

Chemical and Bacteriological Studies of Surface and Subsurface Water Layers in Estuarine Lake Gardno

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Abstract

This paper presents results of chemical and bacteriological examinations of surface and subsurface water layers of estuarine lake Gardno. The obtained data indicate that there are substantial differences in chemical compound concentrations and also bacteria number and activity between the water layers under investigation. Particularly great differences between microlayers and subsurface water refer to concentrations of organic phosphorus and nitrogen. It was found that bacteria number was greater in surface water layers than in subsurface water. Significant differences among the studied chemical and bacteriological parameters were revealed between particular sites across lake Gardno. The number of freshwater, brackish and marine bacteria in the water of lake Gardno was up to chlorides concentration.

Keywords: estuarine lake, water layers, chemical parameters, bacteria occurrence

Introduction

It is characteristic of all water bodies, that on the border of phases of extremely different environments created by water-atmosphere, a specific biotope forms, a microlayer called biofilm [10, 26, 30, 41]. The biotope is a unique physical, chemical and biological environment, completely different to deeper water parts [38]. As the surface microlayer is constantly exposed to such factors as wind, rain and undulation, which disintegrate its structure, a biotope undergoes dynamic changes both in time and space [40, 47]. However, according to some examinations [1, 19], a surface microlayer has the ability to reconstitute quickly on its own and return to its original structure.

Owing to the use of special analysis techniques [20, 38], it was possible to precisely define the structure of a surface microlayer. It was found that the microlayer is formed by the accumulation of lipids, proteins and polysaccharides on the water surface [30]. Hence a surface microlayer has a heterogenic structure and is composed of a 1-10 nm lipid layer and a polysaccharide-protein one of 10-30 nm [29, 36].

There exist many mechanisms such as adsorption, diffusion, flotation, convection, precipitation, and anthropogenic pollution, which causes an accumulation of many types of various inorganic and organic chemical compounds [21, 27, 35]. Many of those compounds are also introduced into the surface microfilm on air bubbles, which move around within the water column [3]. Once the air bubbles with absorbed chemical compounds have reached the water surface they fall apart, ejecting some of

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the compounds into the atmosphere, whereas the majority of them accumulate in the surface film [30, 42]. According to study results available presently, this is the reason why organic matter concentration in a biofilm is much greater than in subsurface water, which consequently given optimal conditions for many aquatic organisms in such a biotope [11, 35, 47]. Those organisms are called neuston and create a biofilm within the surface layer. The surface layer makes a unique home to such organisms as fungi, algae, flagellate, ciliates, amoeba and protozoa [30, 41]. Bacterioneuston is particularly active in colonising the biofilm, forming a 1-50 μm thick layer [10, 29, 33].

Over recent years, more and more hydrobiological studies have been focusing on the explanation of the role which the surface film plays in the water ecosystems functioning. Hence, the present paper has attempted to contribute to that subject and has presented results of studies on chemical and bacteriological parameters that are characteristic of surface and subsurface layers of the estuarine water of lake Gardno.

Material and Methods

The Study Area

The studies were carried out in an estuarine lake Gardno (Figure 1) situated in the World Biosphere Reserve in Słowiński National Park (Poland). The lake is very shallow (1.3 m average depth) but covers a large area (2.500 ha). The shallow depth and large area as well as the lack of shielding winds enables a full mixing of water in both vertical and horizontal profiles. As a result, the lake can be regarded as a polymictic basin in which no thermal or oxygen stratification is observed. The emergent macroflora covers 4% of the surface of lake Gardno, forming a 20-200 m wide offshore belt, which constitutes a residence for many bird species. The main species of macrophytes are: *Typha angustifolia*, *Phragmites australis*, *Scirpus lacustris* and *Schoenoplectus lacustris*. Lake Gardno is characterised by intermediate conditions between

marine and inland environments. On one hand it is supplied by water of the river Łupawa, on the other hand it is connected via a 1.3-km channel with the Baltic Sea, whose large volumes of seawater abundantly penetrate the lake. Therefore, water of that lake (or its parts) acquire seawater quality, resulting in 2-5‰ salinity. Consistent with the Venetian system, lake Gardno can be classified as mixo-oligohaline type (0.5-5.0‰) [8].

Sampling

Water samples were taken from three stations (Figure 1): one near the river inflow (freshwater zone) (station 1), one near-sea part (seawater zone) (station 3) and at one station in mid-lake (mixed zone) (station 2). Water samples for chemical and bacteriological analyses were taken from three layers. Film layer (FL) samples (thickness of $90 \pm 17 \mu\text{m}$) were taken with a 30 x 30 cm glass plate [24], surface layer (SL) samples (thickness of $242 \pm 40 \mu\text{m}$) were collected with a 40 x 50 cm polyethylene Garrett net (24 mesh net of 2.54 cm length) [15]. Glass plate and polyethylene net were rinsed with ethyl alcohol prior to sampling. Water from subsurface layer (SUB) was taken with sterile glass pipettes at a depth of about 10-15 cm. The water samples for bacteriological analyses were collected into sterile glass bottles and stored in an ice-box, where the temperature did not exceed $+7^\circ\text{C}$, until they were taken for analysis. The time between sample collection and performance of chemical and bacteriological analyses usually did not exceed 6-8 h.

Chemical and Microbiological Determinations

Total nitrogen was determined using Kjeldhals method (digestion in sulphuric acid) and total phosphorus concentration was determined by digestion in perchloric acid [17]. Ammonium nitrogen concentration was measured using the phenate method and inorganic phosphorus, by the ascorbic acid method [42]. Nitrate nitrogen was determined by cadmium reduction to nitrites and then by measuring nitrite concentration using a sulphanilic acid method [18]. Concentration of organic forms of nitrogen and phosphorus were calculated as a difference between the concentration of total and mineral forms. Chlorides were determined by argentometric titration.

The total bacterial number (TBN) in the water samples was determined by acridine orange staining and epifluorescence microscopy according to Hobbie et al. [25] using polycarbonate membranes (0.2 μm pore size) for filtration and a New Porton Grid in the eyepiece. Bacteria in 20 fields or a total of 200 bacteria were counted per sample.

To determine the number of heterotrophic bacteria (colony forming units - CFU), the collected samples were diluted with sterile buffered water (pH 7.2) prepared according to Daubner [7]. Following dilution these were inoculated on iron-peptone agar medium [13] in three replications using the spread method. After 10 days' incubation at 20°C , bacterial colonies were counted and results were recalculated per 1 cm^3 water.

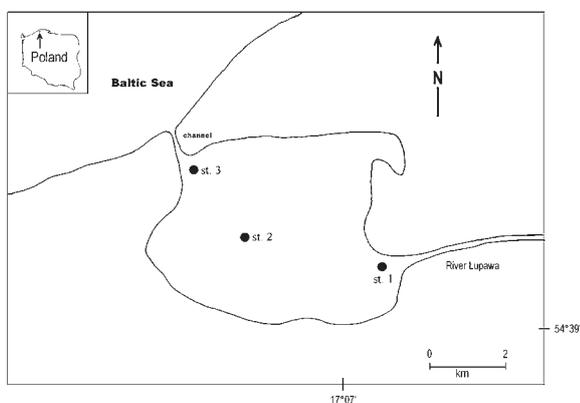


Fig. 1. Lake Gardno, northern Poland, with location of sampling stations.

For the detection the number of freshwater, brackish water and marine bacteria, agar V [45] was used adjusted to 0‰, 2‰, 8‰ salinity. After 10 days' incubation at 20°C, bacteria growing on a non-salted V medium were counted and defined as freshwater, according to Vääänen [45], at 2‰ salinity they were described as brackish water ones, and those developing at salinity 8‰ were classified as marine bacteria.

The secondary production of bacteria (BP) in the water samples was determined by measuring the rate of incorporation of [³H- methyl] thymidine into the bacterial DNA [14]. In order to determine this parameter, 20µl [³H- methyl] thymidine (Amersham 60 Ci/nmol specific activity) was added to 10 cm³ water samples in three replications and a control sample with final concentration of 15-20 nM. The control was treated with formaldehyde before the addition of thymidine in order to obtain blank

values. Samples were incubated at 20°C for 30 min. After this period incubation was interrupted by the addition of 200µl 37% formaldehyde to the samples. The samples were filtered on a 10-station filtering device on 25 nm diameter polycarbon filters (pore diameter 0.2 µm). The filters were rinsed 5 times with 1 cm³ 10% cold trichloroacetic acid (TCA). The filters were then transferred to scintillating vessels containing 5 cm³ of scintillating cocktail and the reading was taken on a Beckman LS 6000 IC scintillation counter. The calculation of bacterial production was based on the thymidine incorporation using a factor of $1.1 \cdot 10^{18}$ cells mol⁻¹ [39] and a carbon content of 0.35 pgC µm⁻³ [4].

Statistical tests (SD, CV, CD) used in this analysis were from Velji and Albright [44]. Simple linear regression was used to investigate the general correlation between studied parameters.

Table1. Chemical and bacteriological characterisation of the surface layers and subsurface water in lake Gardno.

Layer	Parameters	Dimension	Range	Mean	SD	CV (%)	CD
FL	N-NH ₄	µgN · dm ⁻³	23.00 – 185.00	90.37	45.39	50.23	22.80
	N-NO ₃	µgN · dm ⁻³	20.00 – 174.00	76.48	38.90	50.86	19.79
	N-T	mgN · dm ⁻³	2.04 – 8.80	5.16	1.86	35.96	0.67
	P-PO ₄	µgP · dm ⁻³	16.00 – 104.00	51.96	23.70	45.61	10.81
	P _{org}	µgP · dm ⁻³	81.00 – 224.00	154.04	40.21	26.10	10.50
	Cl	mgCl · dm ⁻³	16.60 – 121.10	58.58	33.31	56.86	18.94
	TBN	10 ⁶ · dm ⁻³	29.30 – 3448.30	1044.83	699.58	66.96	468.42
	CFU	10 ³ · cm ⁻³	0.67 – 228.30	52.19	60.93	116.74	71.13
	BP	µgC · dm ⁻³ · h ⁻¹	0.10 – 423.00	65.09	104.37	160.35	167.37
SL	N-NH ₄	µgN · dm ⁻³	20.00 – 103.00	47.59	23.64	49.66	11.74
	N-NO ₃	µgN · dm ⁻³	26.00 – 166.00	86.56	37.94	43.84	16.63
	N-T	mgN · dm ⁻³	0.85 – 4.01	2.18	0.85	39.04	0.33
	P-PO ₄	µgP · dm ⁻³	16.00 – 98.00	42.15	22.44	53.24	11.95
	P _{org}	µgP · dm ⁻³	10.00 – 89.00	44.81	19.02	42.45	8.07
	Cl	mgCl · dm ⁻³	16.60 – 140.10	59.47	34.94	58.76	20.53
	TBN	10 ⁶ · dm ⁻³	10.00 – 2960.00	931.25	800.18	85.93	687.55
	CFU	10 ³ · cm ⁻³	0.67 – 180.00	31.14	37.41	120.14	44.95
	BP	µgC · dm ⁻³ · h ⁻¹	0.58 – 967.00	103.53	240.32	232.13	557.85
SUB	N-NH ₄	µgN · dm ⁻³	6.00 – 58.00	21.07	12.23	58.06	7.10
	N-NO ₃	µgN · dm ⁻³	5.00 – 60.00	28.22	15.65	55.45	8.68
	N-T	mgN · dm ⁻³	0.36 – 1.75	0.76	0.36	47.80	0.17
	P-PO ₄	µgP · dm ⁻³	5.00 – 26.00	11.96	5.47	45.70	2.50
	P _{org}	µgP · dm ⁻³	2.00 – 30.00	12.93	6.42	49.67	3.19
	Cl	mgCl · dm ⁻³	20.30 – 405.50	156.25	128.89	82.49	106.33
	TBN	10 ⁶ · dm ⁻³	5.00 – 60.00	27.96	23.07	129.78	87.82
	CFU	10 ³ · cm ⁻³	0.66 – 160.00	23.71	35.81	151.06	54.10
	BP	µgC · dm ⁻³ · h ⁻¹	1.25 – 566.00	76.57	129.38	168.96	218.60

Explanations: FL - film layer, SL - surface layer, SUB- subsurface layer, SD - standard division, CV - coefficient variations, CD - coefficient dispersion.

Results

Table 1 presents data concerning the studied chemical and microbiological parameters in particular water layers of lake Gardno. Those results indicate considerable differences in the concentration of chemical parameters between the three water layers under study. The greatest differentiation between microlayers and the subsurface water was recorded in the case of organic phosphorus concentration (P_{org}) and total nitrogen (NT). Some minor differences between those water layers were recorded in the case of ammonia nitrogen (NH_4-N) and phosphate phosphorus (PO_4-P). A reverse situation was observed in the case of chloride ions. Their concentration was much greater in subsurface water than in surface water layers.

The data shown in Table 1 makes clear that total bacteria number (TBN) ($931.25 - 1044.83 \cdot 10^6 \cdot dm^{-3}$) in microlayers (FL, SL) was over 30-fold that of subsurface water layers (SUB) ($27.96 \cdot 10^6 \cdot dm^{-3}$). Maximum number of heterotrophic bacteria ($52.19 \cdot 10^3 \cdot cm^{-3}$) was recorded in the film layer while their minimum number ($23.71 \cdot 10^3 \cdot cm^{-3}$) in subsurface water layer. The highest level of secondary production ($103.53 \mu g C \cdot dm^{-3} \cdot h^{-1}$) was recorded in the surface layer, the lowest one in the film layer ($65.09 \mu g C \cdot dm^{-3} \cdot h^{-1}$).

Figures 2 and 3 present data referring to the concentration of nitrogen and phosphorus compounds and chlorides in the water layers under investigation at particular sampling stations. The data presented in Fig. 2 makes it clear that as a rule, the maximum concentration of all examined nitrogen forms was recorded at the Łupawa river mouth into lake Gardno (St.1), while the minimum of the concentrations were recorded in the vicinity of the channel connecting the lake to the Baltic Sea (St.3). The data indicates a fact that the concentration of nitrogen compounds in surface and subsurface water layers declined with water salinity. It was noted at the same time that the least concentration of nitrogen compounds at all stations was recorded in the subsurface water.

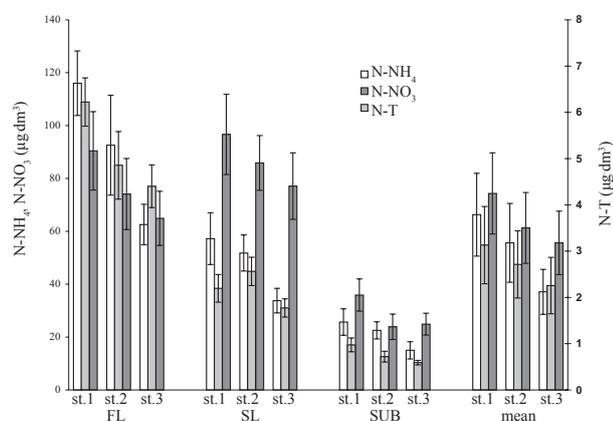


Fig. 2. Various forms of nitrogen in surface layers and in subsurface water of lake Gardno. Vertical bars indicate standard errors.

Figure 3 presents data referring to phosphorus compounds and chloride concentrations in the examined water layers with regard to particular sampling stations. In all the studied layers, owing to seasonal marine water inputs, the highest chloride ions concentrations within lake Gardno were found in the coastal zone (St.3). It was between the film and the subsurface water where particularly great differences of that parameter were recorded. At the same time, in the surface film, the chloride ions concentration in that same water layer in the channel joining the lake with the sea (St.3), was over two times greater than at the Łupawa river mouth (St. 1). At the same time, the data in Figure 3 shows that as a rule the concentration of phosphorus compounds declined along the river current going through the lake. This is particularly true for the phosphate phosphorus (PO_4-P), the concentration of which dropped twice between the river exit into the lake (St.1) and the coastal area (St.3).

Data presented in Figure 4 points to significant differences within the studied bacteriological parameters at particular sampling stations. The greatest total bacteria number was recorded at station 3 in the film layer, while the minimum of the parameter was found in the subsurface layer at stations 1 and 2. Heterotrophic bacteria were most abundant in the film at the station near the river mouth into the lake (St.1), whereas their lowest number in all water layers occurred mid-lake (St.2). Data concerning secondary production gave evidence of the process being most active in the surface microlayer at mid-lake (St.2), while the slowest course of the process was recorded in subsurface layers near the Łupawa mouth into the lake (St.1).

Figure 5 presents results of studies on freshwater, brackish water and marine bacteria occurrence in lake Gardno, which depended on chlorides concentration. The results showed that a change in water salinity triggered oscillations in the number of three bacteria groups under investigation. At the station located at Łupawa river

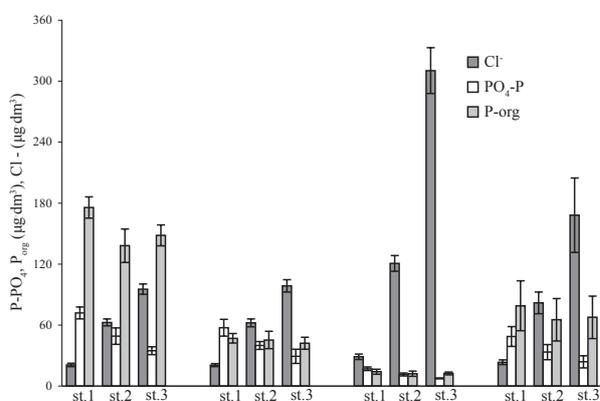


Fig. 3. Concentration of chlorides and different form phosphorus in surface layers and in bulk water in different stations. Vertical bars indicate standard errors.

mouth into the lake (St.1), where the chlorides concentration was at its lowest ($27.0 \text{ mg} \cdot \text{dm}^{-3}$), freshwater bacteria dominated. At mid-lake (St. 2) the number of all three groups of bacteria under study stayed at a similar level. In the area of the channel connecting lake Gardno to the Baltic Sea (St.3) where the chloride concentration was at its highest ($177.2 \text{ mg} \cdot \text{dm}^{-3}$), marine bacteria dominated.

According to statistics factors (CV and CD) (Table 1) the concentration changeability of the phosphorus and nitrogen compounds in Lake Gardno was much higher in the biofilm than in subsurface water. Still greater changeability was found with bacteriological parameters. That confirms high dynamics of transformations that biogenic substances and bacterial microflora undergo in surface water microlayers of the studied lake. Statistical analysis of the examined chemical and bacteriological parameters, which was based on linear regression (Fig.6), showed that there was a firm positive correlation between CFU and P_{org} as well as TBN and $N\text{-NH}_4$. A negative statistical correlation was found between chlorides and CFU, and also between bacterial production as well as P_{org} and also $N\text{-NO}_3$.

Discussion

Processes in the interface between land and water basins are of importance for the flow of material from terrestrial to aquatic ecosystems. Usually, only horizontal transport is considered. However, exchange mechanisms in the interface between water and air and the influence of surface microlayers of different properties and composition have also to be recognised [29, 41].

The present study shows that the concentration of inorganic and organic compounds of phosphorus and nitrogen in lake Gardno was much greater in surface than in subsurface water layers. Many authors [28, 30, 35, 47] have mentioned a similar phenomenon. As with Danos et al. [6] and Hilbricht-Ilkowska et al. [27] it is strictly up to biological processes going on in that specific biotope.

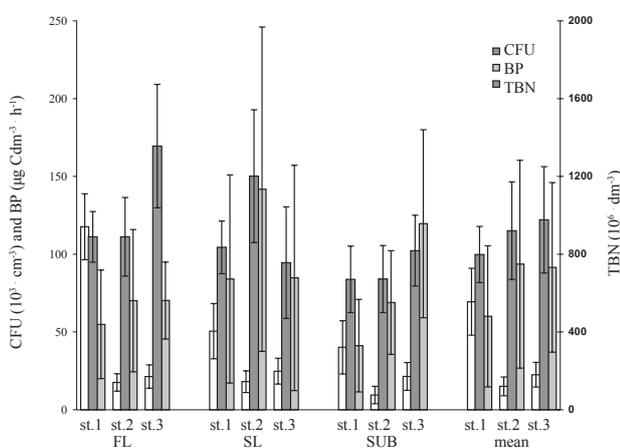


Fig. 4. Total number bacteria (TBN), heterotrophic number bacteria (CFU) and bacterial production (BP) in different water layers in lake Gardno. Vertical bars indicate standard errors.

Great accumulations of biogenic substances generate optimal conditions for the development of photo-synthesising neuston organisms. Falkowska [12] indicates that phytoneuston production in the surface microlayer is 20-40 times more than that of phytoplankton in deep water, and consequently the concentration of organic carbon, in the surface microlayer exceed that in subsurface water by 2 to 17 times [37]. Those differences result from other types of biologic activity of neuston and plankton organisms and a much faster pace of organic matter degradation in the surface layers of the water, which is firmly linked to the intensity of the transformations of nitrogen and phosphorus compounds, heavily influencing them [41].

The decline in the concentration of nitrogen and phosphorus compounds, their inorganic forms in particular occurring along the Łupawa river current through the lake, stays firmly in connection with an increase in water salinity. This is probably owing to the marine water being poorer in the biogenic substance contents mixing with the lake water, resulting in the diluted concentration of nitrogen and phosphorus concentrations. Results of earlier studies by Trojanowski et al. [43] carried out in lake Gardno seem to confirm these findings.

The results of the presented studies indicate that in lake Gardno bacteria occur more frequently in the surface microlayers than subsurface water. Similarly, the investigations carried out for other, both marine [5, 16, 32] and inland basins [10, 26, 35, 46] demonstrated that the greatest abundance of bacteria occurred in the surface layers and decreased with depth. Much accumulation of such organic compounds within the surface layer like proteins, carbohydrates and lipids, which form phyto- and zooplankton excretion because the energy of rich organic substances generate optimal conditions for the development and accumulation of aerobic bacteria, thus forming bacterioneuston [27, 38]. Besides, bacteria occurring in the surface microlayer are characterised by the presence in the external cell structures of mucopolisaccharides,

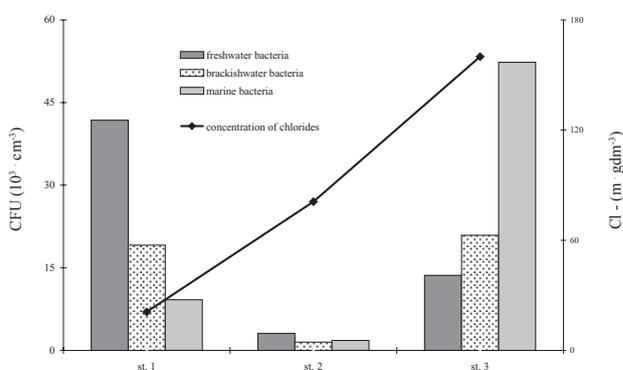


Fig. 5. Number of freshwater, brackish water, marine bacteria and concentration of chlorides in lake Gardno.

glycoproteides, phosphatoline and lecithin polymers which, being hydrophobic, show properties of active attachment to the surface layer [9,31]. At the same time, many much flagellate bacteria are able to move from deeper water parts to the surface layer by way of chemotaxis. They settle in that area by adhering to various organic particles, especially starch grain drops of lipids or cellulose fibres. Another form of bacteria transport

possible is by upwelling, i.e. being carried up from the deeper parts of the water body. At the same time, pigmentation and plasmids present in bacterioneuston cells with a coded UV resistance protect these organisms against the harmful influence of these rays [23, 29].

In lake Gardno the secondary production of bacteria was usually higher in the surface layer and subsurface water than in the film layer. This is due to the higher

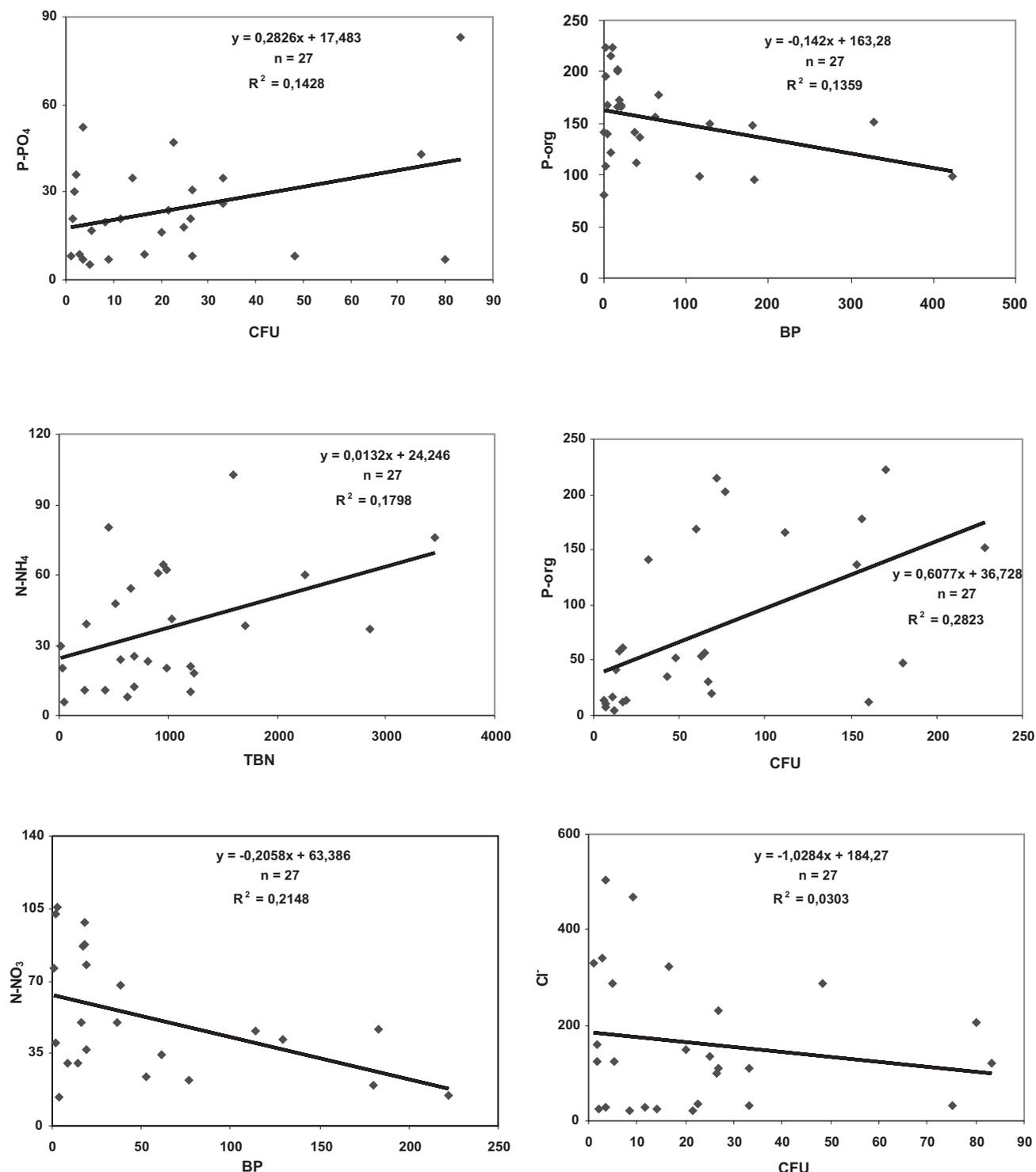


Fig. 6. Correlation between chemical and bacteriological parameters in lake Gardno. The solid line represents linear regression including all data.

metabolic activity of the individual cells of the bacteria in the surface layer and subsurface water, understood as generation time and rate of synthesis of cell proteins. Similar results were obtained by Bailey et al. [2], Hermansson and Dalhbäck [22], Donderski et al. [9], Mudryk et al. [34]. Lower secondary production by bacteria inhabiting film layer could have been caused by stressful effects of many environmental factors, mainly sun radiation, unstable salinity and temperature conditions [47]. Also, a relatively high accumulation of heavy metals, polychloride biophenols and pesticides in the film layer could have an inhibiting influence on metabolic activity of bacteria [30].

In summary, it must be concluded that the air-water interface, like the bottom-water one, is the specific site of intensive autotrophic production and heterotrophic processes of decomposition and transfer of organic resources originating from both sides of the water ecosystem. Simultaneously, the microlayers are influenced by material of terrestrial origin, which passes the ecotone between the aquatic and terrestrial ecosystems. Hence the surface microlayer represents a microenvironment rich in nutrients and organic compounds with a potential to maintain a higher bacterioneuston number and production than bacterioplankton. Thus, a study of the composition and properties of the microlayer may reveal a horizontal transport of material via lake ecotones, which up to now has not been fully recognised.

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