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ORIGINAL RESEARCH ARTICLE

Microbial enzymatic activity and its relation to organic matter abundance on sheltered and exposed beaches on the Polish coast

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Principal component analysis

Summary The activity of lipase, aminopeptidase, α-glucosidase, β-glucosidase was correlated and assessed according to an abundance of organic matter and total forms of nutrients in beach sediments characterized by different strength of anthropopressure and degree of sheltering. 76% of the data variance was explained by six factors identified by the use of principal component analysis: (1) anthropogenic rich in N, (2) microbial enzymatic activity, (3) labile organic matter, (4) bacterial growth, (5) anthropogenic rich in P and (6) hydrolytic. Differences in secondary bacterial production according to the distance from the water line, vertical cores and seasonality are limited by the accessibility of biochemical compounds (lipids, proteins, carbohydrates, total organic carbon), total phosphorus and nitrogen. Sediments collected in exposed beaches were not as rich in organic matter as these collected in sheltered ones due to the impact of sea waves of higher energy and backward current facilitating cleaning. The highest microbial enzymatic activity was observed in the beach infilled prior to the tourist season with well-aerated sand mined from the main harbor canal. Microorganisms induce α-glucosidase synthesis to decompose hardly assimilable COM during deficit of easily assimilable PRT and CHO. The lack of easily assimilable matter activates stronger hydrolytic activity in lower layers of core sediments.

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1. Introduction

Sea coasts are contact zones between the land and the sea or the ocean. One of the few possible types of coasts are beaches, which are the most common form of littoral accumulation. Sandy beaches being a buffer zone between the land and the sea are characterized by wide spectrum of sizes, morphologies and ranges of exposure to oceanographic conditions (Mudryk et al., 2011; Novitsky and MacSween, 1989; Rodil and Lastra, 2004). Those environments are very dynamic, as they are shaped by wind, sand and water remaining in constant motion (Germán Rodríguez et al., 2003; McLachlan et al., 1996; Rodil and Lastra, 2004; Schoeman et al., 2000). Marine beaches, sandy ones in particular, are often subjected to considerable anthropogenic pressure due to recreational and economic functions (Antonowicz et al., 2015; Węsławski et al., 2000). Diverse forms of organic matter including variety of its constituents (lipids, proteins, carbohydrates, total organic carbon, total phosphorus and total nitrogen) transform beaches into specific ecosystems inhabited by microorganisms which participate in the transformation and mineralization of the matter (Koop and Griffiths, 1982; Phillips et al., 2011), and hence sandy beaches play an important role in energy flow and organic matter turnover. Being considered an important component of sandy beach community, bacteria mineralize about 70% of organic matter. Beaches can also be considered huge water filters (approximately 10—70 $m^3\ m^{-1}\ d^{-1}$) (Brown and McLachlan, 1990; Heymans and McLachlan, 1996; Nair and Loka Bharathi, 1980). During water permeation, a large amount of organic matter is adsorbed by the sand grain surface as particulate (POM) and dissolved (DOM) organic matter (Mudryk and Podgórska, 2006).

Productivity of sandy beaches is ultimately limited by the nutrient load (Khiyama and Makemson, 1973). The rate of DOM and POM decomposition depends on the availability of nutriment, physiological properties and bacteria metabolic activity. According to Boetius (1995), production and activity of bacterial hydrolytic enzymes depend on availability, distribution and concentration of organic substrates. Therefore, the activity of enzymes in vertical profiles reflects the distribution of organic matter in water basin sediments. Organic matter accumulated in sediments is further utilized by interstitial organisms and returns to the sea in the form of nutrients. Therefore, on most beaches an interstitial system acts as a biological filter that enhances the mineralization of organic matter and purifies water. Heterotrophic bacteria inhabiting coastal ecosystems are not a homogeneous group of organisms. They represent the population of various physiological groups which is characterized by the ability to carry out the processes of depolymerization of a wide spectrum of macromolecular compounds (Krstulović and Solić, 1988; Mudryk et al., 1999, 2011).

Quality and quantity of organic matter in surface sediments have been considered a major factor in determining the amounts of material potentially available to consumer organisms, thus affecting community structure and benthic metabolism (Buchanan and Longbottom, 1970; Graf et al., 1983; Graf, 1989; Grant and Hargrave, 1987; Thompson and Nichols, 1988). Organic matter (OM) in the marine environment consists of labile and refractory compounds whose relative importance may have profound implications for OM diagenesis and organic carbon turnover (Danovaro et al., 1993; Daumas et al., 1983; Fabiano et al., 1995; Fichez, 1991a; Rowe and Deming, 1985). The labile portion contains mainly simple sugars, fatty acids and proteins that are rapidly mineralized. On the contrary, the refractory matter, which consists of substances like humic and fulvic acids and complex carbohydrates, is characterized by lower degradation rates (Biddanda and Riemann, 1992; Buscail et al., 1990; Danovaro et al., 1999a; Fabiano and Danovaro, 1994; Handa et al., 1972; Robinson et al., 1982; Sargent et al., 1983; Wilson et al., 1986). Sandy beaches usually receive large input of organic matter, which comprises an important source of nutrients for offshore production (Brown and McLachlan, 1990; Jędrzejczak, 1999). Local changes of sedimentary organic matter in the marine environment affect spatial distribution, metabolism and dynamics of all benthic components, from bacteria to macrofauna (Cividanes et al., 2002). Quantitative information on vertical fluxes of particulate proteins, carbohydrates and lipids is extremely rare (Danovaro et al., 1999b). In general, it is expected that labile carbon flux is coupled with surface productivity and decreases with depth (Carney, 1989).

Despite the fact that a range of studies have been conducted worldwide on microbial enzymatic activity on various beaches (Cividanes et al., 2002; Danovaro et al., 1993, 1999a,b; Danovaro, 1996; Dell'Anno et al., 2002; Fabiano et al., 1995, 2004; Fernandes et al., 2012; Fichez, 1991a,b; Graf and Meyer-Reil, 1985; Khripounoff et al., 1985; Meyer-Reil, 1983), only a few of them were focused on the comparison of microbial enzymatic activity. Few papers have attempted to analyze microbial enzymatic activity comprehensively according to biochemical composition of the sedimentary organic matter and specific characteristic of the beach. Moreover, to the best of our knowledge, in the case of the Polish coast only two scientific papers concern microbial enzymatic activity on sandy beaches (Mudryk and Podgórska, 2006; Perliński and Mudryk, 2016). Therefore, the aims of this study were to investigate: (1) the biochemical composition variability of the sedimentary organic matter and microbial enzymatic activity on 3 beaches subjected to a different degree of exposure and anthropopression, (2) the temporal changes in the quantity of sedimentary organic matter composition and microbial enzymatic activity in sheltered and exposed beaches, and (3) the variation of the above mentioned parameters according to the distance from the water line and vertical core depth.

2. Material and methods

2.1. Study area and sampling sites description

The study was carried out in three spots on 130 km long section of the Polish coast, between 232nd and 102nd km of the Polish sea border where the widest, the most beautiful and attractive, according to touristic activity, sandy beaches are located (Fig. 1).

Samples were collected in Ustka (54°34′N/16°51′E) on the eastern side of the mouth of the Słupia river, in Czołpino (54°43′N/17°14′E) and in Puck (54°44′N/18°24′E). Ustka, Czołpino and Puck are situated in northern Poland. They

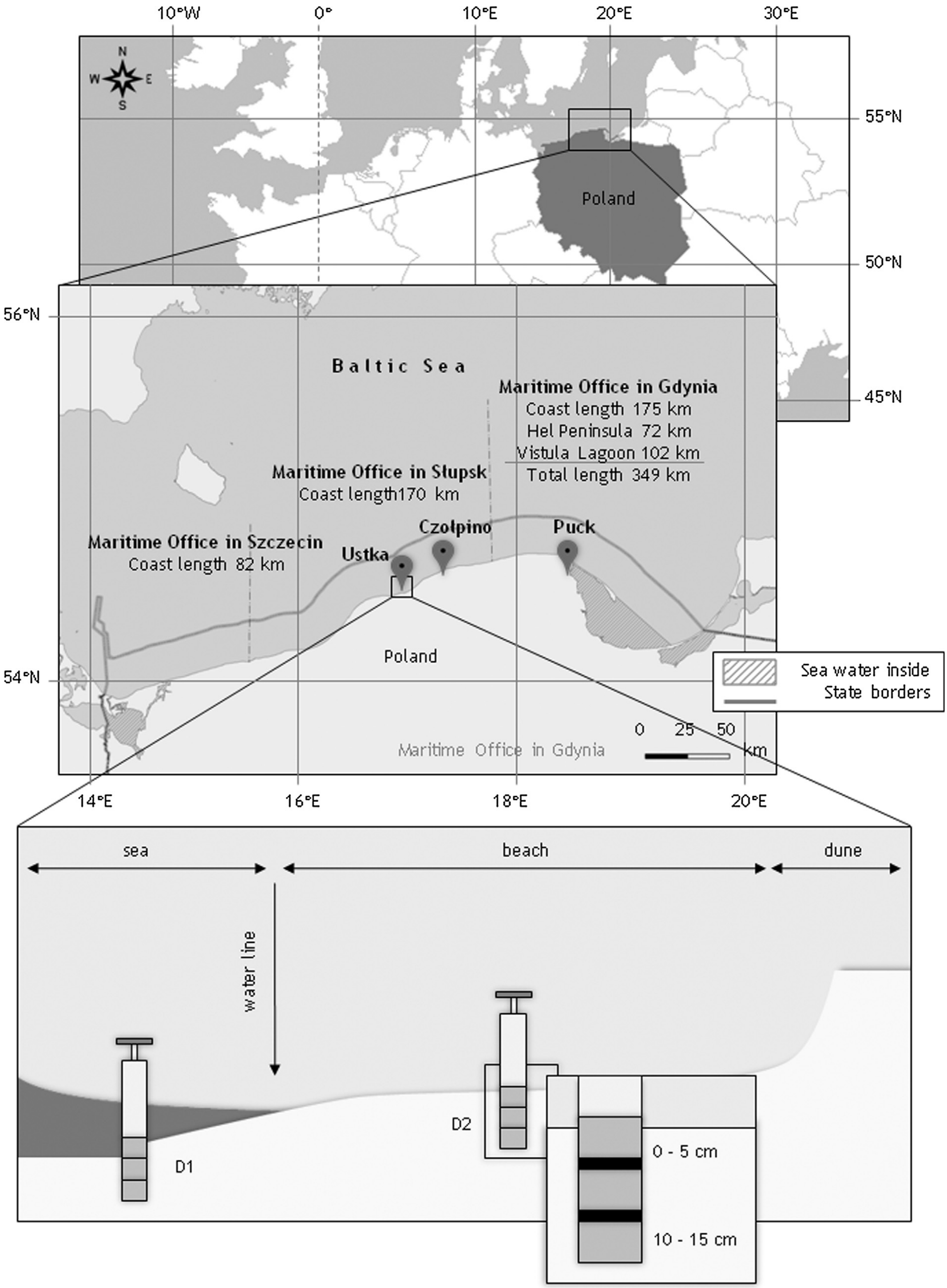

Figure 1 Location of sampling sites and presentation of the horizontal profile of one of the beaches.

differ in terms of degrees of anthropogenic impact and exposition. To avoid repetition, readers are kindly referred to our previous paper presenting very detailed description of the locations according to geomorphology of deposits and anthropogenic impact (Bigus et al., 2016). The only information worth emphasizing here is that beaches located in Ustka and Czołpino are exposed, while the beach located in Puck is a sheltered one. Moreover, the strength of anthropogenic impact decreases in the following order: Puck (heavy) > Ustka (moderate) > Czołpino (light) since the beach located in Czołpino is the part of the Słowiński National Park which is registered on the World List of Biosphere Reserves, the beach in Ustka is located close to the mouth of the slightly polluted Słupia River and the harbor used mainly by fishing and tourist boats while the beach located in Puck belongs to the Gdańsk Bay which is one of the 27 ecologically endangered areas in Poland and one of 132 pollution "hot spots" in the Baltic (Szefer, 2002).

2.2. Sampling and analytical methods

Sand samples were collected seasonally (winter (W), spring (S), summer (Su) and autumn (Au)) in 2011 and 2012 from two sites different in terms of environmental parameters on each beach. Site D1 (sea) — was located approximately 3 m offshore, at a depth of about 1 m underwater while site D2 (beach) — was around halfway up the beach, 30 m from the water line.

Sand cores were taken with a hand-operated Morduchaj-Boltowski sampler (length — 30 cm, inner diameter — 15 cm). 15 cm long sand cores were collected in three replicates and divided into 2 terminal sections in the field (0—5 cm and 10—15 cm). An intermediate section was skipped since previous studies showed that the highest differences in microbiological parameters could be observed between surface and subsurface layers of the marine beach (Mudryk and Podgórska, 2007; Olańczuk-Neyman and Jankowska, 1998; Perliński and Mudryk, 2016). Sand samples were placed in polyethylene bags and put in a special container of temperature not exceeding 8°C and then transported to the laboratory. Since the accomplishment of full microbiological analysis was not possible in a relatively short period of time, samples were frozen to −60°C, however, just before analysis, the batch of samples subjected for treatment was defrosted. Several microbiological and chemical parameters were determined in sand sediments: lipase, aminopeptidase, α-glucosidase, β-glucosidase, secondary bacterial production (SBP), total bacteria number (TBN), lipids (LIP), proteins (PRT), carbohydrates (CHO), organic matter (OM), total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN), biopolymeric carbon (BPC) and complex organic matter (COM). Sand samples were only mixed prior to the chemical analysis, while they were diluted tenfold in a sterile water buffer (pH = 7.2) and sonicated (Ellery and Schleyer, 1984) to separate bacteria from sediment prior to the microbiological analysis. Sonication was done using ultrasonic sonicator Bandelin SONOPLUS HD 2070 (Bandelin, Germany) for 1 min at the frequency of 20 kHz. Methodological details of the above-mentioned settings are depicted in Table 1. Measurements of absorption for LIP, PRT and CHO were done using Hitachi U-5100 (Hitachi, Japan) against deionized water produced by HLP 10 (Hydrolab, Poland), while spectrofluorimetric measures for lipase, aminopeptidase, α-glucosidase and β-glucosidase were done using Hitachi F-2500 (Hitachi, Japan). Enzyme activities in core sediments were determined using substrate proxies: MUF (4-methylumbelliferone) and MCA (L-leucine-4-methyl-coumarinyl-7-amide) for lipase/α-glucosidase/β-glucosidase and aminopeptidase, respectively.

2.3. Statistical methods

Two types of statistical procedures were applied in this study. Differences in median values of microbiological and chemical parameters determined in the sand cores collected on three different beaches were analyzed by the use of nonparametric *H* Kruskal—Wallis's and *U* Mann—Whitney's tests, while in order to discover seasonal differences as well as vertical and horizontal variation of microbiological and chemical parameters of dredge material collected in Ustka, Czołpino and Puck, including their mutual relations, factor analysis was applied along with the method of Principal Component Analysis (PCA) (Massart and Kaufman, 1997; Vandeginste et al., 1997). PCA enables reduction of the dimensionality of the space of the variables in the direction of the highest variance of the system. New variables, called principal components being linear combinations of the previous variables, replace the old coordinates of the factor space. Very often primary PCA solution is additionally rotated using one of the possible strategies (orthogonal rotations: varimax, biquartimax, quartimax, eqauamx; skewed rotations: oblimix, promax, etc.). Rotation strategy simplifies the structure of factors and therefore makes its interpretation easier and more reliable since it strengthens the role of the latent factors with a higher impact on the variation explanation and diminishes the role of PCs with a lower impact (Cattell, 1978; Thurstone, 1947). Prior to running PCA, Spearman's correlation matrix was calculated while Bartlett's test (Bartlett, 1951) was applied to check if the correlation matrix is an identity matrix (null hypothesis). An identity matrix is a matrix in which all of the diagonal elements are 1 (correlation of a given parameter with itself) and all of the diagonal elements are 0 (all parameters are orthogonal). Basing on Bartlett's test results, the null hypothesis was rejected and hence the use of PCA was validated. All calculations were performed by the use of the software package STATISTICA 12.0 (Statsoft Inc., USA).

3. Results

Seasonal, horizontal and vertical variations were not considered in the initial stage of data analysis since it was the target planned for multidimensional analysis. This is why the overall basic statistics concerning microbiological and chemical parameters measured in the beach sediments are shown in Table 2.

The average concentration of PRT decreases in the following order: Ustka (453 $\mu g\ g^{-1}$) > Puck (372 $\mu g\ g^{-1}$) > Czołpino (268 $\mu g\ g^{-1}$). Whereas beach sediments collected in Puck were the most abundant with LIP (250 $\mu g\ g^{-1}$) overtaking Ustka (176 $\mu g\ g^{-1}$) and Czołpino (112 $\mu g\ g^{-1}$). Similar order was observed for CHO: Puck (582 $\mu g\ g^{-1}$) > Ustka (509 $\mu g\ g^{-1}$) > Czołpino (338 $\mu g\ g^{-1}$) and for OM: Puck

Table 1 The list of microbiological and chemical parameters determined in sand samples collected from three Polish beaches.

Parameter	Method	Details	Reference
Lipase Aminopeptidase α-Glucosidase β-Glucosidase	Hopp's	MUF and MCA being derivatives of coumarin are fluorescent markers used to determine enzyme activity. These markers attach to an appropriate substrate and remain fluorescently inactive at this moment. Enzymatic action causes the release of markers and increase of fluorescence. The higher fluorescence the higher enzymatic activity.	Hoppe (1984), Misic and Fabiano (2005)
SBP	Modified Fuhrman's and Azam's	Determination of tritium tagged thymidine incorporated to bacterial DNA using liquid scintillation counter Canberra Packard Tri-Carb 2100TR	Allen et al. (2002), Fuhrman and Azam (1982), Jugnia et al. (2000)
TBN	DAPI	Using epifluorescence microscope Olympus BX41 equipped with excitation-barrier cube UV-2A (excitation λ = 365 nm, emission λ = 420 nm)	Porter and Feig (1980)
LIP	Zöllner's and Kirsch's	Extraction fatty compounds using chloroform-methanol mixture, absorption measure using wave length 530 nm	Zöllner and Kirsch (1962)
PRT	Markwell's	Absorption measure using wave length 480 nm	Markwell et al. (1978)
CHO	Dubois's	Absorption measure using wave length 480 nm for hexose and 490 for pentose	DuBois et al. (1956)
OM	By weight	Comparison of sample mass before and after roasting	Januszkiewicz (1978)
TOC	Tiurin's	Combustion with H_2SO_4 and $K_2Cr_2O_7$ and titration using $Fe(NH_2)SO_4{\cdot}6H_2O$	Myślińska (2001)
TP	Molybdate method	After preliminary mineralization using concentrated H_2SO_4 and 30% H_2O_2, ascorbic acid as a reducer	Bednarek et al. (2005)
TN	Kjeldahl's	After preliminary mineralization using concentrated H_2SO_4 and 30% H_2O_2 using Büchi Distillation Unit K — 350	Bednarek et al. (2005)
BPC	Computational	As the sum or organic carbon present in lipids, proteins and carbohydrates	Fabiano et al. (2004)
COM	Computational	TOC-BPC	Cividanes et al. (2002)

(42 mg g^{-1}) > Ustka (19 mg g^{-1}) > Czołpino (11 mg g^{-1}). In all presented orders core sediments from Czołpino were least abundant with OM proving that increasing anthropopression is positively correlated with an increase of OM abundance.

To evaluate the differences in values of microbiological and biochemical parameters in the sand cores collected in three different beaches results were statistically assessed by the use of nonparametric *H* Kruskal—Wallis (K—W) test (Table 3).

Data listed in Table 3 indicate that statistically significant differences in median values of chemical parameters as LIP, CHO, OM, TOC, TP, TN, BPC and COM prevail over microbiological ones. In the group of microbiological parameters, the only difference was found for α-glucosidase in Czołpino and Ustka. Secondary bacterial production and total bacteria number are comparable in core sediments of all the beaches. This is because of the similarity of the geomorphology of sediments and seawater chemistry. The highest number of differences was found between Czołpino and Puck. They concern median values of LIP, CHO, OM, TOC, TP, TN, BPC and COM and all values were much higher in Puck than in Czołpino (from 71% for BPC to 403% in the case of TN).

As mentioned above, Spearman's correlation matrix was calculated prior to running PCA (Table 4).

An analysis of correlations among microbiological and chemical parameters revealed that 28 out of 105 correlation coefficients were statistically significant at $p = 0.01$. Only 2 out of 28 coefficients were negative. Negative coefficients were found between CHO, TP and the activity of α-glucosidase (respectively $r_s = -0.37$ and $r_s = -0.38$). The activity of lipase was positively correlated with SBP ($r_s = 0.30$) and activity of aminopeptidase ($r_s = 0.49$). Single positive correlation ($r_s = 0.33$) between the activity of aminopeptidase and SBP was found. α-glucosidase activity was positively correlated with OM ($r_s = 0.38$), TOC ($r_s = 0.39$) and COM ($r_s = 0.39$). The highest number of positive coefficients ranging between 0.32 and 1.00 was found for LIP, CHO, OM, TOC, TN, BPC and COM.

The consecutive step consisted in a multidimensional analysis since the correlation matrix was not an identity one. Six independent factors, obtained as an effect of a varimax rotated solution of PCA explaining almost 76% of the variance of the entire data set, were distinguished (Table 5).

Factor 1explaining nearly 25% of the total variance indicates strong positive correlation between OM, TOC, TN and COM. Positive correlation between OM, TOC and TN is not surprising and rather evident since total organic carbon is

Table 2 Basic statistics of microbiological and chemical parameters in the beach sediments from three Polish beaches (northern Poland) (S.D. — standard deviation).

	Location	*N*	Mean	Median	Minimum	Maximum	S.D.
Lipase [nM MUF g^{-1} h^{-1}]	Ustka	32	230	143	19	951	227
Aminopeptidase [nM MCA g^{-1} h^{-1}]			60	37	2	300	64
α-Glucosidase [nM MUF g^{-1} h^{-1}]			51	24	2	237	61
β-Glucosidase [nM MUF g^{-1} h^{-1}]			27	21	2	99	24
SBP [μg C g^{-1} h^{-1}]			70	41	4	257	69
TBN [cell g^{-1}]			6,421,421	6,400,096	1,509,553	12,019,297	2,207,518
LIP [μg g^{-1}]			176	149	51	542	115
PRT [μg g^{-1}]			453	269	78	1782	404
CHO [μg g^{-1}]			509	387	121	2985	493
OM [mg g^{-1}]			19	18	5	34	10
TOC [mg g^{-1}]			11	11	3	20	6
TP [μg g^{-1}]			70	57	8	226	54
TN [μg g^{-1}]			251	251	88	469	71
BPC [μg g^{-1}]			549	410	251	1749	322
COM [mg g^{-1}]			11	10	3	20	6
Lipase [nM MUF g^{-1} h^{-1}]	Czołpino	32	138	112	2	715	133
Aminopeptidase [nM MCA g^{-1} h^{-1}]			39	25	3	273	52
α-Glucosidase [nM MUF g^{-1} h^{-1}]			26	10	1	145	37
β-Glucosidase [nM MUF g^{-1} h^{-1}]			18	11	1	59	17
SBP [μg C g^{-1} h^{-1}]			41	27	2	219	50
TBN [cell g^{-1}]			5,768,562	5,553,173	1,913,988	14,236,203	2,687,490
LIP [μg g^{-1}]			112	95	6	324	80
PRT [μg g^{-1}]			268	218	86	804	181
CHO [μg g^{-1}]			338	335	53	650	134
OM [mg g^{-1}]			11	9	5	21	5
TOC [mg g^{-1}]			9	6	1	20	5
TP [μg g^{-1}]			99	64	8	232	72
TN [μg g^{-1}]			95	94	49	208	32
BPC [μg g^{-1}]			345	313	222	595	97
COM [mg g^{-1}]			8	6	1	19	5
Lipase [nM MUF g^{-1} h^{-1}]	Puck	32	119	104	4	416	88
Aminopeptidase [nM MCA g^{-1} h^{-1}]			42	35	1	108	33
α-Glucosidase [nM MUF g^{-1} h^{-1}]			37	17	1	165	48
β-Glucosidase [nM MUF g^{-1} h^{-1}]			38	9	1	252	62
SBP [μg C g^{-1} h^{-1}]			52	31	2	421	77
TBN [cell g^{-1}]			5,820,068	5,416,809	2,903,134	16,147,673	2,458,823
LIP [μg g^{-1}]			250	190	41	1428	264
PRT [μg g^{-1}]			372	224	92	3862	656
CHO [μg g^{-1}]			582	531	177	1864	305
OM [mg g^{-1}]			42	30	5	183	39
TOC [mg g^{-1}]			30	21	2	139	30
TP [μg g^{-1}]			260	152	63	855	205
TN [μg g^{-1}]			478	329	177	1739	378
BPC [μg g^{-1}]			590	476	278	2688	433
COM [mg g^{-1}]			30	21	2	136	30

usually the carbon stored in soil organic matter, while nitrogen is one of the major components of OM. Similar mutual increase of TOC, COM and TN was observed before by others (Cividanes et al., 2002; Fabiano et al., 1995; Rodil et al., 2008). Because of this, based on source oriented interpretation of the factor, its meaning reflects communal wastes discharged by high-rate (with elevated removal of nutrients) wastewater treatment plants (as is in the case of Słupsk and Ustka wastewater treatment plants) and surface run-off from agriculture. This is why it could be conditionally named as "anthropogenic rich in N".

Factor 2 explains a lesser part of the total variance (about 10%) and shows a directly proportional correlation between lipase, aminopeptidase and SBP. It could be accepted as "microbial enzymatic activity" and informs about the activity of microorganisms toward oil spills (Ziervogel et al., 2012, 2016).

Factor 3 indicates a strong positive correlation between PRT, CHO and BPC. It explains about 14% of the total variance

Table 3 Statistical assessment of the differences of median values of microbiological parameters, enzymatic activity and concentration of total forms of elements according to the location of the beach by the use of multiple Kruskal—Walli's test ($p = 0.05$).

Parameter	*H* value of K—W test (insignificant *p* value)	*H* value of multiple K—W's test (insignificant *p* value)		
		Czołpino vs Ustka	Czołpino vs Puck	Ustka vs Puck
Lipase	4.71 ($p = 0.0948$)	—	—	—
Aminopeptidase	3.48 ($p = 0.1747$)	—	—	—
α-Glucosidase	8.21	2.83	1.03 ($p = 0.9061$)	1.78 ($p = 0.2159$)
β-Glucosidase	2.57 ($p = 0.2759$)	—	—	—
SBP	4.62 ($p = 0.0989$)	—	—	—
TBN	3.28 ($p = 0.1939$)	—	—	—
LIP	12.35	2.37 ($p = 0.0535$)	3.43	1.06 (0.8627)
PRT	2.63 ($p = 0.2679$)	—	—	—
CHO	20.29	2.00 ($p = 0.1346$)	4.50	2.49
OM	25.64	2.82	5.05	2.23 ($p = 0.0772$)
TOC	17.73	1.77 ($p = 0.2300$)	4.19	2.42
TP	30.84	1.68 ($p = 0.2773$)	3.74	5.42
TN	65.52	5.52	7.87	2.36 ($p = 0.0541$)
BPC	21.81	3.45	4.45	1.00 ($p = 0.9510$)
COM	17.32	1.68 ($p = 0.2799$)	4.14	2.46

Table 4 Spearman's correlation coefficients ($p = 0.01$) between microbiological and chemical parameters determined in core sediments collected in three Polish beaches.

	Lipase	Amino-peptidase	α-Glucosidase	β-Glucosidase	SBP	GA	LIP	PRT	CHO	OM	TOC	TP	TN	BPC	COM
Lipase	1.00														
Aminopeptidase	**0.49**	1.00													
α-Glucosidase	0.12	0.20	1.00												
β-Glucosidase	0.11	0.08	−0.12	1.00											
SBP	**0.30**	**0.33**	0.17	−0.01	1.00										
GA	0.17	0.11	0.08	−0.22	0.09	1.00									
LIP	0.12	0.00	0.11	0.06	−0.09	0.21	1.00								
PRT	0.03	0.07	0.16	−0.01	0.00	−0.16	−0.17	1.00							
CHO	−0.04	0.04	**−0.37**	0.18	−0.12	0.00	0.00	0.10	1.00						
OM	−0.05	0.11	**0.38**	0.07	−0.15	0.03	**0.32**	0.15	0.10	1.00					
TOC	−0.03	0.08	**0.39**	0.05	−0.21	−0.00	**0.33**	0.17	0.06	**0.96**	1.00				
TP	0.05	0.10	**−0.38**	−0.05	0.16	−0.06	0.01	−0.23	**0.32**	−0.06	−0.08	1.00			
TN	0.05	0.11	0.08	0.12	0.09	0.08	**0.38**	−0.06	**0.40**	**0.41**	**0.32**	**0.34**	1.00		
BPC	0.03	0.07	0.02	0.16	−0.12	0.01	**0.39**	**0.59**	**0.57**	**0.39**	**0.38**	−0.02	**0.37**	1.00	
COM	−0.03	0.08	**0.39**	0.04	−0.21	−0.00	**0.32**	0.15	0.04	**0.96**	**1.00**	−0.08	**0.32**	**0.36**	1.00

Bold values are statistically significant on $p = 0.01$.

and, being a reflection of mutual relations between proteins and carbohydrates, can be conditionally named as "labile organic matter". Strong contribution of biopolymeric carbon within factor 3 is in agreement with expectations since BPC is the sum of lipid, protein and carbohydrate carbon.

Factor 4 (8% of the variance) indicates the proportional relation between TBN and LIP. It is evident that moist and warm environment of beaches abounding with lipids from many sources (UV filters, tanning oils and lotions, fat from wastewater treatment plants, etc.) makes a favorable habitat for bacteria growth. This is why the fourth factor could be accepted as "bacterial growth".

Factor 5 accounts for 9% of the total variance and contains only one parameter — TP. Since it suggests an impact of phosphates from raw domestic wastes or dumps from low-rate (without increased removal of nutrients) wastewater treatment plants, it was called "an anthropogenic rich in P".

Factor 6 accounts for 8% of the total variance and contains inversely correlated α-glucosidase and β-glucosidase. Similarly as in the case of factor 2 it reflects microbial enzymatic activity, however to distinguish it from factor 2, it was called "hydrolytic". α-glucosidase breaks down starch and disaccharides to glucose and is located in the brush border of the small intestine that acts upon 1,4-alpha bonds. This is in contrast to β-glucosidase which catalyzes the hydrolysis of the glycosidic bonds to terminal non-reducing residues in beta-D-glucosides and oligosaccharides, with the release of glucose. An inversely proportional correlation between α- and β-glucosidase refers to the ability of microorganisms to

Table 5 Factor analysis solution after normalized varimax rotation.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Lipase	−0.07	**0.72**	0.01	0.20	−0.18	0.19
Aminopeptidase	0.01	**0.70**	−0.08	−0.07	0.08	0.12
α-Glucosidase	0.27	0.03	−0.30	−0.06	−0.45	**−0.52**
β-Glucosidase	0.19	0.09	−0.09	−0.15	−0.07	**0.81**
SBP	−0.16	**0.70**	−0.01	−0.13	0.26	−0.21
TBN	−0.09	0.13	−0.03	**0.75**	−0.00	−0.29
LIP	0.38	−0.15	0.09	**0.69**	0.11	0.10
PRT	0.41	0.09	**0.73**	−0.11	−0.28	−0.16
CHO	0.04	−0.09	**0.78**	−0.07	0.23	0.12
OM	**0.96**	−0.04	0.18	0.03	0.04	0.03
TOC	**0.96**	−0.05	0.18	0.04	0.04	0.04
TP	0.18	0.07	−0.08	0.04	**0.83**	−0.03
TN	**0.59**	0.01	0.06	0.07	0.48	−0.02
BPC	0.44	−0.04	**0.86**	0.16	−0.05	−0.02
COM	**0.96**	−0.05	0.17	0.03	0.04	0.04
Eigenvalue	3.80	1.57	2.09	1.18	1.39	1.16
% of the explained variance	25%	10%	14%	8%	9%	8%
% of the cumulated variance	25%	35%	49%	57%	68%	76%

Note: Values in bold are the factor loadings higher than 0.5 and those, which were further interpreted; varimax rotation was applied as indubitably the most popular rotation method by far (Hervé, 2010).

decompose OM. When easily assimilable matter (i.e. proteins, monosaccharide) is present in the environment, a growth of microorganisms synthesizing β-glucosidase and hence an increase of its activity is observed. In the meantime an activity of α-glucosidase decreases. After the source of easily assimilable matter is exhausted or when fresh and hardly assimilable matter appears in the environment, mutual dependence between both forms of glucosidase switches in the direction of α-glucosidase activity increase (to decompose hardly assimilable matter). This is why the activity of both forms of glucosidase can not increase or decrease simultaneously. Higher activity of α-glucosidase or β-glucosidase is conditioned by the kind of OM present in the environment (Zeng et al., 2010).

To verify and evaluate the differences in median values of microbiological parameters, enzymatic activity and a concentration of total forms of elements according to a location of the sampling point, core depth and seasonality, the *U* Mann–Whitney test was applied and its results are depicted in Table 6.

As ensues from data presented in Table 6 CHO, PRT and BPC abundance differs seasonally in Ustka and Czołpino, while in Puck CHO and BPC abundance differs only according to the distance from the water line. Besides the winter period, an increase in the concentration of all labile forms of organic matter is observed in Ustka (winter: PRT – 291.99 $\mu g\ g^{-1}$; CHO – 456.27 $\mu g\ g^{-1}$; BPC – 502.06 $\mu g\ g^{-1}$; spring: PRT – 232.76.99 $\mu g\ g^{-1}$; CHO – 273.44 $\mu g\ g^{-1}$; BPC – 344.74 $\mu g\ g^{-1}$; summer: PRT – 569.87 $\mu g\ g^{-1}$; CHO – 403.19 $\mu g\ g^{-1}$; BPC – 530.24 $\mu g\ g^{-1}$; autumn: PRT – 716.19 $\mu g\ g^{-1}$; CHO – 902.99 $\mu g\ g^{-1}$; BPC – 817.47 $\mu g\ g^{-1}$) and Czołpino (winter: PRT – 138.34 $\mu g\ g^{-1}$; CHO – 364.70 $\mu g\ g^{-1}$; BPC – 299.48 $\mu g\ g^{-1}$; spring: PRT – 290.70 $\mu g\ g^{-1}$; CHO – 256.39 $\mu g\ g^{-1}$; BPC – 353.35 $\mu g\ g^{-1}$; summer: PRT–- 287.96 $\mu g\ g^{-1}$; CHO – 365.98 $\mu g\ g^{-1}$; BPC – 365.80 $\mu g\ g^{-1}$; autumn: PRT – 356.87 $\mu g\ g^{-1}$; CHO – 363.96 $\mu g\ g^{-1}$; BPC – 361.07 $\mu g\ g^{-1}$). The observed increase of labile organic matter abundance in spring, summer and autumn matches the increase of biological activity in the corresponding seasons in the coastal zone of the southern Baltic Sea (Håkanson and Bryhn, 2008; Smayda, 1997; Vu, 2016). In Puck significant seasonal changes of PRT, CHO and BPC are not observed while sediments dredged from location D1 are at least two and a half times more abundant with CHO (691.51 $\mu g\ g^{-1}$) and BPC (732.32 $\mu g\ g^{-1}$) than these dredged from D2 (CHO – 472.96 $\mu g\ g^{-1}$; BPC – 447.80 $\mu g\ g^{-1}$). The lack of seasonal changes in this case is probably caused by the stability of sea water characteristics (temperature, aeration, etc.) in Puck Bay while variation along a horizontal transect of the beach is caused by analogical reasons as were presented for F1 interpretation (sheltered beach, low slope, energy of waves and hydrodynamics, etc.).

4. Discussion

The concentration of OM found on exposed locations in Ustka (19 $mg\ g^{-1}$) and Czołpino (11 $mg\ g^{-1}$) is similar to OM concentration found on exposed beaches in Germany: 5.9 $mg\ g^{-1}$ (Meyer-Reil et al., 1980), 16.80 $mg\ g^{-1}$ (Meyer-Reil, 1983) and on the Ligurian coast: 19.25 $mg\ g^{-1}$ (Fabiano et al., 1995). A comprehensive comparison of dredge material collected in various world-wide spread locations according to the abundance of proteins, carbohydrates and lipids is presented in Fig. 2.

Human activity, emission of pollutants in particular, increases the total budget of labile and refractory OM in the environment (Dell'Anno et al., 2002). However, it must be recalled that a simple division into anthropogenic and natural origin of OM according to its labile or refractory characteristic is not an easy task. As an example, easily mineralized PRT, LIP and CHO are components of the majority of living organisms. They can be both of “marine” and “terrestrial”

Table 6 Statistical assessment of the differences of median values of microbiological parameters, enzymatic activity and concentration of total forms of elements according to sampling points' location along the beach, depth of the core and seasonality by the use of Mann—Whitney's and multiple Kruskal—Walli's tests ($p = 0.05$).

Parameter	*U* value of Mann—Whitney test (according to location and depth) and *H* value of multiple K—W's test (according to seasons) (insignificant *p* value)		
	Ustka	Czołpino	Puck
Lipase	99 ($p = 0.2828$)^{l} 108 ($p = 0.4624$)^{d} 3.32 (=0.3439)^{s}	86 ($p = 0.1178$)^{l} 87 ($p = 0.1269$)^{d} 1.90 ($p = 0.5921$)^{s}	103 ($p = 0.3558$)^{l} 77 ($p = 0.0570$)^{d} 0.56 ($p = 0.9043$)^{s}
Aminopeptidase	60^{l} 102 ($p = 0.3365$)^{d} 2.20 ($p = 0.5316$)^{s}	91 ($p = 0.1689$)^{l} 123 ($p = 0.8653$)^{d} 8.80^{s}	97 ($p = 0.2503$)^{l} 64^{d} 4.30 ($p = 0.2299$)^{s}
α-Glucosidase	90 ($p = 0.1575$)^{l} 92 ($p = 0.1809$)^{d} 4.06 ($p = 0.2551$)^{s}	85 ($p = 0.1092$)^{l} 122 ($p = 0.8358$)^{d} 3.46 ($p = 0.3278$)^{s}	107 ($p = 0.4397$)^{l} 59^{d} 1.03 ($p = 0.7937$)^{s}
β-Glucosidase	124 ($p = 0.8950$)^{l} 107 ($p = 0.4397$)^{d} 5.38 ($p = 0.1461$)^{s}	109 ($p = 0.4856$)^{l} 97 ($p = 0.2503$)^{d} 12.29^{s}	126 ($p = 0.9549$)^{l} 97 ($p = 0.2503$)^{d} 3.32 ($p = 0.3443$)^{s}
SBP	55^{l} 105 ($p = 0.3964$)^{d} 4.86 ($p = 0.1823$)^{s}	74^{l} 95 ($p = 0.2206$)^{d} 9.99^{s}	49^{l} 64^{d} 0.07 ($p = 0.9954$)^{s}
TBN	127 ($p = 0.9849$)^{l} 108 ($p = 0.4624$)^{d} 9.11^{s}	97 ($p = 0.2503$)^{l} 103 ($p = 0.3558$)^{d} 10.80^{s}	91 ($p = 0.1689$)^{l} 123 ($p = 0.8653$)^{d} 11.26^{s}
LIP	117 ($p = 0.6923$)^{l} 118 ($p = 0.7203$)^{d} 4.39 ($p = 0.2219$)^{s}	115 ($p = 0.6375$)^{l} 102 ($p = 0.3365$)^{d} 8.62^{s}	101 ($p = 0.3179$)^{l} 124 ($p = 0.8950$)^{d} 7.07 ($p = 0.0697$)^{s}
PRT	115 ($p = 0.6375$)^{l} 109 ($p = 0.4856$)^{d} 8.28^{s}	114 ($p = 0.6109$)^{l} 125 ($p = 0.9249$)^{d} 9.05^{s}	94 ($p = 0.2067$)^{l} 100 ($p = 0.3000$)^{d} 7.13 ($p = 0.0679$)^{s}
CHO	86 ($p = 0.1178$)^{l} 119 ($p = 0.7487$)^{d} 9.32^{s}	65^{l} 96 ($p = 0.2351$)^{d} 3.36^{s}	70^{l} 82 ($p = 0.08663$)^{d} 3.01 ($p = 0.3903$)^{s}
OM	111 ($p = 0.5340$)^{l} 115 ($p = 0.6375$)^{d} 3.20 ($p = 0.3624$)^{s}	104 ($p = 0.3758$)^{l} 120 ($p = 0.7774$)^{d} 3.74 ($p = 0.2908$)^{s}	0^{l} 109 ($p = 0.4856$)^{d} 1.85 ($p = 0.6042$)^{s}
TOC	113 (0.5847)^{l} 118 ($p = 0.7203$)^{d} 3.14 ($p = 0.3707$)^{s}	117 ($p = 0.6923$)^{l} 126 ($p = 0.9549$)^{d} 4.41 ($p = 0.2200$)^{s}	0^{l} 111 ($p = 0.5340$)^{d} 1.71 ($p = 0.6353$)^{s}
TP	107 ($p = 0.4397$)^{l} 124 ($p = 0.8950$)^{d} 1.86 ($p = 0.6012$)^{s}	116 ($p = 0.6647$)^{l} 126 ($p = 0.9549$)^{d} 2.04 ($p = 0.5636$)^{s}	112 ($p = 0.5591$)^{l} 114 ($p = 0.6109$)^{d} 13.01^{s}
TN	121 ($p = 0.8210$)^{l} 98 ($p = 0.2660$)^{d} 4.16 ($p = 0.2451$)^{s}	99.5 ($p = 0.2913$)^{l} 123.5 ($p = 0.8801$)^{d} 6.25 ($p = 0.1001$)^{s}	46^{l} 113 ($p = 0.5847$)^{d} 1.21 ($p = 0.7499$)^{s}
BPC	113 ($p = 0.5847$)^{l} 120 ($p = 0.7774$)^{d} 10.96^{s}	81 ($p = 0.0796$)^{l} 88 ($p = 0.1365$)^{d} 3.89 ($p = 0.2737$)^{s}	73^{l} 102 ($p = 0.3365$)^{d} 0.85 ($p = 0.8376$)^{s}
COM	109 ($p = 0.4856$)^{l} 117 ($p = 0.6923$)^{d} 3.02 ($p = 0.3877$)^{s}	119 ($p = 0.7487$)^{l} 128 ($p = 0.9849$)^{d} 4.62 ($p = 0.2017$)^{s}	0^{l} 110 ($p = 0.5095$)^{d} 1.71 ($p = 0.6353$)^{s}

l — location (sea vs beach), d — depth (0—5 cm vs 10—15 cm), s — season (winter, spring, summer, autumn).

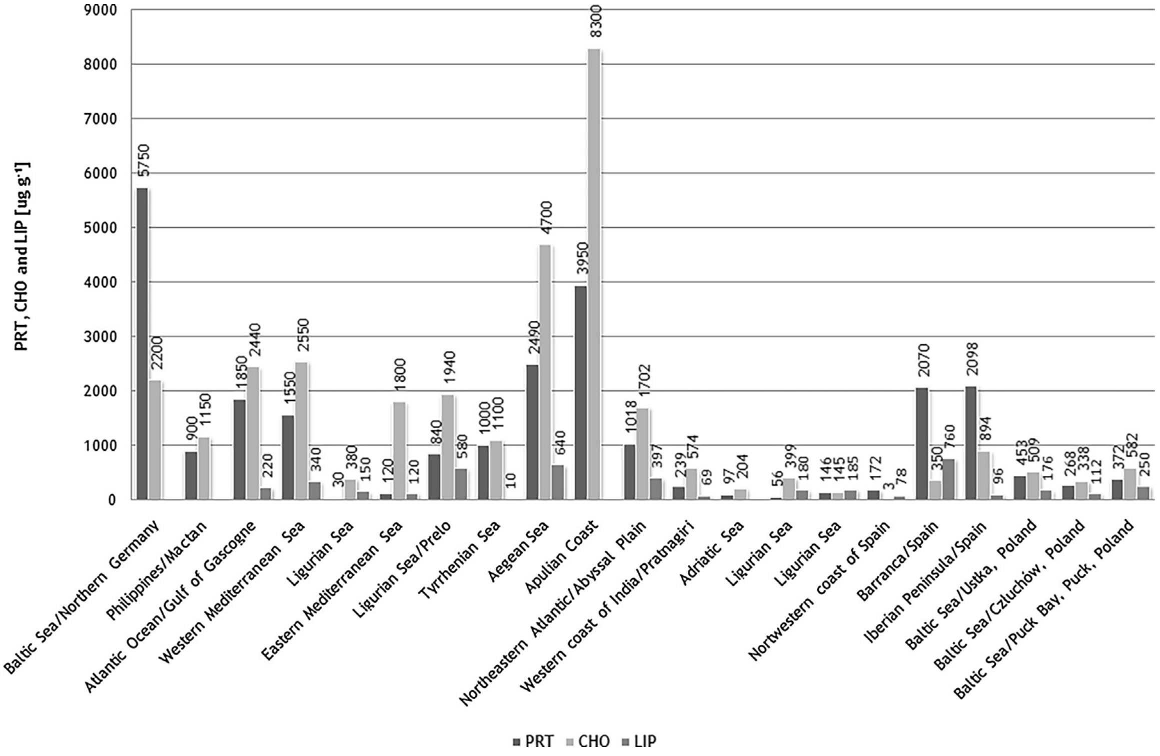

Figure 2 Comprehensive comparison of dredge material collected in various world-wide spread locations according to abundance of labile forms of organic matter (proteins, carbohydrates and lipids).

origin, since i.e. CHO can be photosynthesized by producers in the sea or be a product of decomposition of organic matter present in the debris of animals and plants (Penna et al., 2009). On the other hand, humic and fulvic acids and lignin are of natural origin despite the fact that they belong to refractory OM. Having in mind the facts mentioned above, it could be generalized that OM present in beach sediments can be both of natural or anthropogenic origin with a different contribution of labile or refractory forms, however the higher labile form contribution, the higher the naturalness of OM.

Anthropogenic domination of sources of OM in Puck and Ustka was positively verified by a calculation of contribution of labile forms of OM in the total OM budget. For Ustka and Puck it was 6% and 3%, respectively, while for Czołpino it was 65%. For Puck there are two simultaneous factors causing higher accumulation of organic matter. As mentioned above, one of them is anthropopression while the other concerns the degree of sheltering. Grain size should not be considered a differentiating factor since sediment from the beaches in Ustka, Czołpino and Puck indicates a predominance of fine-grained sands with little variation in all the locations (Bigus et al., 2016). Sheltered (covered) beaches like in Puck, characterized by fine-grained sediments, have a much higher capacity of pollutant accumulation (McLachlan et al., 1996) than exposed beaches characterized by similarly grained sediments due to poorer oxygenation and lower drainage. Limited hydrodynamics of the sheltered beach favors the accumulation of sedimentary organic matter due to a limited renewal of interstitial water (Incera et al., 2003). In addition, low energy of the surge permits the formation of fine and stable sediments that allowed the settlement of a higher amount of fauna (Nordstrom, 1992). On the contrary, stronger hydrodynamics of the exposed beaches permits deposition of coarser sediments through which water runs easily, preventing the accumulation of organic matter while hydrodynamic stress concerning the impact of waves limits biological richness (McLachlan et al., 1996).

As is shown in Fig. 2, dredged material collected on the Polish coast is characterized by a similar concentration of proteins, carbohydrates and lipids to those collected on the Ligurian coast (Danovaro et al., 1993; Fabiano et al., 1995, 2004), Adriatic beaches (Danovaro et al., 2001), northwestern coast of Spain (Rodil et al., 2007) as well as on the western coast of India (Fernandes et al., 2012). Beach sediments collected in Ria de Arousa (Cividanes et al., 2002), in the northwestern part of the Iberian Peninsula (Incera et al., 2003), in the Gulf of Gascogne (Khripounoff et al., 1985), in the area of the Porcupine Abyssal Plain (Danovaro et al., 2001), in the Philippines (Graf and Meyer-Reil, 1985) and on the Tyrrhenian (Fabiano and Danovaro, 1994) and the Ligurian coasts close to Prelo Bay (Danovaro et al., 1999b) as well as in the western (Fichez, 1991a) and eastern (Danovaro et al., 1993) parts of the Mediterranean Sea were at least four to five times more abundant with PRT, CHO and LIP. Concentrations of labile forms of organic matter were significantly higher (more than tenfold) in dredge material collected in the Aegean Sea (Danovaro et al., 1999a), on the Apulian coast (Dell'Anno et al., 2002) and in northern Germany (Meyer-Reil, 1983) than in the samples collected in Poland.

Comprehensive comparison of microbial enzymatic activity on the Polish coast with other locations is problematic due to the limited number of papers dealing with this topic (Danovaro et al., 2001; Misic and Fabiano, 2005), however activity of aminopeptidase did not exceed 6.65 and

3.7 nM MCA g^{-1} h^{-1} in beach sediments collected in the Ligurian Sea (Misic and Fabiano, 2005) and the Adriatic Sea (Danovaro et al., 2001), which is almost tenfold lower than in dredge sediments collected in Ustka, Człuchów and Puck. Similarly, tenfold lower activities of α-glucosidase and β-glucosidase were found in the warmer ecosystem of the Mediterranean beaches (Danovaro et al., 2001; Misic and Fabiano, 2005). Other results concerning microbial enzymatic activity in core sediments of Polish beaches indicate comparable results for lipase (183.75 nM MUF g^{-1} h^{-1}), aminopeptidase (94.38 nM MCA g^{-1} h^{-1}), α-glucosidase (24.40 nM MUF g^{-1} h^{-1}) and β-glucosidase (28.10 nM MUF g^{-1} h^{-1}) activities (Perliński and Mudryk, 2016).

Basing on results depicted in Table 3 it could be unequivocally concluded that beach sheltering joined with strong anthropogenic impact results in huge deposition of both labile and refractory organic matter, particularly those rich in nutrients. As presented in Fig. 1, the beach in Puck is sheltered from large and long period waves by the Hel Peninsula, which separates the land from the Baltic Sea almost precluding an exchange of water. Moreover, the Vistula River flowing through Żuławy Wiślane, being the most important agricultural area in northern Poland, discharges huge loads of N and P to the Baltic Sea, causing significant pollution in the entire region of Puck Bay. According to recent environmental reports in 2009, the Vistula River discharged 92.1 kt of N and 6.44 kt of P (Report, 2010) to the Baltic Sea. In 2011, the total load of N and P increased to 102.1 kt and 7.0 kt, respectively (Report, 2012). The other important source of OM in the vicinity of Puck beach, LIP and CHO in particular, is wastewater from treatment plants located close to the coastline in Puck Bay and the marina located in Puck. Vessels and yachts docking in harbors and marinas are a common source of many organic substances such as fuel, paints, anti-fouling agents, oils and soaps. Communal wastes are the dominant source of phosphorus compounds in Puck Bay, and hence in core sediments collected in Puck. Such an explanation seems logical since significant differences between median values of CHO, TOC, TP and COM were found for Ustka and Puck. In Puck an increase ranging from 14% (CHO) to 271% (TP) in comparison with Ustka was observed. Apart from α-glucosidase statistically different median of concentrations between core sediments collected in Ustka and Czołpino was found for OM, TN and BPC. An increased abundance of nitrogen-rich compounds in core sediments collected in Ustka can be explained by the load of the Słupia River which flows through the agricultural areas in the middle Pomeranian Voivodship, while an increase of OM and BPC abundance can be explained by the impact of two wastewater treatment plants for Słupsk and Ustka located close to the mouth of the Słupia River. According to recent environmental reports, coastal rivers (including the Vistula River) in the Pomeranian region in Poland in 2011 discharged 105.35 kt of N and 7.16 kt of P, however, the contribution of the Słupia and Łeba rivers were much lower. In 2009 the Słupia River discharged 0.812 kt of N and only 0.05 kt of P (Report, 2010) to the Baltic Sea while the Łeba River discharged 0.739 kt of N and 0.046 kt of P. In 2011 the total load of N and P compounds due to the Słupia and Łeba rivers was as follows: Słupia — 1.231 kt N and 0.068 kt P; Łeba — 0.936 kt N and 0.044 kt P (Report, 2010, 2012).

The relation between the above characterized factors and locations (including the distance from the water line and vertical core depth) of the particular beach as well as seasonality were identified by the visualization of factor score values. The unanimous sign of factor scores and factor loadings corresponds to a high influence of a given factor (and hence high concentration of an analyte or high value of the measured parameter) on core sediment sample, while reverse sign corresponds to low influence. Moreover, the higher factor score value, the higher the influence. The plot of scores for the first factor visualized according to the location of the beach is presented in Fig. 3.

It could be easily observed that core sediments collected in Ustka and Czołpino create relatively homogenous groups, while those collected in Puck are spread. The location of samples along F1 axis (OM, TOC, TN, COM) proves that dredge material collected in Ustka and Czołpino is not as rich in organic matter and its constituents as those collected in Puck. This phenomenon reflects a mutual impact of two factors. On the one hand, the beach in Puck is sheltered by the Hel Peninsula and impacted by low energy waves in comparison to exposed locations in Ustka and Czołpino. On the other hand, Puck Bay consists of waters which are highly polluted with organic matter and nutrients due to load discharged by the Vistula River and three wastewater treatment plants located on the coast (Dębogórze, Wschód, Swarzewo). According to Incera et al. (2003), low hydrodynamics of sheltered beaches favors the accumulation of sedimentary organic matter due to a scarce renewal of interstitial water. Additionally, low energy surge permits formation of stable sediments which allow settlement of large amounts of fauna (Nordstrom, 1992). The beaches in Ustka and Czołpino are exposed and endangered by high energy and long period waves. Stronger hydrodynamics and reverse current facilitate mixing of water column and hence limit OM deposition on the beach (McLachlan et al., 1996), however slightly higher factor scores in Ustka compared with Czołpino confirm differences in median concentration values for OM and TN (Table 3) indicating a stronger impact of anthropogenic sources poor in P (the load carried out by the Słupia River waters).

Dispersion for a point set for Puck and compact form of a point sets for Ustka and Czołpino suggests different variability according to seasonal changes, the distance from the water line and vertical core depth. For Puck, much higher factor 1 scores were obtained for samples collected approximately 3 m offshore. Hence, they are richer in OM, TOC, TN and COM than these collected halfway up the beach.

It was statistically proved that only for Puck, location plays an important role and differentiates core sediments collected in the sea from these collected in the middle of the beach according to OM and its constituents. Because of high energy waves, core sediments in Ustka and Czołpino are seasonally stable and homogeneous along the beach as well as along vertical depth. Contrarily, the concentration of OM, TOC, TN and COM in dredge material collected 3 m offshore in Puck is higher (68 mg g^{-1}, 51 mg g^{-1}, 656 $\mu g\ g^{-1}$ and 50 $\mu g\ g^{-1}$, respectively) than in the middle of the beach (15 mg g^{-1}, 9 mg g^{-1}, 299 $\mu g\ g^{-1}$ and 9 $\mu g\ g^{-1}$, respectively). Again, such dependence can be explained by the impact of waves of low hydrodynamics observed in Puck. In locations with a low slope (i.e. sheltered beaches), low wave action

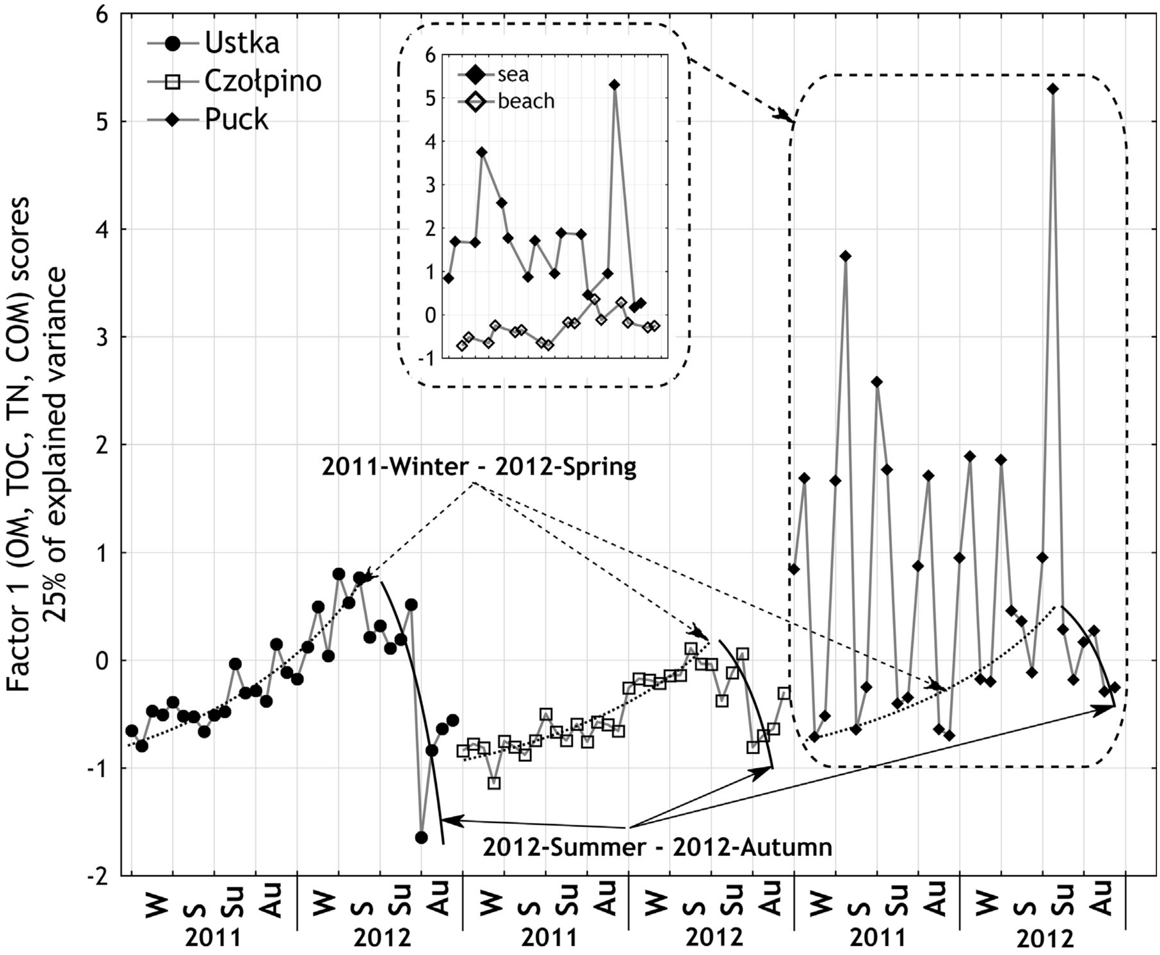

Figure 3 Factor 1 (anthropogenic rich in N) scores according to the location of the beach and horizontal transect in Puck (on *x* axis W, S, Su and Au states for winter, spring, summer and autumn, respectively).

facilitates OM enrichment and deposition within the narrow belt of shallow water since waves do not have enough energy to transport OM along a vertical transect of the beach (Incera et al., 2003).

As could be seen in Fig. 3, time series revealed another unique pattern confirmed by statistical testing (Table 5). Although there is a lack of seasonality in the period between winter 2011 and autumn 2012, a clear exponential increase of the concentration of OM, TOC, TN and COM in core sediments collected in all Polish beaches can be observed. This occurs even in Puck for samples collected in the middle of the beach. However, it breaks down during the last two seasons: summer 2012 and autumn 2012. Since the mentioned observation concerned the entire Polish coast (Ustka, Czołpino and Puck), it was assumed that accidental changes in OM, TOC, TN and COM abundance were caused by a sudden and extensive phenomenon related to biological activity. In the southern Baltic Sea two algae blooms are usually observed. The first of them caused by algae takes place in spring, while the other of much less intensity caused by cyanobacteria takes place in the summer season. The spring bloom is due to an increase of temperature after the winter period and intensive runoff discharge of nutrients (mainly N and P) from farmlands. Algae bloom usually finishes prior to the highest peak of touristic season when the temperature rises, oxygen deficiency appears and an abundance of nutrients has been depleted (Forsberg, 1991). Such explanation fits with a sudden drop of the concentration of OM, TOC, TN and COM observed at the turn of the spring 2012 and summer 2012 seasons, however as ensues from Fig. 3, corresponding algae bloom did not take place in 2011.

The plot of scores for the factor presenting "microbial enzymatic activity" is shown in Fig. 4.

All core sediments create spread groups indicating substantial variation. Basing on factor 2 scores, the highest microbial enzymatic activity (lipase, aminopeptidase and SBP) was observed in core sediments collected in Ustka (Table 2). However, an existence of extreme values in all locations caused the disappearance of statistically significant differences (Table 3) in the activity of lipase, aminopeptidase and secondary bacterial production between the beaches. Despite this, a careful inspection of the data presented in Table 5 revealed the distance from the water line as a factor which differentiates microbial enzymatic activity in Ustka, and vertical core depth in Puck. An average concentration of aminopeptidase and SBP in samples from location D1 (sea) was 36 nM MCA g^{-1} h^{-1} and 54 µg C g^{-1} h^{-1}, respectively, while in samples collected in D2 (beach) it was 84 nM MCA g^{-1} h^{-1} and 87 µg C g^{-1} h^{-1}, respectively. The highest microbial enzymatic activity in Ustka is probably

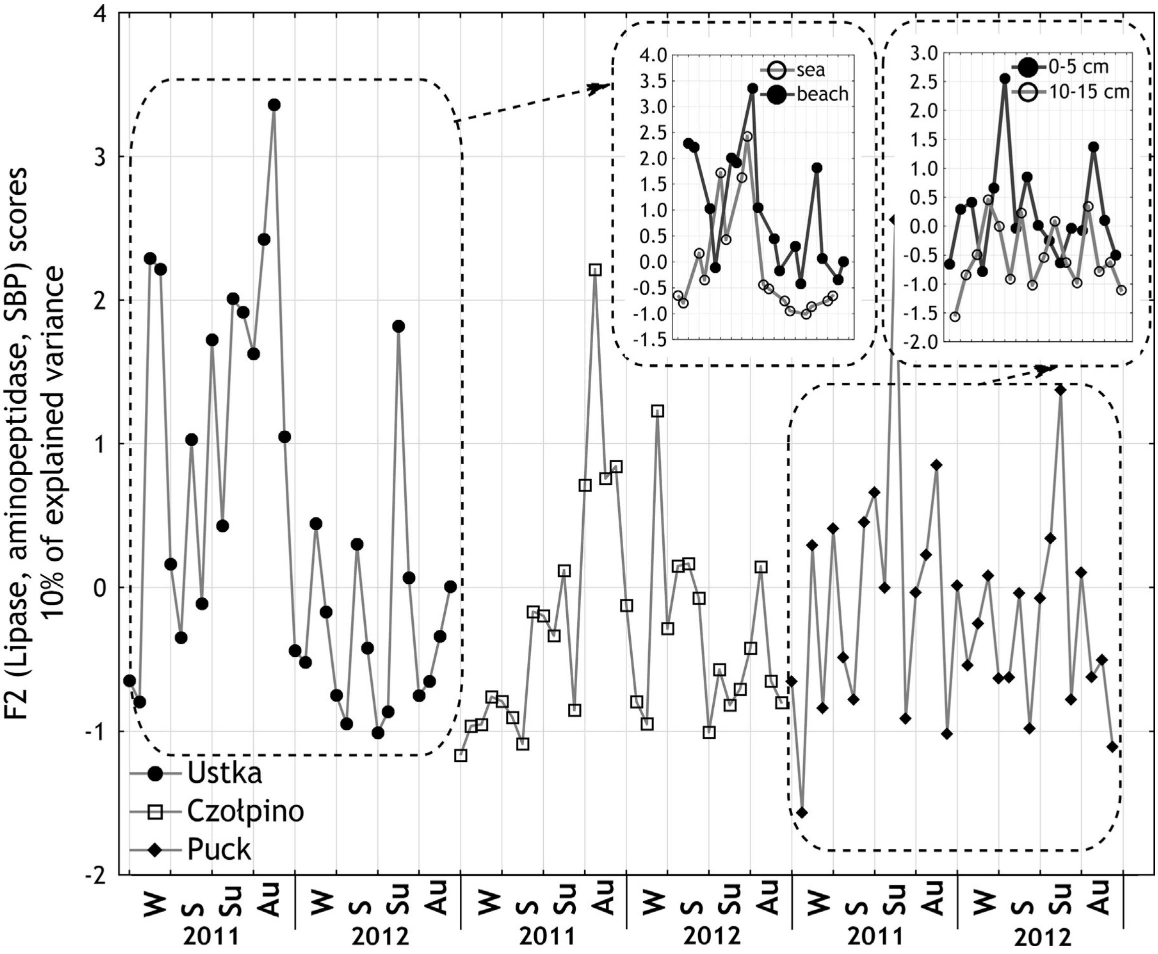

Figure 4 Factor 2 (microbial enzymatic activity) scores according to the location of the beach as well as horizontal transect in Ustka and vertical core depth in Puck (on *x* axis W, S, Su and Au states for winter, spring, summer and autumn, respectively).

caused by infilling with well-aerated sand mined from the main harbor canal carried out to protect unique ecosystem of dunes against substantial abrasion of the coast as well as to assure extraordinary touristic features of this health resort (Bigus et al., 2016). Statistically higher secondary bacterial production observed on the beach (82 μg C g^{-1} h^{-1}) in comparison with the sea (23 μg C g^{-1} h^{-1}) in Ustka confirms that infilling campaigns increase mixing and aeration, and hence facilitate the enzymatic activity of microorganisms in the middle of the beach in comparison with location D1. Due to the lack of seasonal and even annual stability of beach sediments in Ustka caused by infilling actions, the depth-dependent variation of microbial enzymatic activity was not observed. Contrarily, in the case of beach characterized by low slope, low hydrodynamic sand seasonal stability depth-dependent variation in microbial enzymatic activity could be noticed. In the upper layer of beach sediments dredged in Puck the activity of lipase, aminopeptidase, as well as, SBP was almost twice as high (147 nM MUF g^{-1} h^{-1}, 55 nM MCA g^{-1} h^{-1} and 75 μg C g^{-1} h^{-1}, respectively) as in the lower layer (90 nM MUF g^{-1} h^{-1}, 29 nM MCA g^{-1} h^{-1} and 30 μg C g^{-1} h^{-1}, respectively). An increased microbial enzymatic activity in the upper layer of the beach sediments reflects a higher total bacteria number in it, and hence higher SBP. In general, better mixing (also caused by tourist movement), aeration and higher temperature observed in the upper layer of beach sediments facilitate microbial enzymatic activity (Halliday and Gast, 2011; Piggot et al., 2012). However, it should be noticed that organic matter mineralization is not only caused by psammon organisms. The other important factor which facilitates high molecular compounds degradation is photodegradation dependent on insolation. Photodegradation is the most intensive on beaches strongly exposed to sunlight and this is why mineralization of organic matter runs fastest in sediments at a depth of 0—5 cm (Kaiser and Herndl, 1997).

Inverse time variation, in comparison with “anthropogenic rich in N” factor, was observed for an abundance of the labile organic matter. A plot of scores for the third factor visualized according to the location of the beach is presented in Fig. 5.

Similarly, as it was reported by Incera et al. (2003), BPC was dominated by PRT and followed by CHO on all Polish beaches. Starting from winter 2011 to spring 2012, a slight decrease of the abundance of a labile organic matter was observed in all the locations, with the highest variation in Puck. However, statistical assessment (Table 3) proved that only in the case of CHO and BPC some significant differences appeared in the pairwise arrangement of the beaches (Czołpino vs Puck and Ustka vs Puck). Concentrations of carbohydrates were the highest in Puck (582 μg g^{-1}), moderate in

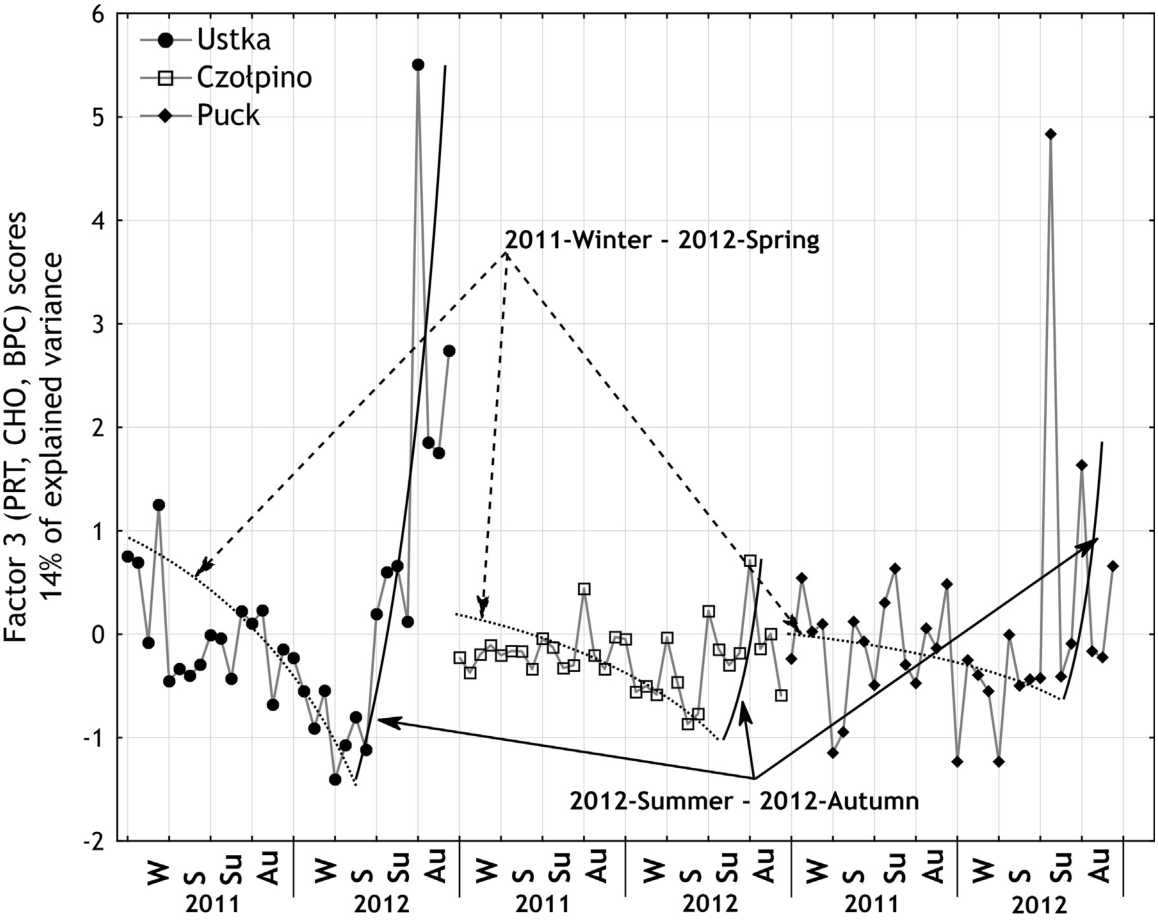

Figure 5 Factor 3 (labile organic matter) scores according to the location of the beach (on *x* axis W, S, Su and Au states for winter, spring, summer and autumn, respectively).

Ustka (509 $\mu g\ g^{-1}$) and the lowest in Czołpino (338 $\mu g\ g^{-1}$). The lack of statistically significant differences in average CHO concentration in core sediments was found between Ustka and Czołpino. The similar sequence of the concentration of BPC was found for the three investigated locations: Puck (590 $\mu g\ g^{-1}$) > Ustka (549 $\mu g\ g^{-1}$) > Czołpino (345 $\mu g\ g^{-1}$), however in this case the lack of statistically important differences was related to Ustka and Puck. Observed differences in abundance of labile organic matter seem to be a mixed effect of two factors: anthropogenic pressure and degree of sheltering and generally decrease in the following order: highly polluted and sheltered > moderately polluted and exposed > lightly polluted and exposed being in agreement with the results presented before by Incera et al. (2003).

As mentioned above, the time-dependent phenomenon of the inverse characteristic in comparison with factor 1 scores was observed for an abundance of labile forms of organic matter in the entire belt of the Polish coast. In Ustka, Czołpino and Puck a sudden and unexpected increase of abundance of CHO, PRT and BPC was observed in summer 2012 and autumn 2012. Increasing concentration of labile forms of OM correlated with decreasing concentration of nutrients (mainly N and P) could be associated with the algae blooms mentioned above usually taking place in Spring in the zone of the southern Baltic Sea. Due to carbohydrate and protein production potential, many microalgae significantly increase their cellular neutral biochemical form content when present in an environment rich in nutrients as is typical in coastal zones (Håkanson and Bryhn, 2008; Smayda, 1997; Vu, 2016).

Total bacteria number and concentration of lipids are almost independent of the degree of beach sheltering or anthropogenic pressure on the Polish coast. Such conclusion arises from the analysis of Fig. 6 where spread and range of factor 4 scores in Ustka, Czołpino and Puck are comparable.

An application of multiple K—W's test (Table 3) confirmed the lack of statistically significant differences in TBN in cores dredged in Ustka, Czołpino and Puck and indicated only one pairwise difference between Czołpino (112 $\mu g\ g^{-1}$) and Puck (250 $\mu g\ g^{-1}$) concerning LIP concentration. The overlapping pattern of points presented in Fig. 6A and B interpreted together with K—W's test results (Table 5) suggests that neither location of the sampling point in a vertical transect of the beach nor vertical core depth discriminates sediment samples according to bacterial growth. The only difference refers to the seasonal variation of TBN. During winter TBN is almost twice as high (around 7 mln cell g^{-1}) as during summer (around 4 mln cell g^{-1}), while during autumn and spring it is comparable (around 6 mln cell g^{-1}). Such phenomenon could be easily explained by beach freezing during winter season. When the external temperature drops down, the upper layer of the beach as well as the shallow part of the coast freeze creating a tight ice cover which usually reaches

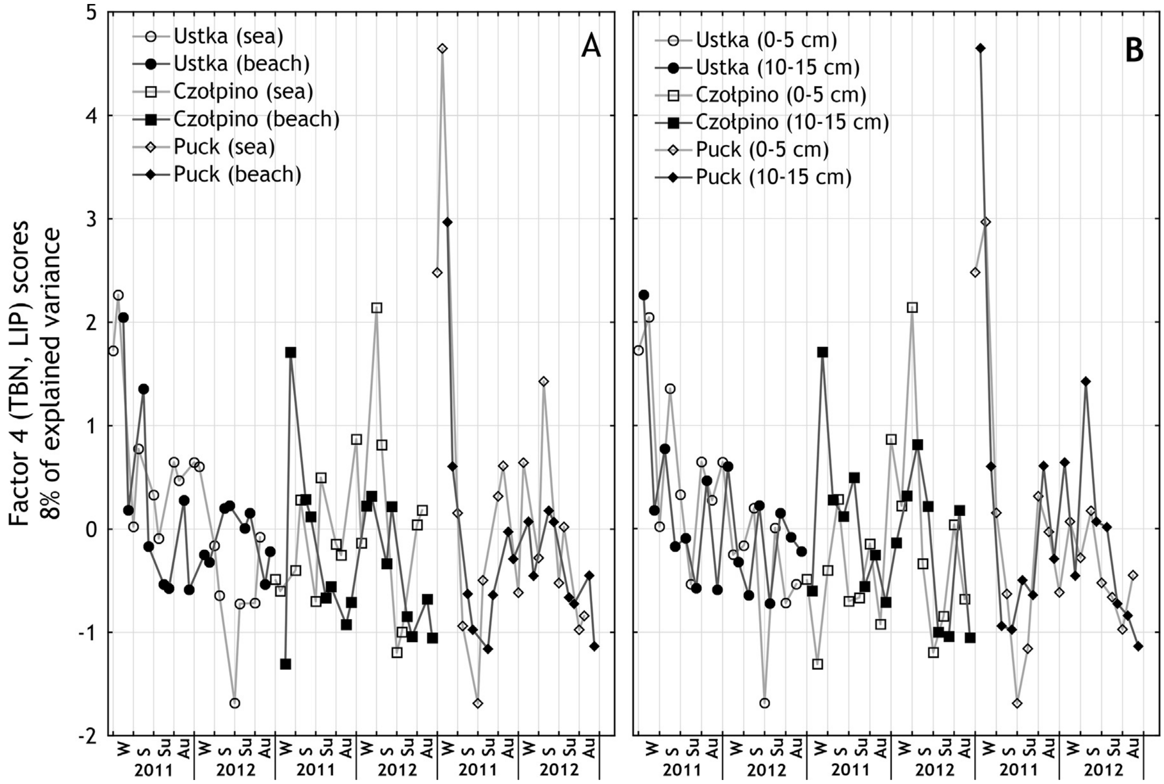

Figure 6 Factor 4 (bacterial growth) scores according to the location of the beach as well as (A) the distance from the water line and (B) vertical core depths (on *x* axis W, S, Su and Au states for winter, spring, summer and autumn, respectively).

the end of the breakwater (up to several dozens of meters). Below the frost zone, the processes of bacterial multiplication still take place, however their intensity is much lower than during summer time and limited by periodic defrosting caused by occasional temperature increase during sunny days. Limited access of sea water to the beach due to elongated ice cover makes removal of organic matter and bacteria difficult and results in bacteria accumulation (Węsławski et al., 2005).

Terrestrial and even more precisely anthropogenic origin is evident when one analyzes factor 5 scores presented in Fig. 7.

A huge load of phosphorus carried out by the Vistula River through Żuławy Wiślane as well as the impact of local low-rate wastewater treatment plants reflect higher factor 5 scores in Puck. High factor 5 scores correspond with statistical difference of TP concentration median values (Table 3) and refer to the highest concentration of TP (260 μg g^{-1}) in comparison with the respective value in Ustka (70 μg g^{-1}) and Czołpino (99 μg g^{-1}). Simple comparison proves that the middle Pomeranian region is relatively slightly impacted by phosphorus compounds carried out by surface flow. Moreover, the impact of the open sea, and waves of high energy in particular, facilitate the cleaning effect as well as the biodegradation of phosphorus compounds (HELCOM, 2009). Nevertheless, it seems that in the period of one year (winter, 2011—winter, 2012) slightly increasing trend is observed for TP concentration in Ustka and Czołpino. Due to the lack of huge variation in Ustka and Czołpino an additional interesting phenomenon was discovered. As can be seen in Fig. 7, an increasing trend of TP changes in Ustka has a zigzag form and all minimal values concern the upper layer (0—5 cm) of sediments collected in the middle of the beach. It suggests that phosphorus in the lower layers of beach sediments could be immobilized in the form of calcium or aluminum phosphates, and hence become inactivated and not easily accessible (Forsberg, 1991; HELCOM, 2009).

Similarly as in the case of factor 1 ("anthropogenic rich in N") and factor 3 ("labile organic matter"), some interesting although unexpected time-dependent change (possibly caused by algae bloom) was observed in the period between spring 2012 and autumn 2012. In all the locations, a huge drop in the load of TP was observed with minimal values in spring and summer 2012. It corresponds proportionally with a drop of OM, TOC, TN and COM which was discussed above. After the end of summer 2012 the concentration of TP dynamically increased once more, reaching the pre-drop level of concentration similarly as in the case of factor 3 ("labile organic matter"). As mentioned above, algae bloom usually ceases prior to the highest peak of touristic season when the temperature rises, oxygen deficiency appears and the abundance of nutrients has been exhausted (Forsberg, 1991). Exhaustion of easily accessible N source causes termination of algae blooms in this case, while anthropogenic P load carried out constantly by coastal rivers causes its dynamic increase prior to the winter season. The last factor analyzed was "hydrolytic" one and its factor scores are presented in Fig. 8.

As ensues from Fig. 8, a majority of factor scores for all investigated Polish beaches range between −1 to +1, indicating similar variation. The lack of statistically important

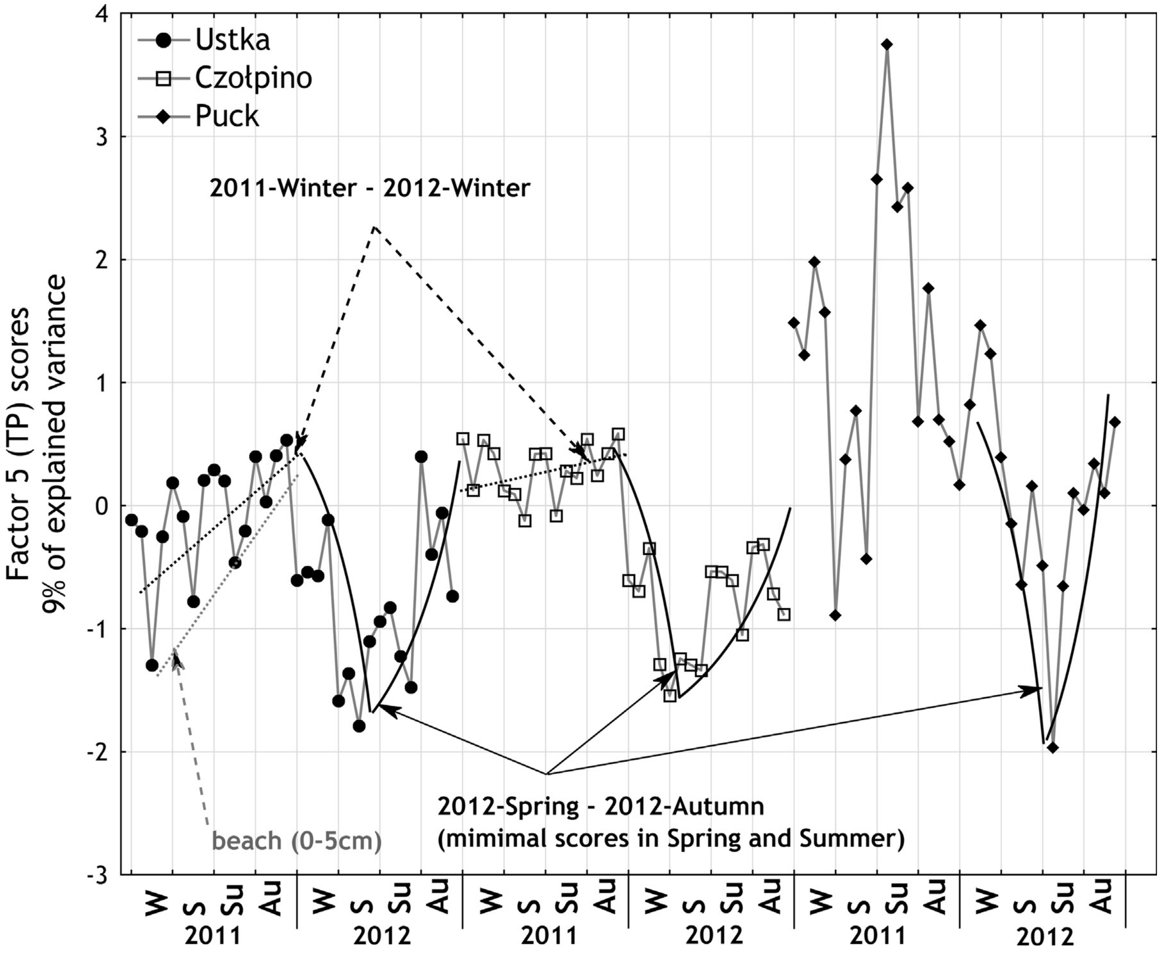

Figure 7 Factor 5 (anthropogenic rich in P) scores according to the location of the beach (on *x* axis W, S, Su and Au states for winter, spring, summer and autumn, respectively).

differences between the median activity of α-glucosidase and β-glucosidase was confirmed in pairs: Czołpino-Puck and Ustka-Puck. The only difference appeared between α-glucosidase activity in Ustka and Czołpino (Table 3). It appears that in Ustka twice as high activity of α-glucosidase is observed which is confirmed by corresponding mean and median values (Table 2) as well as a slightly higher spread of extreme factor 6 scores. As mentioned above, the activity of α-glucosidase increases when hardly assimilable matter appears in the environment, which is much more predictable in a location of moderate anthropogenic impact (Ustka) than in a clean area (Czołpino). A careful inspection of Fig. 8 revealed some other interesting details. During Spring 2012 some significant drop of factor 6 scores is observed in Ustka and Czołpino. For the "hydrolytic" factor,the observed drop relates to an increase of α-glucosidase activity (due to a negative factor score value, Table 4). Increased α-glucosidase activity in Ustka and Czołpino fits with maximal factor 1 scores (Fig. 3) and minimal factor 3 scores (Fig. 5) in these locations. Such simultaneous assessment proves that microorganisms induce α-glucosidase synthesis to decompose hardly assimilable COM during deficit of easily assimilable PRT and CHO. Moreover, in both locations (Ustka, Czołpino) the mentioned drop consists of two pairs of scores. Among them, lower and upper scores correspond with lower and upper layer of sediment cores respectively. It suggests that the lack of easily assimilable matter activates stronger hydrolytic activity in the lower layers of core sediments.

5. Conclusions

The microbial enzymatic activity and its relation to various forms of organic matter abundance in beach sediments still remain an underdeveloped field of research. The results of the presented research prove the occurrence of essential changes in microbial enzymatic activity in sediments dredged from beaches diversified according to the anthropogenic impact and the degree of sheltering. The dredged material collected on the Polish coast is generally characterized by lower concentrations of PRT, CHO and LIP, as well as higher microbial enzymatic activity as compared to other coasts spread in southern Europe. Biochemical analysis of sediments derived from three different locations enables identification of various factors (anthropogenic rich in N, microbial enzymatic activity, labile organic matter, bacterial growth, anthropogenic rich in P and hydrolytic) impacting their microbial activity according to seasonality as well as horizontal and vertical profiles of the beach. Therefore, it can be concluded from the present study that anthropogenically originated OM dominates over natural

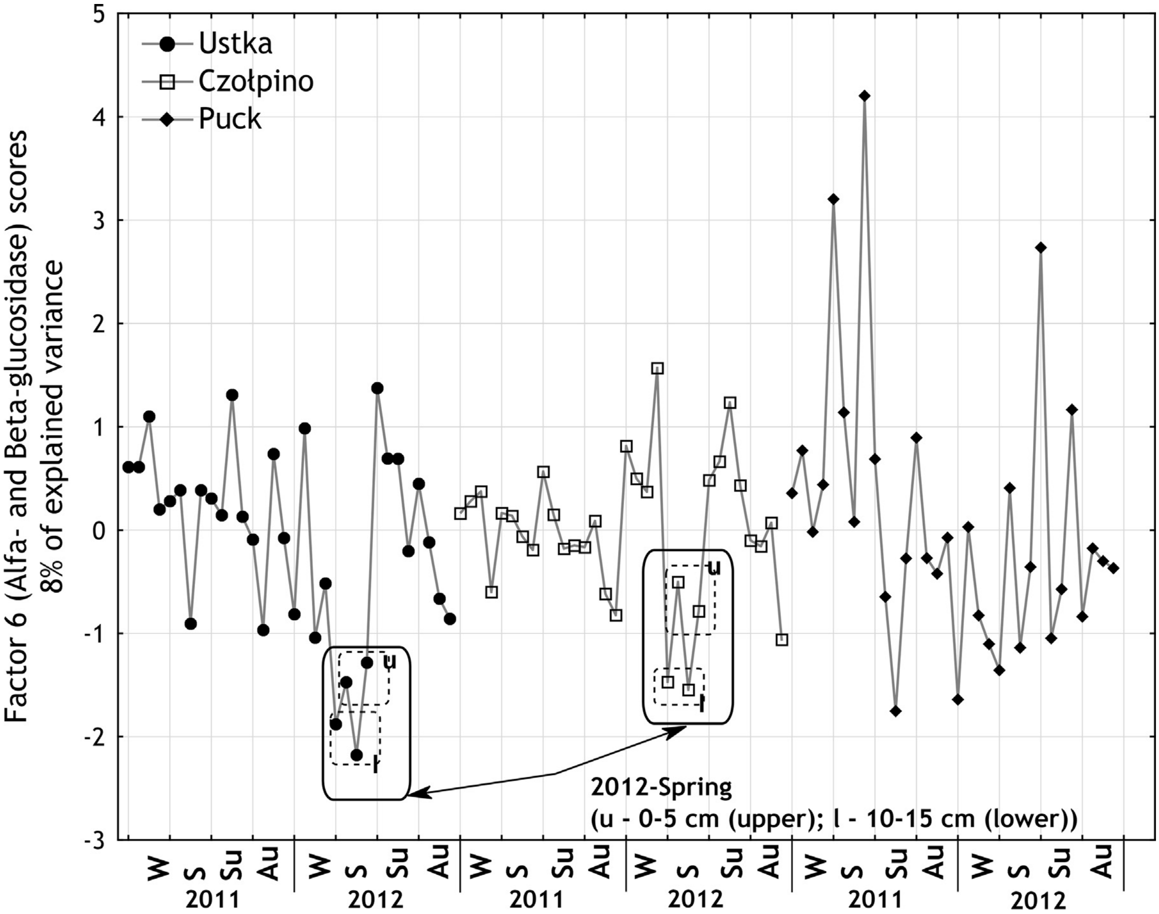

Figure 8 Factor 6 (hydrolytic) scores according to the location of the beach (on *x* axis W, S, Su and Au states for winter, spring, summer and autumn, respectively).

one on the Polish coast. The highest impact of artificial pollution takes place in Puck Bay, where several factors related to pollution sources as well as hydrodynamics of the sea create negative interactions for the environment. Microbial enzymatic activity depends on beach management, while hydrolytic activity varies according to the accessibility of hardly or easily assimilable forms of organic matter.

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