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PHYSIOLOGICAL PROPERTIES OF BACTERIA INHABITING POLLUTED AND UNPOLLUTED MARINE SANDY BEACHES (SOUTHERN BALTIC SEA)

ABSTRACT: The occurrence of bacteria displaying particular physiological properties was studied in polluted (Sopot) and unpolluted (Czołpino) marine sandy beaches (southern Baltic Sea). All eight isolated physiological groups of bacteria were much more numerous in polluted than in unpolluted beach. In polluted beach, bacteria hydrolyzing uric acid (32.5 cells 10^3 g^{-1} dry w. of sand) and ammonifying bacteria (32.3 cells 10^3 g^{-1} dry w. of sand) were the most numerous, while nitrifying bacteria were the least numerous (0.014 cells 10^3 g^{-1} dry w. of sand). In unpolluted beach, bacteria hydrolyzing uric acid (0.66 cells 10^3 g^{-1} dry w. of sand) and reducing methylene blue (0.18 cells 10^3 g^{-1} dry w. of sand) were the most numerous, while no bacteria producing hydrogen sulphide from organic compounds or bacteria decomposing urea were isolated. In both beaches, considerable differentiation in the distribution of physiological groups of bacteria was found in a horizontal profile i.e. from the waterline to the middle of beach (~60 m). Data concerning horizontal distribution of the physiological groups of bacteria in the sand of the polluted beach show that the majority of those groups was most numerous in the dune. No clear regularity in the distribution of physiological groups of bacteria was found in the horizontal profile of the unpolluted beach. Results of the present study indicate differences in the distribution of the physiological groups of bacteria in the surface (0–5 cm) and subsurface (5–10 cm) sand layers. Generally, in both studied beaches all physiological groups of bacteria were much more numerous in the surface than in the subsurface sand layer. The exception were bacteria reducing sulphates which in the polluted beach were most numerous at the depth of 5–10 cm.

KEY WORDS: Southern Baltic, marine beach, bacteria, physiological properties

1. INTRODUCTION

Sandy beaches are marine coastal ecosystems constituting a buffer zone between the land and the sea (Novitsky and MacSween 1989). Those environments are very dynamic, as they are determined by wind, sand and water remaining in constant motion (Schoeman *et al.* 2000). In coastal ecosystems, sandy beaches play an important role in energy flow and organic matter turnover. According to Nair and Bharathi (1980), Brown and McLachlan (1990), and Heymans and McLachlan (1996) they can be considered as the huge filters through which large amounts (10–70 $m^3 \cdot m^{-1} d^{-1}$) of water are filtered.

While the seawater is filtered, the organic matter in the dissolved (DOM) and particulate (POM) form is adsorbed on the surface of the sand grains. This organic matter consists mainly of phytobenthos assimilates, products washed and leached out of the seaweed, animal faeces produced mainly by meio- and macrofauna, and remains of marine plants and animals (Koop and Griffiths 1982, Koop *et al.* 1982, Brown and McLachlan 1990, Jędrzejczak 1999). Organic matter reaching marine coastal habitats can also originate from rivers and the surrounding land (Węsławski *et al.* 2000). The organic matter accumulated in marine beaches is further utilized by interstitial organisms and returns to the sea in the form of nutrients. Therefore, in most beaches the interstitial system functions as a biological filter that enhances the mineralization of organic matter and thus purifies the water (McLachlan and Romer 1990, McLachlan and Turner 1994, Urban-Malinga and Opaliński 2001).

The key role in the processes of destruction and transformation of organic matter in marine beaches is played by bacteria (Meyer-Reil *et al.* 1980, Brown and McLachlan 1990, Podgórska and Mudryk 2003). According to Koop and Griffits (1982) bacteria can mineralise about 70% of the organic matter reaching the sea beaches. The rate of decomposition of DOM and POM depends on the physiological properties of bacteria and on their metabolic activity (Krstulović and Solić 1988). To date, physiological properties of bacteria inhabiting marine beaches were rarely studied (Mudryk *et al.* 2001), and the understanding of those problems is still incomplete and fragmentary. Therefore, the aim of this paper was to determine physiological properties of bacteria inhabiting two marine sandy beaches differing in the level of human impact and to asses their role in the transformation of organic matter and self-purification of marine sandy beaches.

2. STUDY AREA AND METHODS

The study was carried out on two sandy beaches differing in the level of human impact. The Sopot beach (Fig. 1) affected stronger is very high, as the beach is located close to a densely populated urban area, and is frequented by holidaymakers whose density in summer reaches 30 persons per 100 m^2; about 3000 people can visit this place daily (Węsławski *et al.* 2000). The beach in Czołpino (Fig. 1) is located in the Słowiński National Park and is rarely visited, therefore the level of human impact there is relatively low. In the paper the beach in Sopot was described as "polluted" beach, while the beach in Czołpino as "unpolluted" one.

Sand samples were taken in July 2001. A transect was marked along a profile formed perpendicularly to the shoreline, and four sampling sites were located along this transect (Fig. 1). Site 1 was located approximately 1–1.5 m from the waterline into the water, at a depth about 1 m, site 2 was situated at the waterline, site 3 lies halfway up the beach, at 30 m distance from the waterline, and site 4 lies in a sheltered place in the dune, 60 m away from the waterline.

Sand core samples were taken with a Morduchaj-Boltowski core scoop (length – 30 cm, inner diameter – 15 cm) five per station. In the field, the sampled sand cores were divided vertically into three sections: 0–1 cm, 1–5 cm, 5–10 cm and placed in sterile glass jars, which were put into containers with ice and transported to the laboratory. The time between sample collection and bacteriological tests did not usually exceed 2–3 h.

Aseptically weighted 10 g sand samples were transferred into sterile marine water for subsequent homogenisation (NPW 120 homogeniser, 5 min. at 23000 rpm) in order to desorb the microorganisms normally stuck to sand grains. The supernatant was serially diluted with sterile seawater to reach final concentration ranging from 10^{-1} to 10^{-5} and each suspension was inoculated on different media.

The following physiological properties of bacteria were considered:

1. The ability to ammonify was examined in a liquid medium prepared according to Seeley *et al.* (1991). NH_3 was detected with Nessler`s reagent.
2. The ability to produce hydrogen sulphide from organic compounds was tested in a liquid medium prepared according to Rodina (1972). The presence of hydrogen sulphide was detected with paper strips

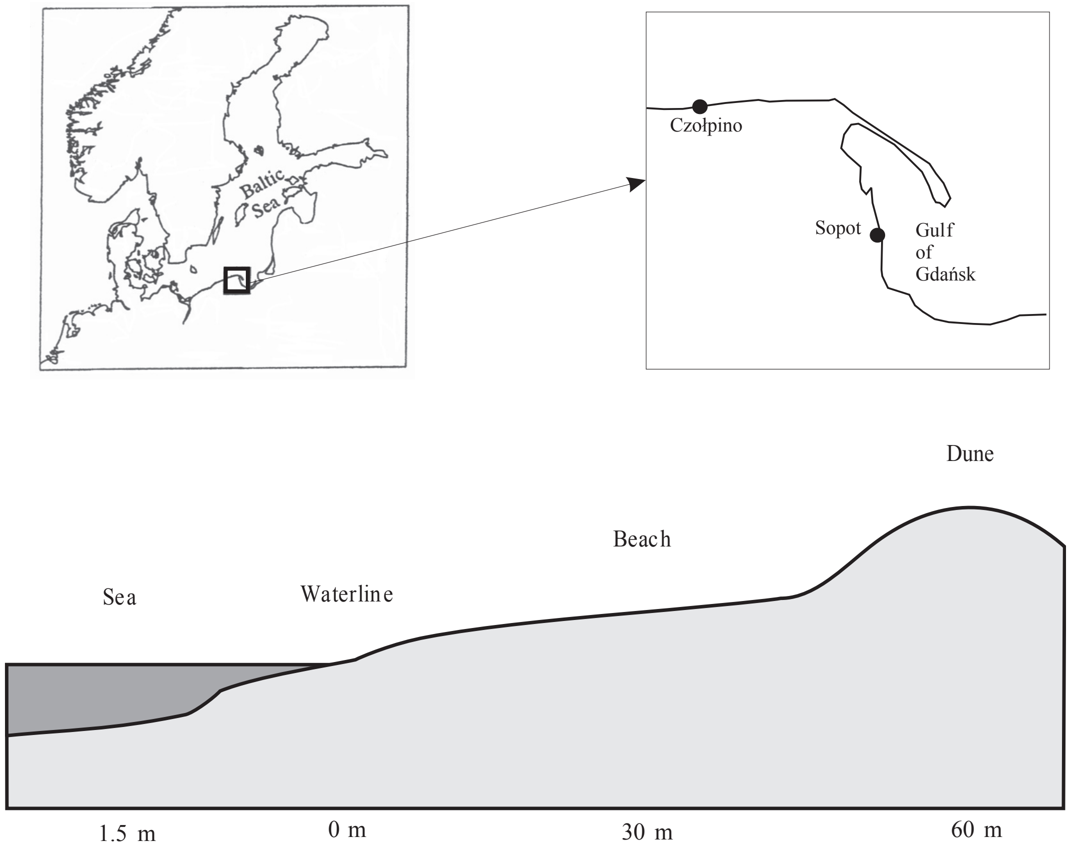

Fig. 1. Map of the study sites: polluted beach in Sopot and unpolluted in Czołpino. Location of the sampling sites in the profile of the beach.

saturated with 10% lead acetate and placed inside test tubes above the medium.

3. The ability to carry out heterotrophic nitrification was tested in a liquid medium prepared according to Kölbel-Boelke *et al*. (1988). Nitrates were detected with α-naphthylamine and sulphanilic acid.

4. The ability to reduce nitrates to nitrites (partial denitrification) was tested according to Kölbel-Boelke *et al.* (1988). Nitrates were determined as described above.

5. The ability to reduce sulphate was tested in a liquid medium prepared according to Postage (1966). The reducing factor (thioglycollic acid), separately sterilised on membrane filters, was introduced into the sterilised medium. Blackening of the medium was a positive result.

6. The ability to reduce methylene blue was tested in a liquid medium prepared according to Rodina (1972) with 0.02% aqueous solution of methylene blue. Medium decolouring was a positive result.

7. Decomposition of urea was tested in a liquid medium prepared according to Seiler *et al*. (1980). Urea was sterilised by membrane filtration. The change of colour from orange to pink was a positive result.

8. Uric acid hydrolysis was tested on agar medium prepared according to Steinmann (1976). Clear zones around the colonies were a positive result.

The pH of all media was adjusted to 7.0–7.4. The media were sterilised at 117^{o}C for 20 min and subsequently inoculated with 1 cm^3 of appropriately diluted solutions of the sand. The incubation was carried out at 20^{o}C. The tests number 1, 2, 4, 6, 7, and 8 lasted 6 days, and tests number 3 and 5 lasted 14 days. The number of bacteria decomposing uric acid was recorded in Petri dishes, whereas the numbers of the other physiological groups were determined with the MPN method, on the basis of Mc Crady´s tables (Seeley *et al.* 1991). Each test was carried out in triplicate. An additional sample of 20 g of sand was weighed and dried at 105°C for 24 h in order to determine the dry weight of the sand.

3. RESULTS

The physiological groups of bacteria were much more numerous in the sand of the polluted beach in Sopot than unpolluted one in Czołpino (Table 1). On the polluted beach, the most numerous were bacteria hydrolyzing uric acid (32.5 cells 10^3 g^{-1} dry w. of sand) and ammonifying bacteria (32.3 cells 10^3 g^{-1}dry w. of sand). Bacteria able to reduce methylene blue and to produce hydrogen sulphide from organic compounds were also relatively numerous. The least numerous were nitrifying bacteria (0.014 cells 10^3 g^{-1}dry w. of sand). On the unpolluted Czołpino beach, the predominant physiological groups were bacteria hydrolyzing uric acid (0.66 cells 10^3 g^{-1} dry w. of sand) and reducing methylene blue (0.18 cells 10^3 g^{-1} dry w. of sand). No bacteria producing hydrogen sulphide from organic compounds or decomposing urea were isolated from the sand of this beach.

On the polluted beach, denitrifying bacteria, bacteria hydrolyzing uric acid and urea, producing hydrogen sulphide from organic compounds, and reducing methylene blue were most numerous in the dune (Fig. 2). The highest numbers of ammonifying and nitrifying bacteria were determined at the waterline, while bacteria reducing sulphates were most numerous in the sea (Fig. 2). On the unpolluted beach, ammonifying and denitrifying bacteria, and bacteria reducing methylene blue were most numerous on the middle beach, while bacteria reducing sulphates, hydrolyzing uric acid and nitrifying bacteria were most numerous at the waterline (Fig. 3).

Data concerning vertical distribution of the physiological groups of bacteria in the sand of the polluted beach show that the majority of them were most numerous at the depth of 1–5 cm (Figs. 4 and 5). Only ammonifying bacteria reached maximum numbers in the surface layers of the sand (0–1cm), and bacteria reducing sulphates were most numerous at the depth of 5–10 cm (Fig. 4). On the unpolluted beach, ammonifying, denitrifying bacteria, and bacteria reducing methylene blue were most numerous in the surface layers of the sand (0–1cm), while bacteria hydrolyzing uric acid, reducing sulphates, and carrying out the process of nitrification were most numerous at the depth of 1–5 cm (Fig. 5).

Table 1. Abundance (cells 10^3 g^{-1} dry w. of sand) (n = 12) of different physiological groups of bacteria inhabiting the polluted (Sopot) and unpolluted (Czołpino) beaches.

Physiological groups of bacteria	Polluted beach		Unpolluted beach	
	mean	SD mean		SD
Amonifying	32.30	78.33	0.022	0.042
Methylene blue reducing	13.58	35.36	0.18	0.33
H_2S producing	3.90	7.69	0.0	0.0
Denitrifying	0.89	2.23	0.012	0.020
Nitrifying	0.014	0.023	0.005	0.007
Sulphate reducing	0.40	0.93	0.001	0.001
Uric acid hydrolysing	32.50	64.90	0.66	0.69
Urea decomposition	0.42	0.61	0.0	0.0

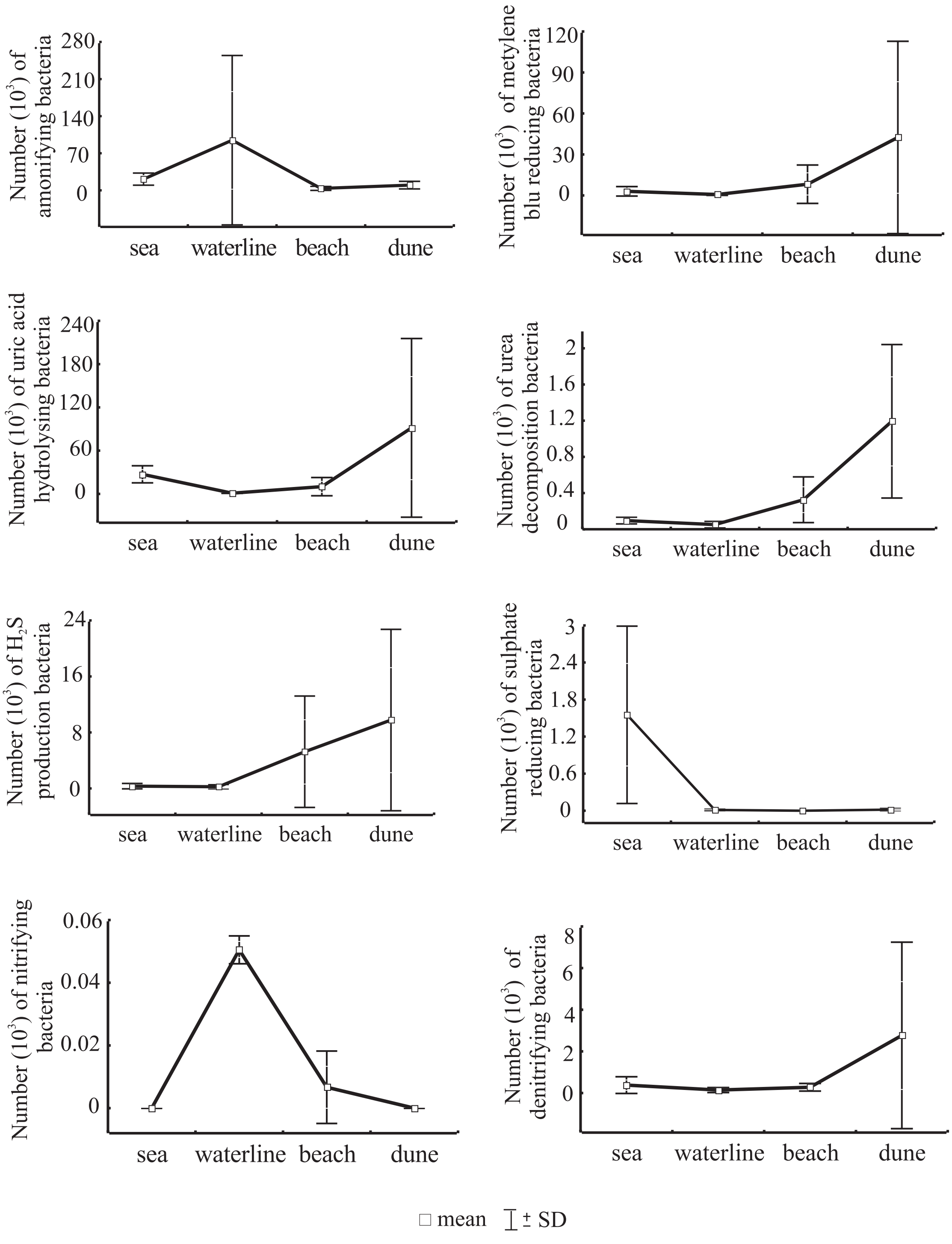

Fig. 2. Distribution of physiological groups of bacteria (along the horizontal profile of polluted beach in Sopot). Number of cell bacteria per g^{-1} dry w. of sand.

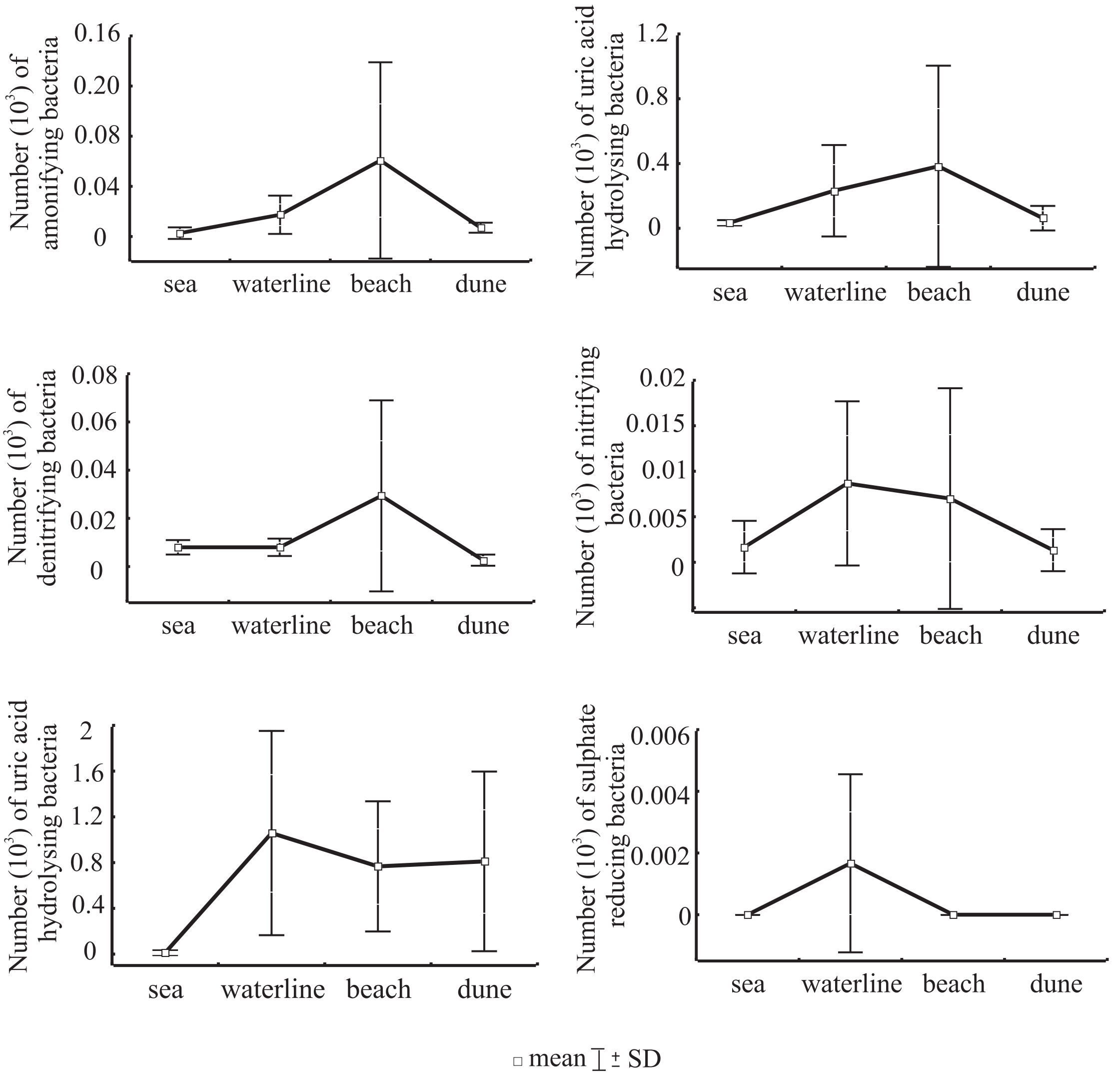

Fig. 3. Distribution of physiological groups of bacteria (along the horizontal profile of unpolluted beach in Czołpino). Number of cell bacteria per g^{-1} dry w. of sand.

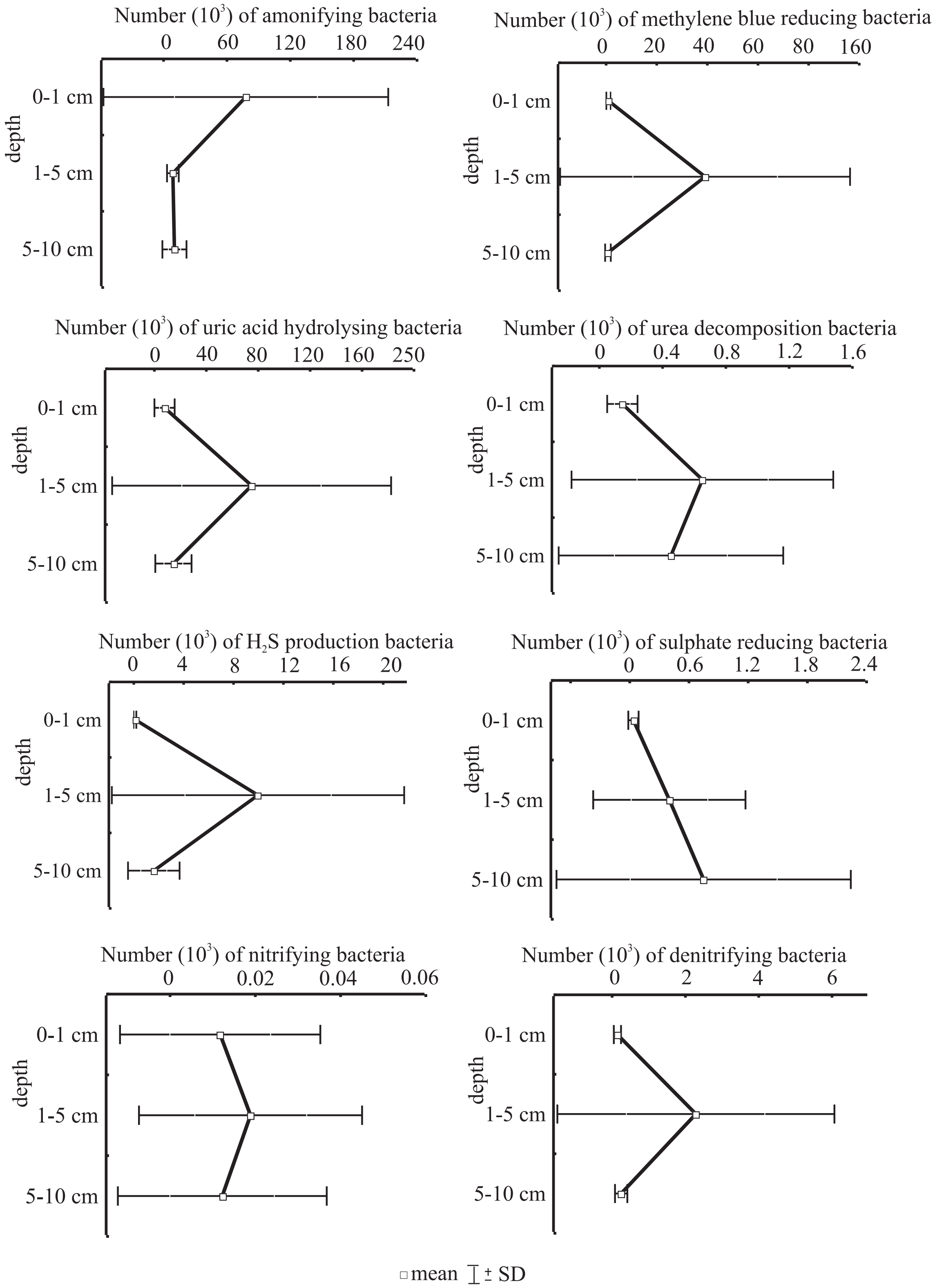

Fig. 4. Vertical distribution of the physiological groups of bacteria in a 10 cm layer in g^{-1} dry w. of sand on the polluted beach in Sopot (the pooled data of all sites).

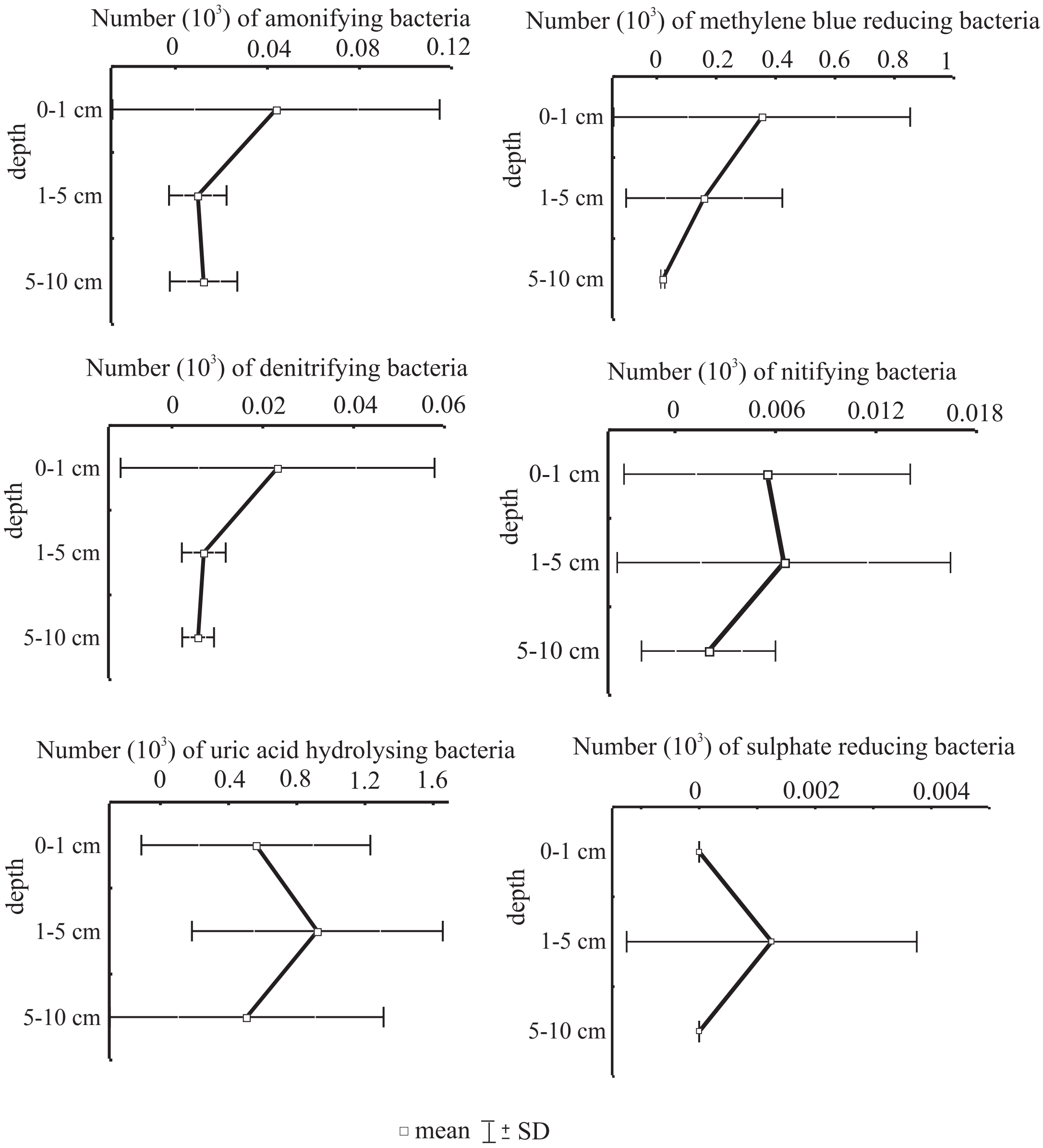

Fig. 5. Vertical distribution of the physiological groups of bacteria in a 10 cm layer in g^{-1} dry w. of sand on the unpolluted beach in Czołpino (the pooled data of all sites).

4. DISCUSSION

In the sand of the studied beaches, bacteria hydrolyzing uric acid were the most numerous physiological group. According to Salas and Ellar (1985) bacteria utilize this compound as carbon and nitrogen source. The main sources of uric acid in the marine costal zones are faeces (guano) of water fowl, often occurring in high numbers in those areas (McLachlan 1983). In birds, ammonia formed during amino acid catabolism is converted to uric acid for excretion in the form of excrements (Singer 2003). Zdanowski *et al.* (2005) draw attention to the very important role of bacteria in guano degradation. Bacteria capable of degrading uric acid are found in gastrointestinal tracts in many species of herbivorous and granivorous birds (Braun and Campbell 1989). End products of microbial degradation of uric acid include short chain fatty acids, ammonia, and carbon dioxide (Mead 1989, Schmidt *et al.* 2004). Steinmann (1976) determined that the abundance of bacteria decomposing uric acid was related to the total abundance of saprophytic bacteria, which means that they may be used as indicators of water and sediment pollution.

In the sand of the polluted beach, bacteria carrying out the process of amino acid deamination constituted a numerous physiological group. The studies carried out by Donderski (1971); Mudryk (1987), Iriberri *et al.* (1988) and Mudryk *et al.* (1991) have shown that ammonifying bacteria play a key role in the processes of mineralization of organic nitrogen in water bodies and constitute one of the most numerous physiological groups of microorganisms. According to Billen and Fontigny (1987) and Brown and McLachlan (1990), the intensity of bacterial ammonification in water bodies depends on the concentration of organic matter. The present study also shows that in the polluted beach, where larger amounts of organic matter were accumulated (4.08 mg g^{-1} dry w. of sand), the number of ammonifying bacteria was considerably higher than in the unpolluted beach, where organic matter concentration was only 1.84 mg g^{-1} dry w. of sand (Podgórska 2002).

Gunkel *et al.* (1990) draw attention to the fact that in the costal zone of the Baltic Sea high concentration of urea is observed; it is carried into this area by numerous rivers, and originates from sanitary pollution. Urea is also excreted into the sea water as a final product of zooplankton and fish metabolism (Eppley *et al.* 1973, Singer 2003). Hence, Steinmann (1976) and Jørgensen *et al.* (1999) have shown that Baltic Sea is inhabited by large numbers of bacteria characterised by the ability to hydrolyse urea. This has not been shown in the polluted beach where bacteria hydrolyzing urea were not numerous; from the sand of the unpolluted beach no bacteria synthesizing urease were isolated. These results may indicate that concentration of urea accumulated in the sand of both beaches was low.

Microbiological nitrification is an important process of nitrogen regeneration in estuaries and seas (Caffrey and Miller 1995). Nitrifying bacteria compete with phytoplankton and phytobenthos for ammonia, which for algae is the most preferred source of nitrogen (Keil and Kirchman 1991). Usually, the assimilation of NH_4^+ by phytoplankton and phytobenthos is faster than its oxidation by bacteria (Ward *et al.* 1984) which might explain the very low numbers of nitrifying bacteria in the sand of the studied beaches. Similarly, Donderski (1971) and Petrycka *et al.* (1990) have shown that nitrifiers constitute one of the least numerous physiological groups of bacteria in water bodies.

Bacteria reducing sulphates were another physiological group present in low numbers in the sand of both beaches. Low numbers of those bacteria were also observed in the bottom sediments of the German zone of the Baltic Sea (Gast and Gocke 1988, Bussmann and Reichardt 1991). Taking into account the relatively good oxidation of the surface layer of the sediments and anaerobic character of the process of desulphurication, such result is fully understandable.

The zonation of different groups bacteria in sandy beaches is a well known phenomenon in marine ecosystems (McLachlan and Jaramillo 1995, Olańczuk-Neyman and Jankowska 1998 Mudryk *et al.*

2001). Also in both studied beaches, a considerable differentiation in the distribution of physiological groups of bacteria was determined in a horizontal profile. According to Novitsky and MacSween (1989) and Jędrzejczak (1999) such horizontal variations in the number of physiological groups of bacteria is possibly due to different of physiochemical conditions (temperature, oxygen, pH salinity, composition and availability of inorganic and organic matter) along the zones of a marine beach. Therefore, particular zones of a marine beach are populated by specific physiological groups of bacteria carrying out different metabolic processes (Brown and McLachlan 1990, McLachlan and Jaramillo 1995).

According to Koop and Griffiths (1982) and Brown and McLachlan (1990) also bacterivorous organisms mainly meiofauna and macrofauna, for which bacteria are a basic source of food, are important factors affecting numbers and distribution of bacteria on marine beaches. Meiofauna and macrofauna, which, as has been shown by Haque *et al.* (1996) are very numerous in the studied beaches also can significantly influence on the number and distribution of physiological groups of bacteria in a horizontal profile. These organisms can show preference for specific physiological groups of bacteria in their diet.

Results of the present study indicate clear differences in the distribution of the physiological groups of bacteria in the surface (0–5 cm) and subsurface (5–10 cm) sand layers. In both studies beaches physiological groups of bacteria were more numerous in the surface than subsurface sand layers. Similarly, Boucher and Chamroux (1976) and Olańczuk-Neyman and Jankowska (1998) in other marine beaches have also determined a clear tendency for the decrease in the number of bacteria with increasing depth. This pattern of distribution results most probably from the fact that the concentrations of inorganic and organic matter and oxygen which are main stimulators of physiological activity and growth for bacteria, decrease with depth.

In summary, the data presented in this paper indicate that between polluted and unpolluted beaches there is a considerable differentiation in the number of bacteria displaying particular physiological properties. All studied physiological groups of bacteria were much more numerous in polluted than in unpolluted beach. Such results indicate the potential role of bacteria in the processes of self-purification of marine sandy beaches.

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