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Chemical and phycological structure of surface microlayer and subsurface water in a southern Baltic Sea estuary

by Józef Piotr Antonowicz^{1,2} and Anna Kozak³

ABSTRACT

The surface microlayer of water (SML) is a unique ecotone found on all water bodies. It is an interphase for the exchange of matter between the hydrosphere and the atmosphere. This ecotone is capable of accumulating chemicals and microorganisms in amounts as high as 100-fold greater than those observed in the pelagic zone.

Here we report on the accumulation of chemicals and phytoneuston in the SML of the estuarial Łebsko Lake located at the southern coast of the Baltic Sea in the unpolluted region of the World Biosphere Reserve: the Słowiński National Park in Poland. The physicochemical parameters and phytoplankton composition from the SML (thickness of $242 \pm 40 \mu\text{m}$) were compared with those in subsurface water layers (SUB; 15 cm under the surface). A wide spectrum of chemical and microbiological analyses was performed to investigate the capacity to accumulate substances. Almost all analyzed trace metals (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Ba, Pb), biogenic substances such as forms of phosphorus and nitrogen as well as chlorophyll *a*, pheophytin, and heterotrophic bacteria were detected at higher levels in SML than in SUB. Also, a greater number of taxa and higher abundance and biomass of phytoplankton were found in SML than in SUB. In contrast, some chemical parameters such as salinity components (Cl^- , SO_4^{2-} , Na^+ , K^+ , Mg^{2+} , Ca^{2+}) as well as pH occurred at a comparable level in SML and SUB. Physicochemical factors such as components of salinity in freshwater, brackish, and marine areas determined taxonomic composition, abundance, and biomass of phytoplankton and phytoneuston. Canonical correspondence analysis indicated the most significant chemical factors, such as nutrients and essential metals, affecting phytoplankton composition in the analyzed water layers. The degree of water salinity, chemical components such as metals, nutrients of water, and lotic and lentic environments influenced the qualitative and quantitative parameters of the phytoplankton and phytoneuston; hence, the common occurrence of resistant species such as *Desmodesmoum communis* in the studied estuary and those characteristic of freshwater occurring only in the river stand, e.g., *Woronichinia naegeliana* and, for example, *Chaetoceros decipiens* occurring only in the sea.

Keywords: Baltic Sea, estuary, ecotone, surface microlayer, phytoneuston, phytoplankton, metals

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1. Introduction

The air–sea interface is a thin (1–1,000 μm), unique layer with physical, chemical, and biological properties differing from those of subsurface water (Cunliffe et al. 2013; Ebling and Landing 2015). This surface microlayer (SML) is an important boundary between the hydrosphere and the atmosphere and key to a range of global biogeochemical and climate-related processes (Wurl et al. 2017). It is a zone of exchange for matter and energy that both affects and is affected by global climate changes (Gašparović et al. 2007). The SML is a gel-like layer characterized by unique polysaccharidic and proteinaceous composition (Wurl et al. 2009; Engel and Galgani 2015). This layer is capable of accumulating high amounts of chemical pollutants including toxic heavy metals and herbicides (Wurl and Obbard 2004). SML is highly dynamic in nature owing to the numerous non-equilibrium processes such as temperature fluxes, irradiance, salinity gradients, and wind and wave action that influence its biogeochemical properties (Kostrzewska-Szlakowska 2005). The SML is exposed to physical damage caused by wave motion, with the disrupted continuity in the SML being compensated for by the SML capacity of rapid relaxation and reconstruction (Hale and Mitchell 1997; Wurl et al. 2011; Sabbaghzadeh et al. 2017). Under typical oceanic conditions, e.g., moderate wind speeds and presence of breaking waves, the surface microlayer is mechanically more stable than the water column below because of the surface tension created between water molecules and the high concentration of surface active organic components. The relative stability of SML is provided mainly by physical forces such as surface tension, adhesion, and cohesion (Norkrans 1980).

Heavy metal concentrations recorded in SML frequently exceed those in subsurface water (Ebling and Landing 2015; Antonowicz et al. 2017). These frequently toxic metals affect neuston organisms living in SML such as bacteria, algae, and fungi, as well as early larval stages of the nekton (Agogué et al. 2005, Trojanowski and Antonowicz 2011). Chemical pollutants in the surface microlayer are accumulated as a result of biological, physical, and chemical processes, including simple diffusion, turbulent mixing, scavenging, convection, upwelling of underlying waters, atmospheric deposition, transport by bubbles, buoyant particles, and chelation of inorganic components by organic matter (Kostrzewska-Szlakowska 2005; Santos et al. 2011). The composition of the SML may vary horizontally, from day to night, or seasonally (Antonowicz 2008; Perliński et al. 2017).

Studies on an increasingly broad range of chemical elements provide new information on the accumulation capacity of SML, particularly in relation to the phytoneuston and phytoplankton. It is essential to investigate these interdependencies, because changes in water chemistry cause a rapid response of phytoplankton (Kawecka and Eloranta 1994). Communities of phytoplankton and phytoneuston vary in the surface and subsurface layers both in terms of their taxonomic composition and population size (Wang et al. 2014). Studies conducted to date on the surface microlayer have focused on uniform water bodies such as seas (Wurl and Obbard 2004) or lakes (Mudryk et al. 2003; Antonowicz et al. 2015; Kostrzewska-Szlakowska 2005; Walczak 2009). This study describes analyses of biological and chemical dependencies in the SML of an estuarine lake. It also compares the effects of

salinity in the Baltic Sea and a river, indicating differences resulting from the salinity gradient in the estuary marine, brackish, and freshwater areas. Studies of phytoneuston colonization in the SML are scarce and literature sources typically focus on analyses of chlorophyll concentration in relation to chemical parameters. For this reason, research concerning these dependencies in this dynamic ecotone of SML is of great importance and provides novel information.

Studies concerning SML describe the accumulation capacity typically within a narrow range of parameters (for details see, e.g., Knulst et al. 1997; Kostrzevska-Szlakowska 2005; Ebling and Landing 2015). This study comprises analyses of a wide spectrum of physico-chemical parameters such as metallic elements, including heavy metals and macronutrients, nutrients, nonmetals such as halogens, physical parameters such as water temperature, and biological factors such as qualitative and quantitative composition of phytoplankton as well as counts of heterotrophic bacteria. Such an extensive multiparametric analysis of the chemical composition provides greater insight into the dependencies between the investigated parameters that influence water characteristics and therefore would be expected to influence microorganisms.

We hypothesized that regardless of the salinity gradient and nature of the water source (the lacustrine vs. riverine ecosystem) the concentrations of nutrients and trace elements would be higher in the SML than in the SUB. As a consequence, also, the abundance of organisms, i.e., heterotrophic bacteria, cyanobacteria, and algae, were expected to be higher in the surface water layer.

The aim of this study was as follows: (1), determine the taxonomic composition, abundance and biomass of phytoplankton in the SML and SUB layers of the Łebsko Lake estuary in relation to chemical factors and characteristics of two different habitats: SML and SUB; (2) examine the most significant chemical factors affecting phytoplankton composition in SML and SUB using multivariate analysis techniques; and (3) to relate the salinity gradient from marine, brackish to freshwater to the distribution of microbiological and chemical components in the lotic and lentic environments.

2. Materials and Methods

The study area is Łebsko Lake, a unique zone comprising the southern part of the Baltic Sea in the area of the town of Łeba as well as the nearest zones of influence of the Łeba River flowing through the lake. The entire area is situated within the World Biosphere Reserve—the Słowiński National Park with Łebsko Lake also protected within the framework of the Natura 2000 network. The surface area of the water table in Lake Łebsko is 7,020.0 ha, at the shoreline length of 55,880 m. It is a shallow polymictic lake located at an altitude of 0.2 m above the sea level, which facilitates the exchange of waters with the Baltic Sea. The maximum length of the lake is 16,360 m and its width is 7,600 m; the maximum depth is 6.3 m with an average depth of 1.6 m (Trojanowski et al. 1990; Choiński 1995). The coastal climate and the exposed lake shoreline promote rapid wave formation on the lake.

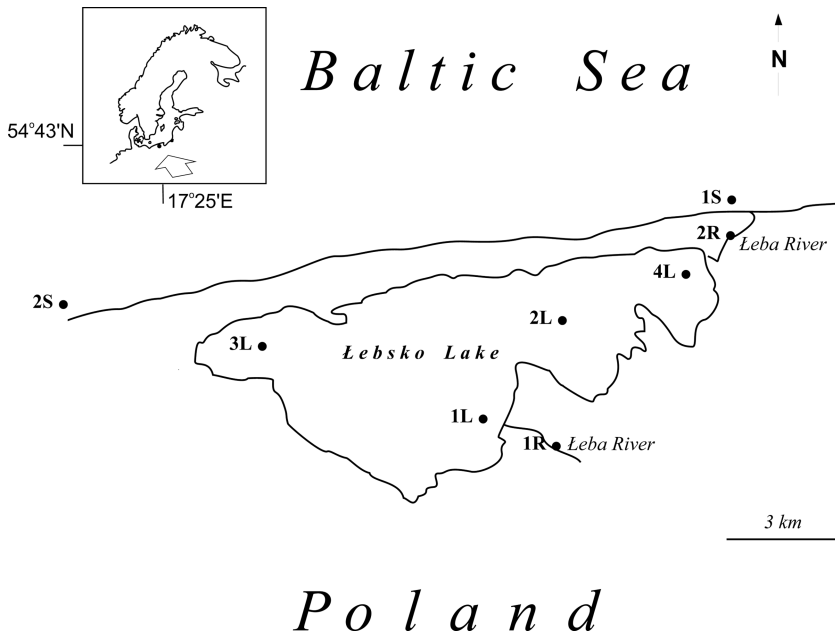


Figure 1. Location of sampling stations in the Łebsko Lake estuary.

For the purpose of this study, eight sampling stations were established (Fig. 1) with four sampling stations situated in Łebsko Lake (1L, 2L, 3L, and 4L), two stations in the Łeba River—one at the inflow to the lake (1R) and one downstream from the outflow from the lake (2R)—and two sampling stations in the coastal zone of the Baltic Sea (1S—in the vicinity of the Łeba mouth to the sea in the town of Łeba, and 2S in the town of Czołpino located at a distance of 10 km, well outside the anthropogenic pollution impact zone). The Łebsko Lake stations 4L, 2L, and 1L were selected so that the salinity was decreasing, whereas station 3L is located in a calm bay and the Klukowe Legi strict nature reserve.

Surface microlayer water samples were collected using the Garrett screen (Garrett 1965), facilitating sampling of a layer of $242 \pm 40 \mu\text{m}$ in thickness. The Garrett screen was placed horizontally over the water surface and the SML was collected from the surface to a container using a transparent wiper. Samples of the subsurface water layer were collected from a depth of 15 cm using a polyethylene container attached to a sampling dipper. All the samples were collected in summer 2012. Collected samples were stored in polyethylene containers. Chemical analyses were conducted using chemically pure polyethylene containers rinsed with the first batch of the sample. Subsamples for trace elements according to Ahlers et al. (1990) were collected following the contamination-free procedure, including etching with nitric (V) acid and then rinsing with demineralized water to remove chemical contamination from glass containers and PET. Water samples for analyses of phytoplankton were fixed

with formalin. A portion of the sample material was not fixed in order to conduct qualitative analyses. For microbiological analyses prior to sampling, the Garret net was rinsed with distilled sterile water (Hydrolab) and ethyl alcohol. The collected samples of water were transported to the laboratory in ice containers at a temperature that did not exceed 7°C. The time between sample collection and microbiological analyses usually did not exceed 3 h (Perliński et al. 2017).

a. Chemical and microbiological analyses

Water samples for analyses of metals were mineralized using an Ertec Magnum II pressure microwave mineralizer in *Suprapur*[®] HNO₃ (US EPA 1998). Analyses of metals Al, Cr, Mn, Fe, Ni, Cu, Zn, Sr, Cd, Ba, Pb, and As were conducted using a mass spectrometer (ICP-MS Thermo Scientific) and applying atomic absorption spectrophotometry (AAS-F, AAS-GF). Standard series were prepared using certified standard solutions. Ultrapure deionized water (Hydrolab) was used to produce dilutions. The accuracy and precision of the method were satisfactory based on a comparison with certified reference material TM-24.3 (water from Lake Ontario, Environment Canada), with recovery rates ranging from 95% to 109% for the assayed metals. In order to verify contamination during sample preparation, tests were conducted on the blank sample (deionized water, electrolytic conductivity < 0.06 µS/cm) and a sample of deionized water subjected to an identical mineralization in *Suprapur*[®] HNO₃ as the tested sample. Concentrations of dissolved Ca²⁺, Mg²⁺, K⁺, Na⁺, F⁻, Cl⁻, Br⁻, and SO₄²⁻ ions were assayed using an ion chromatograph (Methrom) coupled with an automatic sample injector. The calibration curve was established based on a series of dilutions from a certified standard solution (APHA 1992).

Analyses included concentrations of biogenic substances, i.e., phosphate phosphorus assayed using method with ascorbic acid (P-PO₄), ammonia nitrogen (N-NH₄) measured using the indophenol method, and nitrate nitrogen (N-NO₃) after reduction to nitrite was determined using a UV-VIS spectrophotometer (Hitachi). Total phosphorus (T-P) was assayed after mineralization in an Ertec Magnum II microwave mineralizer. Total nitrogen according to Kjeldahl (T-N) was assayed using the Büchi distillation method after mineralization in a microwave mineralizer. Organic nitrogen and phosphorus concentrations were calculated according to APHA (1992). The concentration of dissolved oxygen was measured with a Martini Instruments oxygen meter. Temperature and electrolytic conductivity (EC) were measured by potentiometry using an Elmetron conductometer. Water reaction (pH) was determined using a Martini Instruments multiparameter meter. Abundance of heterotrophic bacteria (CFU; colony forming units) was established using the culture method. The analyses were described in detail in a paper by Mudryk et al. (2003). The concentrations of chlorophyll *a* (chl *a*) and pheophytin (pheo) were determined after extraction in ethanol (Arvola 1981). Water samples were filtered using 0.45-µm pore size filters in Millipore filtration apparatus connected to a AgaLabor vacuum pump. The obtained extracts were analysed in a Hitachi UV-VIS spectrophotometer.

The abundance and taxonomic composition of the phytoplankton and phytoneuston were examined using an inverted microscope. Cylindrical chambers were used for samples sedimentation. The biomass of organisms was calculated by approximating the shape of specimens with geometric figures (Wetzel and Likens 1991; Hutorowicz 2009). Taxa were considered dominant when their percentage share in total phytoplankton abundance/biomass reached at least 5% in one sample.

b. Statistical analyses

Basic statistical parameters including standard deviation (SD), means, t-tests, and correlation coefficients, respectively, were calculated. The type of distribution was determined using the Shapiro–Wilk test. In order to show differences in the level of a given nutrient in SML in relation to its level in SUB the enrichment factors (EF) were calculated using the following formula presented by Santos et al. (2011): $EF = C_{SML}/C_{SUB}$, where C_{SML} is the level of a given nutrient in SML, C_{SUB} that in SUB, respectively. Enrichment factors were calculated for each pair of results and averaged. Differences between individual samples were analyzed using the Kruskal–Wallis rank ANOVA test. Moreover, species diversity was analyzed based on the Shannon–Weaver diversity index (Shannon and Weaver 1949) using the PAST software (Hammer 2016).

Classification and ordination analyses were also carried out. The response of the phytoplankton communities to environmental conditions was investigated using multivariate statistical analyses. Detrended correspondence analysis (DCA) and direct gradient analysis, i.e., canonical correspondence analysis (CCA; ter Braak and Šmilauer 2002; Lepš and Šmilauer 2003), were used to analyse data on phytoplankton abundance. DCA was used first to determine the character of variability in the studied assemblages. The length of the first gradient was greater than 4 standard deviations, providing justification for the further use of CCA analysis (Jongman et al. 1987; ter Braak and Šmilauer 2002). CCA is a direct gradient analysis that summarizes relations between phytoplankton species and environmental parameters (ter Braak 1990). Analyses were conducted separately for each layer (SML and SUB) by testing dependencies between the number of dominant taxa and compounds of nitrogen and phosphorus, Ca, Fe and Mg, and PE, as well as the following metals: Al, Cr, Mn, Fe, Ni, Cu, Zn, Sr, Cd, Ba, Pb, and a metalloid, As. Both DCA and CCA were performed using the CANOCO software package (ter Braak and Šmilauer 2002).

3. Results

Concentrations of Cl^- , SO_4^{2-} , Br^- , F^- , Mg^{2+} , Na^+ , and K^+ , as well as values of EC (Fig. 2) decreased systematically starting from the marine-type locations, through the estuarine canal linking the sea with the lake and the lake sampling stations, to the lowest values of these parameters observed in waters of the Leba River. The highest salinity was recorded at stations 2S and 1S (marine water) located in the coastal zone of the southern part of the Baltic Sea (in SML mean EC of 12.3 mS, 4760 mg Cl^- dm⁻³, 1816 mg Na^+ dm⁻³), with

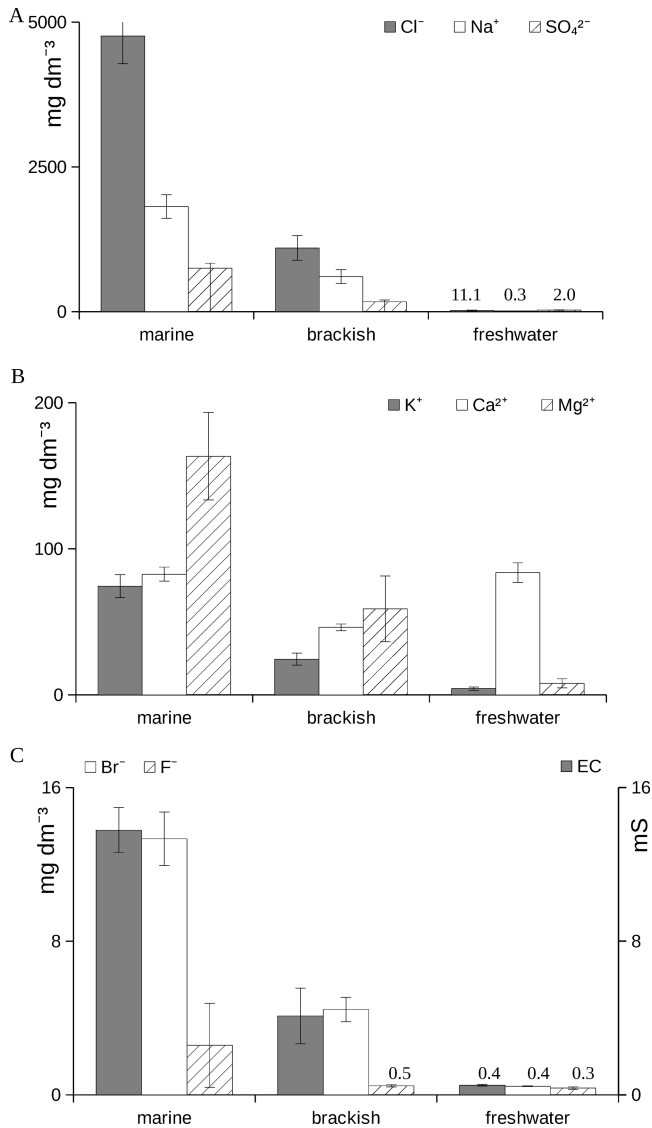


Figure 2. Changes in ion concentrations and values of electrolytic conductivity (EC) in SML at individual stations in the estuary: diagram A presents Cl⁻, Na⁺, and SO₄²⁻; diagram B presents K⁺, Mg²⁺, and Ca²⁺; diagram C presents Br⁻, F⁻, and EC.

the salinity gradient decreasing at stations 2R and 4L–2L (brackish water; EC = 4.1 mS, 1101 mg Cl⁻ dm⁻³, 605 mg Na⁺ dm⁻³), whereas the lowest salinity was recorded in waters of Łebsko Lake at station 1L and in the Łeba before it flows into the lake at sampling station 1R (freshwater; EC = 0.5 mS, 18 mg Cl⁻ dm⁻³, 13 mg Na⁺ dm⁻³). The concentration of

Table 1. Mean enrichment factors (EF) and standard deviations (SD) for chemical and microbiological parameters.

	T-N	N-org	N-NH ₄	N-NO ₃	T-P	P-org	P-PO ₄	chl <i>a</i>	feo	Abund.	Biom.	CFU
EF	2.53	2.82	2.18	1.65	1.93	1.88	1.99	2.51	1.69	1.89	1.63	8.85
SD	0.90	1.54	0.79	0.46	0.30	0.33	0.52	1.60	1.09	1.14	0.69	7.47
	Al	Cr	Mn	Fe	Ni	Cu	Zn	As	Sr	Cd	Ba	Pb
EF	1.55	1.26	1.82	1.32	1.49	1.31	1.45	1.49	1.06	1.29	1.69	1.80
SD	0.7	0.35	1.91	0.38	0.66	0.51	1.72	0.66	0.12	0.35	0.74	1.63
	Cl ⁻	Br ⁻	F ⁻	SO ₄ ²⁻	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	EC	pH		
EF	1.05	1.04	1.03	1.05	1.06	1.06	1.03	0.98	0.94	0.98		
SD	0.70	0.05	0.09	0.05	0.11	0.18	0.11	0.06	0.17	0.08		

chlorine ions at station 2S was 500-fold higher than at station 1R. Calcium concentration was high in marine waters (2S and 1S) and in river water (2R), whereas in lake waters (2L–4L) the levels of this nutrient were 50% lower. Enrichment factors (EF) in SML in relation to SUB for Cl⁻, Na⁺, SO₄²⁻, Br⁻, F⁻, K⁺, Mg²⁺, and Ca²⁺ were very low and ranged from 0.94 to 1.06.

Concentrations of nitrogen and phosphorus compounds in SML were higher than in SUB. Statistically significant differences between SML and SUB were found for T-N, N-org, N-NO₃, N-NH₄, T-P, P-org, and P-PO₄ (the Mann–Whitney U test, $p < 0.05$). This is confirmed by the respective high enrichment factors EF = 1.65–2.82 (Table 1). For chlorophyll, this value was EF = 2.51, abundance of phytoplankton EF = 1.89, and phytoplankton biomass of 1.63, for abundance of heterotrophic bacteria (CFU) EF = 8.85. Concentrations in SML and SUB were highest for N-NO₃, T-N, P-org, and P-PO₄ in fresh water (1L and 1R), whereas the concentration of ammonium ions was highest in marine water (1S and 2S; Fig. 3). The highest mean concentrations of N-org and T-N in SML were recorded at the brackish locations (2R, 2L–4L), whereas in the SUB layer it was in the freshwater stations (1L and 1R). The highest concentrations of chlorophyll *a* and pheophytin as well as biomass and abundance of phytoplankton were recorded in the brackish locations in both investigated layers. The abundance of heterotrophic bacteria (CFU) was highest in marine water in both layers; however, the highest recorded enrichment factors were found at the freshwater sampling stations (1R and 1L). Water temperature ranged from 20.6°C at the brackish locations to 21.5°C at the marine locations. Water oxygenation ranged from 8.8 mg dm⁻³ at the freshwater locations to 12.5 mg dm⁻³ at the marine sampling stations.

Generally, all metals were detected in the surface microlayer at higher concentrations than in subsurface water (Fig. 4). Statistically significant differences between SML and SUB were recorded for the following metals: Cr, Fe, Cd, Al, and Ba, as well as a metalloid, As (the Mann–Whitney U test, $p < 0.05$). The obtained enrichment factors for Cr, Mn, Fe,

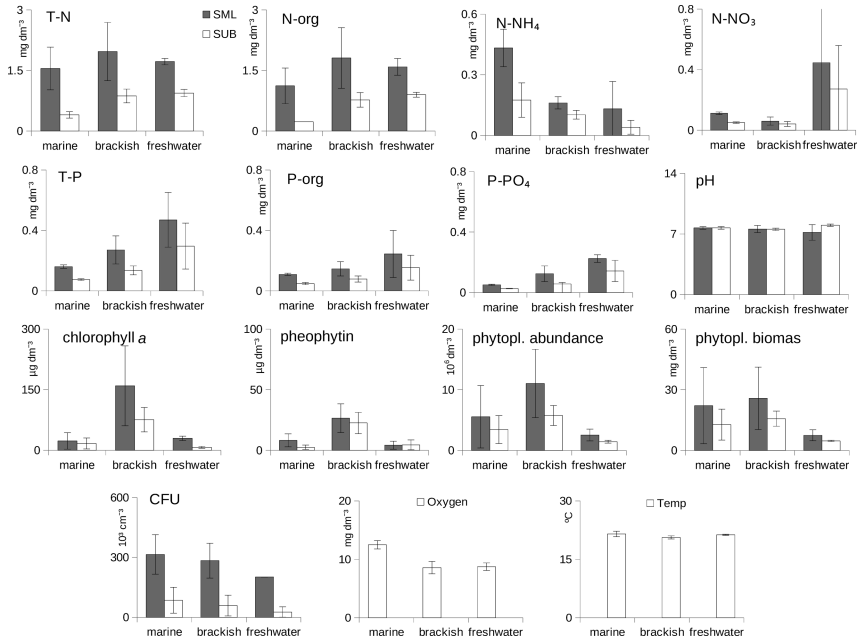


Figure 3. Distribution of concentrations of nitrogen and phosphorus compounds, chlorophyll *a*, pheophytin, biomass, and population size of microalgae in SML and SUB in the Łebsko Lake estuary.

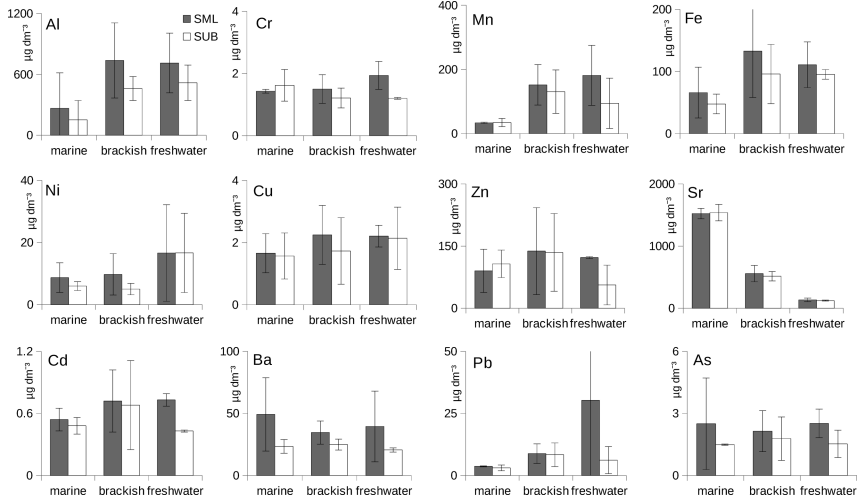


Figure 4. Distribution of concentrations of heavy metals, Al, Sr, and Ba, as well as As, in SML and SUB in Łebsko Lake estuary.

Table 2. The number of taxa from individual phytoplankton groups in SML and SUB layers and in both layers jointly, as well as depending on salinity: marine water, brackish water, and freshwater.

Layer Type of water	SML				SUB				SML and SUB all
	All	Marine water	Brackish water	Fresh water	All	Marine water	Brackish water	Fresh water	
Group	Number of taxa								
<i>Cyanobacteria</i>	22	16	18	0	23	15	10	6	25
<i>Dinophyceae</i>	0	0	0	0	1	1	1	0	1
<i>Bacillariophyceae</i>	54	14	11	36	43	15	6	40	62
<i>Euglenophyceae</i>	1	0	0	1	0	0	0	0	1
<i>Conjugatophyceae</i>	2	0	2	0	3	2	0	1	4
<i>Chlorophyceae</i>	43	12	36	8	37	12	15	8	52
<i>Ulvophyceae</i>	1	0	0	1	0	0	0	0	1
total	123	42	67	46	107	45	32	55	146

Ni, Cu, Zn, Cd, and Pb (heavy metals) fell within the range of $EF = 1.26$ – 1.82 , as well as $EF_{Ba} = 1.69$, $EF_{Sr} = 1.06$, and for the metalloid $EF_{As} = 1.60$, respectively.

Concentrations of metals varied in the SML and SUB layers. It is evident that the highest Sr concentration was observed in marine water, whereas it was lowest in fresh water. Higher concentrations of Al, Mn, Fe, and Cu in both layers were found in brackish and fresh waters, whereas they were lower in marine waters. The highest Ni concentration was recorded in fresh water (1L and 1R). The highest Zn, Cd, and Pb levels were observed in the SUB in brackish water.

Autotrophic plankton was analyzed in the SML (phytoneuston) and SUB (phytoplankton) layers. Overall 146 taxa of algae were found, with a greater number in SML (123 taxa) than in SUB (with 107 taxa; Table 2). They jointly represented 7 systematic groups. *Cyanobacteria*, *Bacillariophyceae*, *Conjugatophyceae*, and *Chlorophyceae* were detected in both layers. In contrast, *Dinophyceae* were found only in SUB at one sampling station, and *Euglenophyceae* and *Ulvophyceae* were reported only in SML (at one sampling station each, 4L and 1S, respectively). In the marine locations a total of 42 taxa were identified in SML and 45 taxa in SUB, in the brackish locations there were 67 taxa in SML and 32 in SUB, and at the freshwater sampling stations 46 taxa were reported in SML and 55 in SUB, respectively. The species composition varied between individual sampling stations and layers. The Kruskal–Wallis test showed statistically significant differences ($p < 0.05$) between the freshwater, brackish, and marine water sampling stations, respectively, for the following: Al, Mn, Fe, Ni, Zn, Sr, Pb, N-org, N-NH₄, N-NO₃, T-P, P-org, P-PO₄, chl *a*, pheo, abundance and biomass of phytoplankton, EC, Na⁺, K⁺, Mg²⁺, Ca²⁺, F⁻, Br⁻, F⁻, and SO₄²⁻. The largest numbers of taxa were recorded among diatoms (42.5% of all taxa, 54 in SML and 43 in SUB), *Chlorophyceae* (35.6%, 43 in SML and 37 in SUB) and *Cyanobacteria* (17.1%, 22 in SML and 23 in SUB).

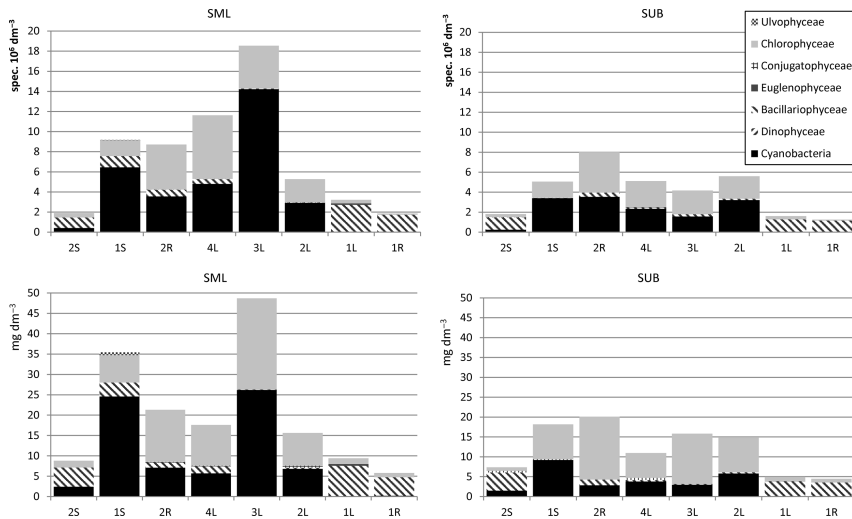


Figure 5. Biomass and abundance of phytoplankton and phytoneuston in taxonomic groups at individual sampling stations.

The abundance of the phytoplankton varied between individual sampling stations and layers, ranging from 1.3×10^6 to $18.5 \times 10^6 \text{ dm}^{-3}$ (Fig. 5). Higher abundance was found in SML (EF = 1.89). The highest abundance of phytoplankton was recorded in SML at the sampling station located within the lake (3L; Fig. 5). Lower abundance levels were reported at the freshwater locations 1R and 1L as well as one typically marine location (2S). The phytoneuston biomass (in SML) was greater than that of phytoplankton (in SUB; EF = 1.63). The Shannon–Weaver indexes of taxonomic diversity calculated based on biomass were statistically significantly higher in SML than in SUB (the t -test, $p < 0.01$, $n = 8$).

At most sampling stations diatoms accounted for a large share of the phytoneuston population, being as high as 81%. At three sampling stations, i.e., 1L, 2S, and 1R, they accounted for over 50%. In that group the highest contribution in abundance and biomass of total phytoplankton were found for *Navicula reichardtiana*, *Asterionella formosa* (particularly in SML), *Skeletonema costatum* (in both layers), and for *Thalassiosira* sp. in SUB. The taxon *Nitzschia sigmaidea*, despite its low abundance, in both layers accounted for a high share of the biomass (station 1L; Table 3).

The share of *Chlorophyceae* in the phytoplankton population was high at all sampling stations, ranging from 6% to 57%. The percentage contents of this group in the total biomass were even higher, ranging from 12% to 80%. Among *Chlorophyceae*, the highest share in the population was recorded for *Willea rectangularis* (in both SML and SUB), whereas in terms of biomass it was *Pseudopediastrum boryanum*, *Pediastrum duplex*, and *Westella botryoides*.

Table 3. Dominant species (> 5% in total abundance) in SML and SUB layers. Explanations: total abundance, *** >15%, ** 11%—15%, * 5%*–10%, ° < 5%.

Cyanobacteria	Dominant taxa	Abbreviation	Surface microlayer								Subsurface water							
			2S	1S	2R	4L	3L	2L	1L	1R	2S	1S	2R	4L	3L	2L	4L	1R
	<i>Anabaena cylindrica</i>	A.cyl	°		*	*	*	**	**					°			*	
	<i>Aphanizomenon flos-aquae</i>	A.flo		*		°	°	***										
	<i>Aphanocapsa incerta</i>	A.inc		°	*	°	°	*					°	***	°	*		
	<i>Dolichospermum flos-aquae</i>	D.flos		*			***											
	<i>Dolichospermum planctonicum</i>	D.pla		°	°	*	°	°	°	*			°	°	°	°	°	
	<i>Dolichospermum spiroides</i>	D.spi		°	°	°	°	°	°	°			°	°	°	°	°	
	<i>Gomphosphaeria aponina</i>	G.apo	*	*	°	°	°	°	°	*								
	<i>Limnococcus limneticus</i>	L.lim									*							
	<i>Merismopedia elegans</i>	M.ele									°			*				
	<i>Merismopedia glauca</i>	M.gla			*	*	*							***				
	<i>Merismopedia sp.</i>	M.sp.															*	
	<i>Merismopedia tenuissima</i>	M.ten		***														
	<i>Microcystis aeruginosa</i>	M.aer	*	°	*	*	*	*	**	°				°				
	<i>Planktolyngba contorta</i>	P.con		*	*	*	*	*	**	*				***	**	**	***	
	<i>Planktolyngba limnetica</i>	P.lim		*														
	<i>Snowella litoralis</i>	S.lit			°	°	°	°	°					°		*	°	
	<i>Woronichinia naegeliana</i>	W.nae									*			°	°	°	°	

(Continued)

Table 3. Continued

Dominant taxa	Abbreviation	Surface microlayer								Subsurface water							
		2S	1S	2R	4L	3L	2L	1L	1R	2S	1S	2R	4L	3L	2L	4L	1R
<i>Bacillariophyceae</i>																	
<i>Achnanthes coarctata</i>	<i>A.coa</i>								o								*
<i>Achnanthyidium minutissimum</i>	<i>A.min</i>							o	*							o	o
<i>Asterionella formosa</i>	<i>A.for</i>						*	*	o							*	*
<i>Aulacoseira granulata</i>	<i>A.gra</i>						***	***								**	**
<i>Chaetoceros decipiens</i>	<i>C.dec</i>	*							o								*
<i>Cocconeis placentula</i>	<i>C.pla</i>							o	o								*
<i>Cyclotella bodanica</i>	<i>C.bod</i>								o								*
<i>Diploneis interrupta</i>	<i>D.int</i>	o							*								*
<i>Diploneis ovalis</i>	<i>D.ova</i>																
<i>Fragilaria capucina</i>	<i>F.cap</i>			*					o								o
<i>Fragilaria crotonensis</i>	<i>F.cro</i>						*	*									
<i>Gomphonema olivaceum</i>	<i>G.oli</i>							o	*							*	*
<i>Melosira varians</i>	<i>M.var</i>				o		*		o							*	*
<i>Navicula tripunctata</i>	<i>N.tri</i>								*							*	*
<i>Navicula lanceolata</i>	<i>N.lan</i>							o	o							*	*
<i>Navicula reichardtiana</i>	<i>N.rei</i>							o	**							*	*
<i>Pauliella taeniata</i>	<i>A.tae</i>	*															**
<i>Skeletonema costatum</i>	<i>S.cos</i>	***	**						o								**
<i>Thalassiosira hyalina</i>	<i>T.hya</i>	**							*								*
<i>Thalassiosira</i> sp.	<i>T.sp.</i>								o								***
<i>Ulnaria acus</i>	<i>U.acu</i>	o						o	o							*	*
<i>Ulnaria ulna</i>	<i>U.uln</i>							o	o							o	o
<i>Acutodesmus acuminatus</i>	<i>A.acu</i>		o	*		o	o	*	*						o	o	*

(Continued)

Table 3. Continued

Dominant taxa	Abbreviation	Surface microlayer								Subsurface water							
		2S	1S	2R	4L	3L	2L	1L	1R	2S	1S	2R	4L	3L	2L	4L	1R
<i>Chlorophyceae</i>																	
<i>Desmodesmus armatus</i>	<i>D.arm</i>			*	o		*	*	o	*	*	*	*	o	*	*	o
<i>Desmodesmus communis</i>	<i>D.com</i>	*	*	*	*	o	*	*	o	*	*	*	*	o	*	*	o
<i>Desmodesmus opolitenis</i>	<i>D.opo</i>			o	o	o	*	*						o	o	o	
<i>Mucidosphaerium pulchellum</i>	<i>M.pul</i>	*	o	o	o	o	o	o	*	*	o	o	o	o	o	o	o
<i>Pseudopediatrum boryanum</i>	<i>P.bor</i>	o	o	o	o	o	o	o	o	*	o	*	*	*	*	o	o
<i>Pediatrum duplex</i>	<i>P.dup</i>		o		o	o	o	o			*	o	o				
<i>Phacotus lenticularis</i>	<i>P.len</i>							o							*		
<i>Scenedesmus arvernensis</i>	<i>S.arv</i>	*		o	o				o			o	*	*			
<i>Scenedesmus obliquus</i>	<i>S.obl</i>												*	*	*		o
<i>Willea rectangularis</i>	<i>W.rec</i>			*	***						*	***	*	***	*	***	

composed of 2S and 1S chemically comprised marine waters of similar salinity (Fig. 2) but differed in the taxonomic composition of the phytoplankton. The typically marine location 2S, in terms of the abundance of phytoplankton and taxonomic composition of the phytoplankton, differed markedly from the others. The most halophilous species recorded at sampling station 2S included *Thalassiosira hyalina*, *Diploneis interrupta*, *Skeletonema costatum*, *Pauliella taeniata*, and *Chaetoceros decipiens*. They were positively correlated with F^- (in SML and SUB), K^+ , and Br^- (in SML) and Ca^{2+} (SML and SUB), as well as EC (Fig. 6, $p < 0.05$). In turn, high abundance of *Lyngbya contorta* and *Dolichospermum spiroides* was recorded at sampling station 1S and the species were found to be numerous only at the brackish sampling stations in both layers (2R, 4L–2L). The second group consisted of brackish water locations (2L–4L, 2R). The taxonomic composition of the groups as well as the abundance of phytoplankton and phytoneuston were comparable at the brackish water sampling stations. Dominant species represented *Chlorophyceae* (*Pediastrum duplex*, *Willea rectangularis*) and *Cyanobacteria* (*Anabaena cylindrica*, *Dolichospermum flos-aquae*, *Merismopedia glauca*). In SML and SUB, *Microcystis aeruginosa*, *Dolichospermum planctonicum*, and *Merismopedia tenuissima* were positively correlated with $N-NH_4$. *Gomphonema olivaceum*, *Navicula tripunctata*, *Achnanthes coarctata*, and *Melosira varians* correlated with $P-PO_4$ (SML and SUB) and $N-NO_3$ (SUB). Sampling stations 1R and 1L were also markedly different from the other locations. In those two sampling stations diatoms predominated (*Asterionella formosa*, *Cocconeis placentula*, *Gomphonema olivaceum*, *Navicula lanceolata*, and *N. reichardtiana*). In terms of hydrochemical parameters, they are freshwater locations. *Aulacoseira granulata* from the group of diatoms was reported only at 1L, where it accounted for 10%–15% of the phytoplankton population. *Cocconeis placentula*, *Achnanthes coarctata*, *Navicula lanceolata*, *N. reichardtiana*, *N. tripunctata*, and *Cyclotella bodanica* were found at sampling station 1R and were positively correlated with Ni (in SUB, $p < 0.05$; Fig. 6).

4. Discussion

Studies on the Łebsko Lake estuary reported a diversity of the phytoplankton and phytoneuston species under varied hydrochemical and hydrological conditions in three closely interrelated environmental systems: SML and SUB marine, brackish water, and freshwater, as well as lentic and lotic. Estuaries, being transitional areas between marine and freshwater environments, are characterized by unique combinations of physical, chemical, and biological properties (Moisander et al. 2002; Telesh and Klebovich 2010). The dynamics and salinity gradient observed in estuaries are significant elements influencing living conditions of the phytoplankton (Nikulina 2003) affecting its quantitative and qualitative composition (Moisander et al. 2002). Electrolytic conductivity (EC), concentrations of Cl^- and Na^+ ions (correlation coefficients for the correlation between EC and Cl^- $r = 0.96$ and between EC and Na^+ $r = 0.97$) as well as such ions correlated with EC as SO_4^{2-} ($r = 0.95$), Mg^{2+} ($r = 0.97$), F^- ($r = 0.84$), and Br^- ($r = 0.97$) showed the effect of salinity caused by marine water in the investigated estuary.

In the investigated estuary such parameters as Na^+ , Cl^- , SO_4^{2-} , Mg^{2+} , K^+ , Br^- , and F^- , being essential components of marine water, as well as the concentrations of Sr and N-NH₄ systematically decreased in this order: marine water > brackish > freshwater in both SML and SUB. An opposite order (freshwater > brackish water > marine water) was observed for changes in the concentrations of N-NO₃, T-N, N-org, P-PO₄, T-P, and P-org as well as metals Pb, Fe, Al, and Cu, which indicates their terrestrial origin. Similar observations for the proportions of nutrient concentrations in the Vistula River waters in comparison to the respective concentrations in the Gdańsk Bay (Poland) were also recorded in the summer period by Wielgat-Rychert et al. (2013).

In most cases chemicals were found to be enriched in SML reaching higher concentrations than observed in subsurface water, which is particularly evident for biogens (Liu et al. 2014; Mudryk et al. 2003) and heavy metals (Wurl and Obbard 2004; Trojanowski and Antonowicz 2011; Antonowicz et al. 2015). Similar observations were also noted in coastal regions worldwide (Table 4). A comparison of enrichment factor values from the Łebsko Lake estuary (Table 1) with the results recorded in various regions worldwide confirms the statement that both heavy metals and nutrients are systematically accumulated in SML. The enrichment factors recorded in the estuary are consistent with the data reported in other studies. No publications have been found in world literature on the phenomenon of accumulation in SML within such a wide range of simultaneous analyses as it is presented in this paper on the estuary of Łebsko Lake. This study comprises extensive quantitative and qualitative chemical and phycological analyses, providing insight into the enrichment capacity in SML. Analyses of a wide spectrum of environmental elements such as metals and nonmetals, including biogenic substances, as well as physical parameters in the same water samples provide a broader picture of the estuary ecological status.

Macronutrients such as Cl^- , Ca^{2+} , and Na^+ are typically found at comparable concentrations in SML and SUB or are enriched sporadically (Knulst et al. 1997; Antonowicz 2016). Similar regularities for enrichment processes in SML for individual groups of chemicals were found in the presented study of the Łebsko Lake estuary. Here, nitrogen compounds were enriched in SML with EF values ranging from 1.65 to 2.82, phosphorus compounds with EF of 1.88–1.99, and heavy metals with EF = 1.26–1.82, whereas Na^+ , K^+ , Ca^{2+} and Mg^{2+} , Cl^- , F^- , SO_4^{2-} , and Sr were detected at comparable concentrations in both layers (EF = 0.98–1.06). Enrichment in the case of nitrogen and phosphorus compounds in SML is an element promoting phytoneuston development in SML (Kostrzevska-Szlakowska 2005; Antonowicz 2013). Reproduction of algae is most frequently associated with enrichment of the aquatic environment with phosphorus (Pliński et al. 1997; Nikulina 2003) and nitrogen (Nikulina 2003). Heavy metals such as Mn, Fe, Zn, Ni, Cu, and Co also belong to the group of bioactive substances essential for phytoplankton growth and their deficit may limit plankton production (Brand et al. 1983; Morel et al. 1991). Thus, the presence of these metals in SML may promote phytoneuston development, provided that it is at concentrations within the physiological requirements of these organisms (Tchounwou et al. 2012). Phytoplankton absorbs metals depending, e.g., on the metal ion species and solution conditions

Table 4. A comparison of enrichment factors (EF) of metals and nutrients in SML in selected studies on coastal regions worldwide.

Coastal region	Enrichment factor													Reference	
	Al	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb	T-N	N-NH ₄	N-NO ₃	T-P		P-PO ₄
Lourtoygos, Gulf of Elefsis, Mediterranean Sea, Greece	-	1.0-2.2	-	-	1.2-3.0	1.2-2.9	-	0.7-1.8	1.5-3.3	-	-	-	-	-	Sakellari et al. (2015)
Vourkari, Gulf of Elefsis, Mediterranean Sea, Greece	-	1.1-3.2	-	-	1.5-1.7	1.2-5.4	-	0.3-1.2	1.9-8.7	-	-	-	-	-	Sakellari et al. (2015)
Pahi, Gulf of Elefsis, Mediterranean Sea, Greece	-	0.3-3.2	-	-	1.3-2.0	3.9-23	-	0.2-2.1	1.3-7.8	-	-	-	-	-	Sakellari et al. (2015)
Mandovi Estuary, India	-	-	-	1.5-2.3	0.8-1.7	1.0-2.0	1.3-11.7	-	-	-	1.7-14.0	1.1-1.3	-	1.0-1.9	Singhal and Narvekar (1988)
Atlantic, Mauritanian region	-	-	-	-	-	-	-	-	-	-	9.3	3.7	-	2.2	Reinthalier et al. (2008)
Mediterranean Sea, Algerian basin	-	-	-	-	-	-	-	-	-	-	12.9	94.6	-	3.1	Reinthalier et al. (2008)
Mediterranean Sea, coastal water near Barcelona, Spain	-	0.9-5.0	-	-	1.2-3.1	2.3-35.0	1.7-12.0	0.5-3.7	2.6-21.0	-	-	-	-	-	Fowler et al. (2015)
Benyus sur Mer, France	-	0.5-1.2	-	-	1.1-1.5	1.5-6.5	0.7-2.3	0.4-2.3	1.6-19.0	-	-	-	-	-	Fowler et al. (2015)
Bay of Villefranche, France	1.15-13.5	-	1.1-5.1	1.2-5.3	1.6-3.3	0.5-15.7	1.2-6.1	1.8-6.0	1.3-13.4	-	-	-	-	-	Ebling and Landing (2015)
Pearl River, China	-	0.1-3.0	0.5-2.6	0.5-6.5	0.2-8.0	-	-	-	0.4-1.7	0.6-1.4	0.8-6.3	0.9-1.5	0.5-3.8	0.5-2.0	Liu et al. (2014)
Dolgie Wielkie, lagun Baltic Sea lake, Poland	-	2.6	3.4	-	1.7	2.8	1.4	3.2	4.7	-	-	-	-	-	Antonowicz et al. (2015)
City pond, in coastal of Baltic Sea, Poland	-	1.4	2.1	2.4	1.4	1.5	1.8	1.1	1.9	-	-	-	-	-	Antonowicz et al. (2017)
City ponds, in coastal of Baltic Sea, Poland	-	-	-	-	-	-	-	-	-	1.3-2.2	0.9-1.5	1.4-1.9	1.4-1.6	1.4-1.6	Antonowicz et al. (2016)
Estuarine Gardno Lake, Poland	-	-	-	-	-	2.4	1.7	-	3.2	-	-	-	-	-	Trojanowski and Antonowicz (2011)

(González-Dávila et al. 1995) and it shows metal biosorption capacity significant for the condition of water bodies (Rajfur 2013). Biosorption by microalgae is an element of the natural detoxification mechanism within water bodies, removing most heavy metals and excess nutrients (Joseph and Joseph 2001).

Observed high concentrations of heavy metals in SML in comparison to SUB ($EF = 1.26-1.82$) may be harmful to neuston microorganisms. Concentrations of Cr, Fe, Cd, Al, Ba, and As were statistically significantly higher in SML than in SUB. Accumulation of heavy metals in SML may be potentially harmful to neustonic organisms such as invertebrate larvae and early fish life stages (McFadzen and Cleary 1994; Rumbold and Snedaker 1997; Wurl and Obbard 2004) as well as bacterioneuston (Donderski et al. 1999), phytoneuston, and zooneuston (Hardy 1999). High concentrations of heavy metals do not necessarily cause an immediate toxic effect for the phytoneuston. Thanks to its effective adaptation mechanisms, phytoplankton may tolerate relatively high concentrations of heavy metals observed in SML. A study by González-Dávila et al. (1995) listed mechanisms of algal resistance to heavy metals and reduction of metal toxicity, such as development of energy-driven efflux pumps, oxidation, precipitation, complexing with excreted metabolites, vaporization, binding by protein or polysaccharides, methylation, and complexing by organic matter (González-Dávila et al. 1995). The above-mentioned mechanisms reduce the potential toxic effect of heavy metals (Coale and Bruland 1988; González-Dávila et al. 1995). The effectiveness of these adaptation mechanisms shown in this study may be evidenced by the high accumulation of the phytoneuston in SML ($EF_{abund.} = 1.89$ and $EF_{biom.} = 1.63$), despite the high accumulation of heavy metals ($EF = 1.26-1.82$). Also, in SML a richer taxonomic composition was observed in the phytoneuston when compared with the phytoplankton.

Cyanobacteria and certain diatoms, e.g., *Aulacoseira granulata*, as well as many taxa from the genus *Cyclotella* are considered to be sensitive to such heavy metals as Cu and Zn (Pandey et al. 2015). As it was shown by studies of the above-mentioned authors, these two metals cause morphological changes in the cell structure of algae. Copper has a negative effect on reproduction in *Cyanobacteria* (Brand et al. 1983). The toxic effect of copper on the phytoplankton was described by Debelius et al. (2010). In this study (CCA) the dependence of the phytoplankton (including *Cyanobacteria* recorded at sampling stations 2L and 3L) on Cu levels was statistically nonsignificant. It may be assumed that this is connected with the mechanisms of resistance to heavy metals described in a study by González-Dávila et al. (1995). In our study *Cocconeis placentula* was dominant at 1R in SUB, where it was positively correlated with Ni. At that sampling station a high Ni concentration was recorded, which may have shown a toxic effect on the phytoplankton. In turn, such chlorophytes as, e.g., *Desmodesmus communis* have an extensive heavy metal tolerance (Shanab et al. 2012; Pandey et al. 2015).

Salinity was a significant factor affecting the distribution of taxa. Such diatom taxa as *Thalassiosira hyalina*, *Pauliella taeniata*, and *Skeletonema costatum* were evidently related with higher salinity (a positive correlation with EC). In turn, *S. costatum* and *P. taeniata* are halophilous (marine-brackish) species (Denys 1991), whereas *T. hyalina* is a typically

marine species (Wiktor et al. 1998). The above-mentioned taxa are also examples of eurythermal species (Wang et al. 2014). Those species found to be predominant at 2S were frequently reported in the marine environment (Hällfors 2004). The taxa were also correlated with Ca (CCA), for which concentration at the marine sampling stations was higher than at the brackish locations. The conditions in the brackish environment showed the greatest biodiversity, especially in the case of phytoneuston. It may be assumed that the changing level of salinity has a positive effect on phytoplankton biodiversity. However, for stenotopic species, which are sensitive to large salinity changes, the conditions were probably unfavorable. It was confirmed by a high level of pheophytin (chlorophyll degradation product) from the brackish samples. According to the intermediate disturbance hypothesis, disturbances of intermediate intensities are conducive to maintenance of high species diversity in phytoplankton communities (Hambright and Zohary 2000). Taxa recorded at the sampling stations with the lowest salinity levels, i.e., those preferring low salinity, included *Achnanthes coarctata*, *Cocconeis placentula*, *Navicula tripunctata*, *N. lanceolata*, *N. reichardtiana*, and *Cyclotella bodanica*, predominant in the population at 1R and at the inflow of the river to the lake (1L). The taxa were positively correlated with P-PO₄ (statistically significantly in SUB) and N-NO₃ (statistically significantly in SML; Fig. 6). The Łeba River is characterized by a considerable slope, flow, and eutrophic level, which explains the presence of *Navicula lanceolata* at that location, because it is a species preferring rivers with high flow rates, or *Cocconeis placentula* typical of eutrophic waters (Noga et al. 2014). Dominant taxa reported primarily at the lake sampling stations, e.g., *Aphanizomenon flos-aquae*, *Merismopedia tenuissima*, *Pseudopediastrum boryanum*, *Pediastrum duplex*, *Desmodesmus opoliensis*, and *Microcystis aeruginosa*, were correlated with N-NH₄ and Fe levels.

Greater phytoneuston abundance and biomass in SML, in comparison to SUB, were most probably caused by higher concentrations of biogens in comparison to SUB (Trojanowski et al. 2001; Mudryk et al. 2003). Higher abundance of euglenids and pennate diatoms in SML, as well as greater abundance of dinoflagellates in the subsurface layer, were also reported, e.g., in studies on the coastal lagoon of Baja California (Montes-Hugo and Alvarez-Borrego 2007). In the case of euglenids, positive phototaxis was stated to be a mechanism responsible for the accumulation of these organisms in the subsurface layer.

In the Łebsko Lake estuary, the highest abundance and the greatest biomass of phytoplankton as well as greatest taxonomic diversity were recorded in SML at sampling station 3L in the area of lentic water. This is consistent with reports of Grabowska et al. (2014) stating that an increase in water retention time has a significant effect on the diversity and population size of the phytoplankton. In the phytoplankton and phytoneuston from the locations of lowest salinity, situated at the Łeba River (1R), dominant species represented diatoms and chlorophytes, preferring considerable water flow and turbulence (Hindak et al. 2006). Potamophytoplankton is typically dominated by both these algal groups (Dembowska 2009, Grabowska 2012). At sampling station 2R, i.e., a stretch of the Łeba River downstream from the lake, the taxonomic composition and the population size of the

phytoplankton were markedly different than at the inflow to the lake (1R). This was manifested by a greater abundance of cyanobacteria than diatoms recorded at 1R, resembling rather the composition and abundance reported for the lake. This indicates a strong effect of limnoplankton on the potamoplankton at the stretch of the Łeba at 2R. Similar phenomena were also observed in the case of other large flow-through water bodies, e.g., the Maltański Reservoir in the course of the Cybina River (Kozak 2010), as well as the Siemianówka Reservoir in the course of the Narew River (Grabowska 2012).

The sampling stations located at Łebsko Lake differed from one another not only in terms of their salinity and chemical composition, but also the composition and population size of their phytoplankton. The dominant species in the phytoplankton represented *Cyanobacteria* and *Chlorophyceae* except for the sampling station located at the inflow of the Łeba (1L), for which the species composition to a considerable degree resembled that found in the inflowing river (1R). The dominant groups at the brackish locations included cyanobacteria and chlorophytes, which are frequently dominant in the summer periods in lakes with high trophic levels (Jaworska et al. 2014; Kozak et al. 2013; 2014). A factor promoting growth of *Cyanobacteria* at the sampling stations (1S, 2R, 4L–2L) may have been connected with a higher concentration of ammonium nitrogen, preferred by cyanobacteria, which was also indicated for other water bodies (Kozak et al. 2015; Rosińska et al. 2017). Many *Cyanobacteria* species are also adapted to fluctuating salinity in their environment through production of osmotically active organic substances and active transport of ions outside and into cells, as well as their capacity to produce stress proteins (González-Dávila et al. 1995, Moisander et al. 2002).

This study showed the effect of inland waters on the area affected by marine waters in the vicinity of the port in Łeba (1S). Differences in the taxonomic composition and population size between sampling stations 1S and 2S are considerable. In the case of the sampling station located in the vicinity of the outflow (1S), *Cyanobacteria* predominated, whereas several kilometers to the west (2S), a definite predominance of diatoms was observed in the phytoplankton and phytoneuston. Sampling station 1S, although chemically similar to 2S, is characterized by considerable water inflows from Łebsko Lake; thus, the taxonomic composition of the phytoplankton resembles that found in Łebsko Lake rather than that in the Baltic Sea (sampling station 1S is clustered with 2L–4L in CCA). Microorganisms at the marine sampling station (1S) were to a considerable extent supplied passively from Łebsko Lake with waters of the Łeba River, partly adapting to the much greater salinity than at the brackish water locations, using adaptation mechanisms according to González-Dávila et al. (1995).

5. Conclusions

Studies conducted on the estuarine Łebsko Lake found distinct differences in the chemical and microbiological characteristics in the ecotone of the surface microlayer. The quantitative and qualitative composition of phytoplankton was more diverse in SML than in SUB and

changed in the horizontal and vertical plane depending on chemical conditions. Changes in phytoplankton were evident in waters of different level of salinity as well as in the area of lotic and lentic waters. The greatest phytoneuston biodiversity was observed in the brackish water area. Limnoplankton was dominated by diatoms and chlorophytes, potamoplankton mostly by cyanobacteria. An effect of lake waters at the stretch of the Łeba River was recorded on river sampling station. Analyzed trace metals and biogenic substances, as well as chlorophyll *a*, phytoplankton, and heterotrophic bacteria, were detected in higher concentrations in SML than SUB. On the other hand, significant components of water salinity, such as Cl^- , SO_4^{2-} , Na^+ , K^+ , Mg^{2+} , and, Ca^{2+} , were found to be at comparable levels in both layers. These analyses showed that the accumulation of chemicals was not uniform. Substances found at low concentrations such as trace elements or biogenic substances, particularly organic forms, are accumulated in SML, whereas the investigated salinity parameters in marine water found at high concentrations were not accumulated in SML. The investigated neustonic organisms were accumulated in SML to a greater degree than in SUB, probably because of enhanced availability of essential nutrients, which is confirmed in the results of canonical analyses.

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