depth.

At site II the number of saprophytic bacteria in the sediments ranged within wide limits during the various months. Thus, the number of bacteria on NA at a depth of 10–15 cm fluctuated from 2% in April and October to $> 90\%$ in August in relation to the counts in the surface layer, whereas on

ZO it ranged from $< 1\%$ in April, May and June to 50% in November. The ZO/NA ratio in April–August continued to decline with depth. However, in October and November a slight rise in this value was noted.

At site III in the surface layers (0–5, and 5–10 cm) it was observed that the numbers of saprophytic bacteria growing on both media were similar. Here, a depth-dependent decline in the bacterial count was also noted. Thus, at a depth of 10–15 cm the number of bacteria on NA fell to *ca* 10%, while on ZO it diminished to *ca* 3% in relation to the surface layer count. The ZO/NA ratio here rose along with the depth, but in August and October it decreased.

At site IV at the respective layer depths during the successive months relatively small changes in the numbers of saprophytic bacteria growing on both media were noted. The mean number of bacteria in all layers cultivated on NA ranged between 1.4×10^4 c.f.u. g^{-1} in May and 1.2×10^5 c.f.u. g^{-1} in April and August; on ZO it was from *ca* 3.8×10^3 c.f.u. g^{-1} in May to 2.4×10^4 c.f.u. g^{-1} in April and August. With respect to the total number of bacteria in the surface layer, the bacterial count decreased with depth: from *ca* 50% in May and June to 2.5% in August in the 10–15 cm layer on NA, and from $> 90\%$ in April and October to *ca* 3% in August and November on ZO. ZO/NA in the three layers from 0–5 to 10–15 cm rose together with depth. However, in the lowest layer (50–55 cm) these relationships were different. In May, June, and November the ZO/NA ratio fell, in other months this value rose.

The smallest numbers of spore-forming bacteria were found at sites II, III and IV in May–June (from < 10 to 3×10^2 c.f.u. g^{-1}). In April, October, and November the numbers were the largest – up to about 10^4 c.f.u. g^{-1}. In general, the largest numbers of these bacteria were recorded at site IV, particularly in the 50–55 cm layer. The percentage of spore-forming bacteria in the total number of saprophytic bacteria on NA rose with the depth.

3.2. Assessment of the sanitary state of the coastal water and the sandy beach

The bacteriological assessment of the sanitary state of the coastal water and the sand was based on the presence of faecal coliform bacteria and the number of mesophilic bacteria. Fig. 3 illustrates the counts of mesophilic bacteria and the numbers of faecal coliform bacteria on a semi-logarithmic scale, while Fig. 4 shows the changes in the sanitary quality of the water and sediment samples in the relevant months of the year.

The bacteriological quality of the water and sand covered by water during the investigation period was variable, but mostly class 1 or 2. The water samples were twice classified as class 3 (August, November), the sand

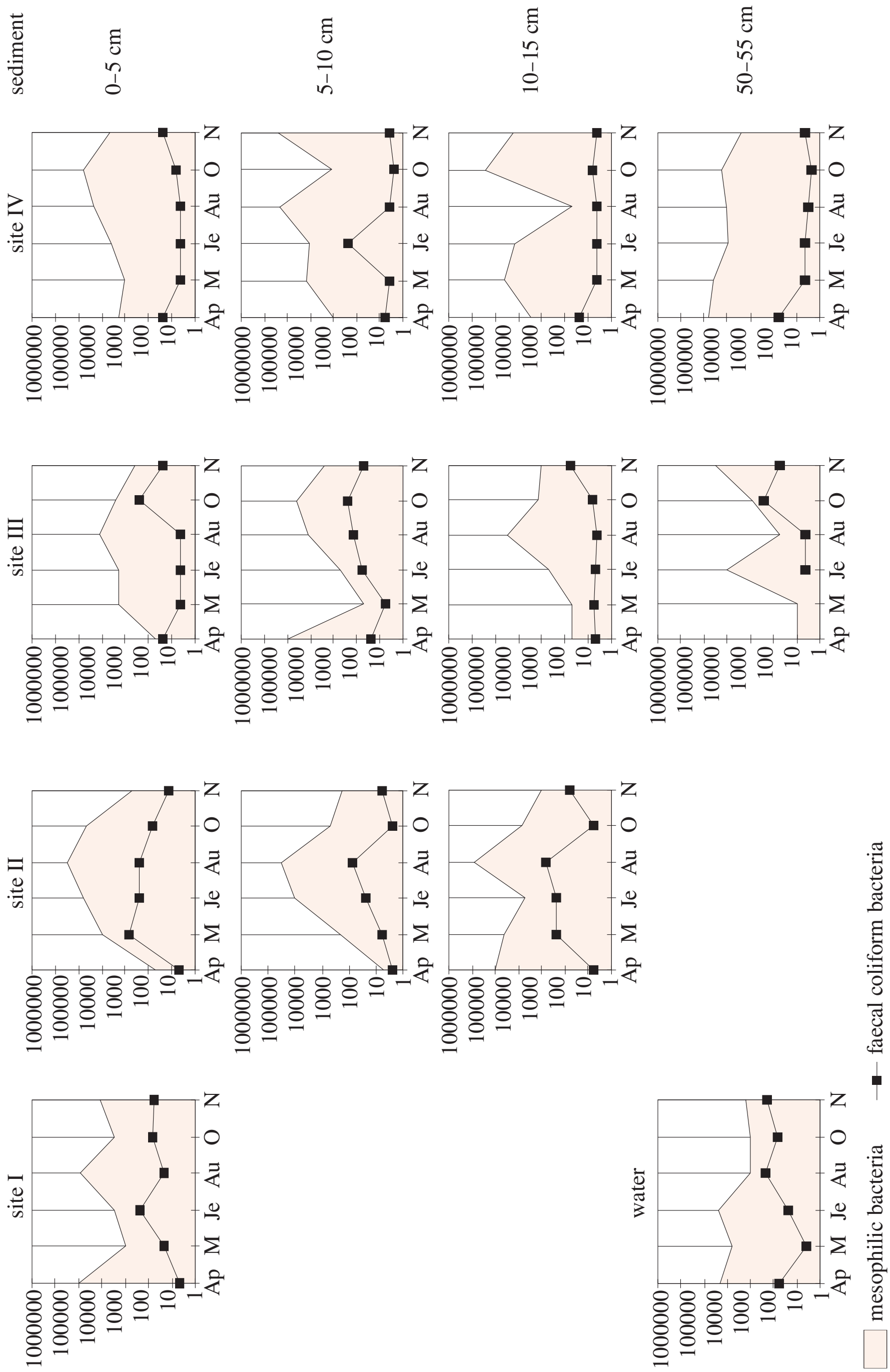

Fig. 3. Seasonal fluctuations in the counts of mesophilic and faecal coliform bacteria

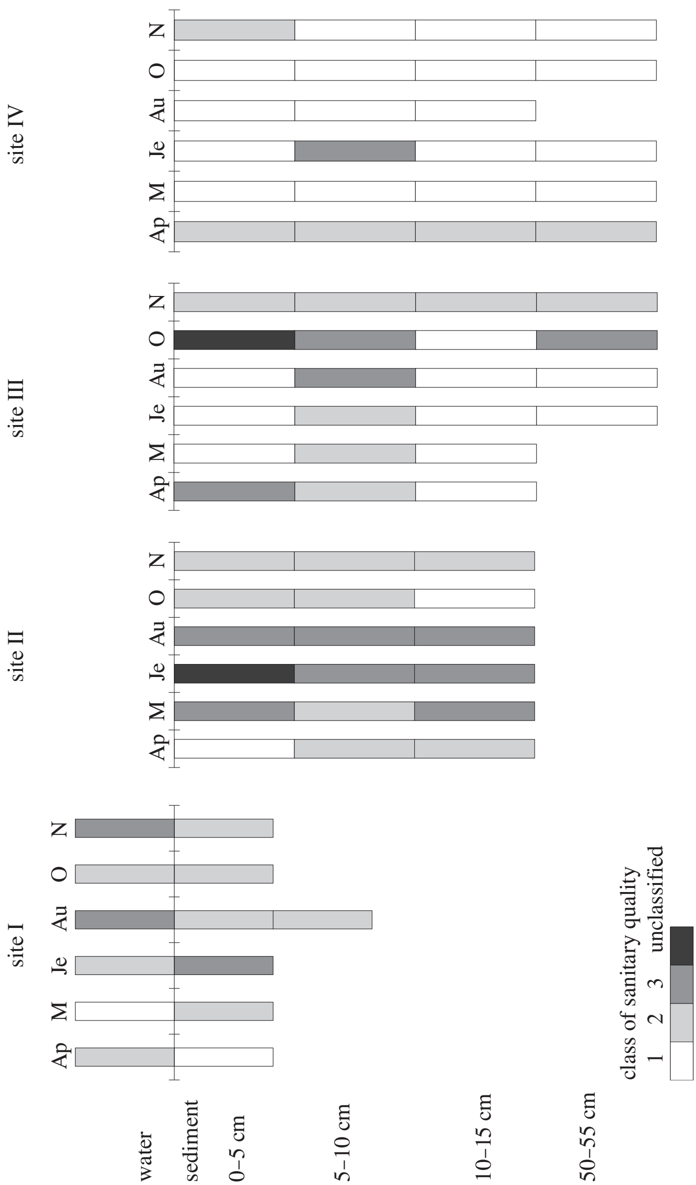

Fig. 4. Seasonal fluctuations in the sanitary quality of water and sediment

– once (June). The sanitary condition of the sand covered by water at site I did not usually correspond to the water quality. In most cases inverse relationships were noted. For instance, in June the number of mesophilic bacteria in the water amounted to 2.3×10^3 c.f.u. g^{-1}, whereas in the sediment there were only 3×10^2 c.f.u. g^{-1}; in August, there were less than 10^2 c.f.u. g^{-1} in the water, but 9×10^3 c.f.u. g^{-1} in the sediment.

The worst sanitary state was indicated by core samples taken at site II between May and August. At this time the sand was heavily polluted in all the layers from the surface to a depth of 15 cm. Quality class 3 was prevalent (class 2 and unclassified only once).

The numbers of mesophilic bacteria varied from 1×10^3 c.f.u. g^{-1} in May to 8.1×10^3 c.f.u. g^{-1} in August. In April, October and November, class 2 was dominant.

In general, with increasing distance from the shore the pollution of sediments by faecal coliform bacteria decreased. At site III, 32 m from the waterline, the sand was only once found to be badly contaminated – in October in the surface layer (0–5 cm).

The sanitary condition of the sand was best at site IV on the dunes. The mesophilic bacteria count there was from < 10 c.f.u. g^{-1} to *ca* 2.5×10^4 c.f.u. g^{-1}. The number of faecal coliform bacteria in most of the samples under investigation did not normally exceed 1 c.f.u. per gram of sand; only once – in June – at a depth of 5–10 cm did it reach 24 c.f.u. g^{-1}.

4. Discussion

The results of this investigation concern the bacteriological pollution of sandy beaches, *i.e.* the numbers of saprophytic and faecal coliform bacteria.

In the first part of the paper attention was drawn to the distribution on the beach of saprophytic bacteria determined on two different culture media: nutrient agar with fresh water (NA), and on ZoBell medium (ZO) with seawater of salinity 7 PSU (corresponding to the environment under test).

The investigation area covered four sites distributed along a transect perpendicular to the shore from the littoral zone (depth of water – 0.5 m) to an area on a dune (65 m from the waterline). The environment at each site was different.

Site I was situated at a distance of about 1–1.5 m from the seashore where the water is approximately 0.5 m deep. The results of the bacteriological tests of the coastal water and sediments reflect the degree of pollution in that zone. Taking into consideration the number of saprophytic bacteria cultured on NA one can conclude that the quality of coastal seawater varied from unpolluted to badly polluted (Cabejszek, 1974). This was confirmed by the wide range (from 0.4% – in August to 100% in April and October) of the percentage of bacteria incubated on ZO in the total number of saprophytic bacteria on NA, as well as large variations in the sanitary quality of the water – from quality class 1 to 3. It is to be expected that the sanitary conditions of the bottom sediments at this site, usually better than those of the water above the sediments, can be attributed to the lower survivability of the indicator bacteria in the presence of an abundant saprophytic microflora.

The observed changes in the counts of saprophytic bacteria and the simultaneous prevalence of fresh water – cultivated bacteria in the coastal water samples should be explained by the variable loads of organic pollutants and the high variation in the numbers of bacteria discharged daily in this area by the waters of the Grodowy Potok (Grodowy Stream) and Kamienny Potok (Kamienny Stream) (Olańczuk-Neyman, 1997) (Fig. 1a), as well as the effect of wind direction in this area. The number of faecal coliform bacteria discharged daily by the Grodowy Potok in the investigation period ranged from 1.2×10^8 to 7.7×10^{10} and by the Kamienny Potok from 6.2×10^8 to 9.3×10^{11} (Czerwińska *et al.*, 1998). It should be stressed that the extent of the streams influence on coastal seawater quality depends on the wind direction. Most often, the prevailing northerly and westerly winds in the spring and summer impedes the distribution of pollution into the coastal zone. The nearby pier may also help to prevent the spread of pollution (Olańczuk-Neyman *et al.*, 1992).

The lowest number of the saprophytic bacteria in the surface layer of sediments was noted at site I (covered with water) in comparison to the other sites, *i.e.* II, III and IV. This can by explained by the occurrence of numerous meiobenthic organisms grazing on bacteria (Elmgren, 1976; Reichelt, 1991). At the same time, the percentage of ZO/NA bacteria slightly exceeded 30%, that is to say, the number of allochthonous bacteria carried, for instance, by the streams was dominant.

In August the percentage of ZO/NA bacteria in the water above the sediments and in the sediment was quite different from the values in other months. This was due to the distinctive environmental conditions in this month: the water contained a large biomass of dead algae, which caused the water to become very turbid and to darken in colour; the characteristic odour of hydrogen sulphide was also noticeable. The sediments at this point were atypically grey-brown in colour.

It should be mentioned that the low proportion (up to 1%) of spore-forming bacteria noted in the coastal water in the total saprophytic bacteria count, increased to about 10% in the sediment. This is due to the relatively poor feeding conditions in the surface layer of sandy sediment at this site.

Located at the waterline, site II was partially flooded and frequently exposed to intense wave action. The largest numbers of saprophytic bacteria in the surface layer occur there in comparison to the other sites, and the mean ZO/NA value indicates a larger number of allochthonous bacteria than marine saprophytic bacteria. Among the allochthonous bacteria in May–August there was also a relatively high number of bacteria indicative of the quality class 3 or worse. The numbers of saprophytic bacteria in the sand decline with depth, and the decreasing percentage of ZO/NA bacteria (excluding October–November) indicate that this environment is still appropriate for saprophytic allochthonous bacteria.

The numbers of spore-forming bacteria, which vary with time and depth, can be attributed to the non-uniform availability of organic substances.

Site III was located between the shore and the edge of the beach at a distance of 32 m from the shore. Lower numbers of saprophytic bacteria were noted in the surface layers (0–5 and 5–10 cm) than at site II. The similar results obtained at site III in the first two layers (0–5 and 5–10 cm) are probably due to their partial mixing caused by the interaction of the environment, people trampling the sand, and wind. At the same time, there was a steady rise in the proportion of ZO bacteria in relation to NA, and even in some periods, an excess of the former. This refers to all the layers examined at this site. Moreover, the sand in the core samples taken at this site indicated that the sanitary condition there was much better than at site II; this indicates that pollutants carried by the coastal seawater reach that site only periodically.

Site IV was located in an enclosed area of dunes 60 m from the waterline, a highly stable environment. Although the number of saprophytic bacteria here varies only within an insignificant range, the rising trend in the proportion of ZO bacteria compared with the total number of NA bacteria is still detectable. The fact that the population of spore-forming bacteria in the deep layers of the dune is greater than in the surface layer proves that their access to nutrients is limited.

In general, the numbers of saprophytic bacteria found in the sandy beach sediments range from 7.8×10^2–4×10^4 c.f.u. g^{-1} on the eastern coast of the Mediterranean (Khiyama, 1973) to 1.3×10^9 c.f.u. g^{-1} on the southern Baltic (Meyer-Reil *et al.*, 1978).

The second part of the paper deals with the sanitary state of the beach, indicated by the numbers of mesophilic bacteria and faecal coliform bacteria. The latter are indicator bacteria used for assessing the sanitary condition of the environment. The presence of faecal coliform bacteria indicates recent faecal contamination, and that the spread of water-borne pathogenic microorganisms is possible.

It is worth pointing out that variations in the sanitary condition of the water during the successive months of the study were caused, among other things, by the variable daily number of bacteria carried by the streams into the coastal waters, and the changeable direction of the wind.

At site II, located as it is in an area of intensive wave action and high rates of filtration and sorption, earlier observations of much larger numbers of indicator bacteria in sand washed by water than in the water itself were corroborated. On the other hand, the sanitary condition of sand not washed by water (sites III and IV) is much better, which also confirms earlier results (Olańczuk-Neyman and Czerwionka, 1994). The lower numbers of indicator bacteria in the surface layer of sand at sites III and IV than at sites I and II, noticeable particularly in the summer months, are probably due to solar radiation and the longer distance from the water-line (Kokina, 1986; Olańczuk-Neyman *et al.*, 1992).

Spore-forming bacteria were present in the largest numbers in the dune region (site IV) and represented a significant percentage of the total saprophytic bacteria count: from 1.1% in June to 10% in October and November in the surface layer, and from 11% in May to over 90% in April and October in the deeper layers.

At site III spore-forming bacteria occurred in the largest numbers in April ($>90\%$). In the surface layer of sediments, the proportion of these bacteria in the total number of the saprophytic bacteria cultivated on NA at sites I and II (the saturation zone) was insignificant. This relationship was particularly evident in May (0–4%), June (0.01–1.1%) and August (0.8–1.8%). Here too, in deeper sediment layers, the proportion of spore-forming bacteria was higher ($>90\%$) in April and November.

5. Conclusions

The results of investigations into sandy sediments at four sites located on a transect perpendicular to the waterline of the beach in Sopot covering a distance from the littoral zone (water depth of 0.5 m) to the dune sand (65 m away from the waterline) lead to the following conclusions:

- The beach area under investigation is located in an area between the mouths of two streams carrying freshwater with temporally variable loads of organic pollutants and different numbers of bacteria per day. The bacteriological quality of the coastal waters thus fluctuates widely.
- There was considerable differentiation in the number of bacteria in the sandy sediments at the test sites representing different ecological conditions. These sediments vary in their content of saprophytic bacteria. At the sites situated in the vicinity of the littoral zone, the

domination of allochthonous bacteria is significant. With increasing distance from the shore, their prevalence declines in favour of autochthonous bacteria, an indication of the diminishing effect of anthropogenic pollutants.

- The most serious bacteriological pollution of sand determined by the numbers of saprophytic and indicator bacteria was found in the zone of sand washed by water; the sand on the dune was found to be of much better sanitary quality.

These results will be subjected to further validation.

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