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Quantitative and Qualitative Composition of Heterotrophic Microflora of Underground Waters of Omulewski Aquifer

I. Gołaś

University of Warmia and Mazury, Department of Environmental Microbiology,
Prawocheńskiego 1, 10-957 Olsztyn-Kortowo, Poland

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Abstract

This work presents the results of research on quantitative and qualitative composition of heterotrophic bacteria in waters from 11 wells and 3 piezometric bore-holes in Omulewski Reservoir Aquifer before liquidation of large cattle and swine farms (in 1989-1991) and after their liquidation (1994-1995). Examinations concerning the number of indicatory heterotrophic microorganisms on TGY medium showed fluctuations in the number (from some to several thousand CFU/1 cm^3 of water) depending on the way of utilization of the soil and the research period. Gram-negative rods (44-79%) dominated in 241 water samples taken from wells and piezometric bore-holes in both research periods. Gram-positive coccus (3-32%) and cells rod-shaped (3-19%) were not so numerous. The strains: *Achromobacter, Flavobacterium* and *Xanthomonas* dominated among Gram-negative bacteria irrespective of the research period and group of wells. Gram-positive bacteria were mainly represented by: *Bacillus, Sarcina* and *Micrococcus.*

Keywords: underground waters, wells, piezometers, heterotrophic bacteria.

Introduction

Increased degradation of the natural environment caused by human activity, urbanization, chemicalization of agriculture and industry are factors which disqualify surface waters as reservoirs of drinking water. In fact, only a reservoir of underground waters can be a source of cheap and good water for consumption. In these specific water ecosystems, which seem not to be threatened by direct contact with sources of contamination, changes concerning physico-chemical properties and biologic composition have lately been observed.

The number and qualitative composition of heterotrophic microflora of underground water reservoirs are strictly connected with their geologic structure and physico-chemical properties of both water and soil environment [2, 14,16, 21]. The depth of water layers and the degree of contamination are a significant factor conditioning the quantitative occurence and percentage participation of different morphological forms [3, 13, 15, 26, 30, 39]. The number and development of this group of microorganisms reflect chemical processes taking place in these specific reservoirs [37, 46].

The research [4, 11, 12, 19, 28, 32, 39, 42] of different underground water reservoirs shows that this microflora is represented by the following bacteria: *Achromobacter, Xanthomonas, Micrococcus, Flavobacterium, Pseudomonas, Acinetobacter, Moraxella, Alcaligenes, Aeromonas, Escherichia, Klebsiella, Citrobacter, Proteus, Campylobacter, Arthrobacter, Agrobacterium, Pasteurella, Staphylococcus, Vibrio, Salmonella, Clostridium, Cytophaga, Nocardia, Bacillus, Streptococcus, Enterobacter,* and *Legionella.* They are auto- or allochtonic microflora of undergroud water reservoirs which may significantly influence their chemism as they are capable of decomposing organic matter [3, 5, 9, 17, 44, 45]. They may be useful for removing chemical contaminations as well [2]. Knowledge about qualitative and quantitative composition of this microflora has got a significant importance in planning recultivational procedures of underground water reservoirs especially in areas where there is no isolation from the surface. Omulewski Reservoir

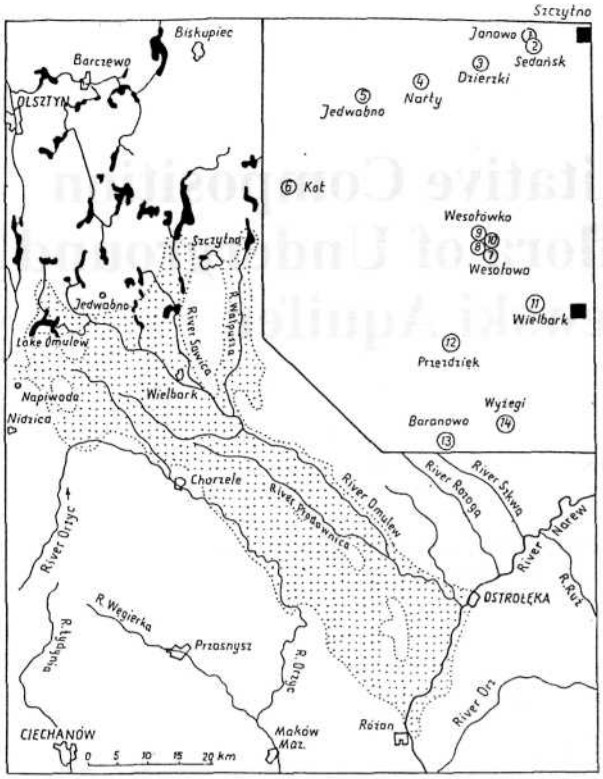

Fig. 1. Situational draft of the Omulewski Reservoir according to Szczepkowski [41]. 1, 2, 3,... 14 sampling sites (wells and piezometric bore-holes).

Aquifer is such a hydrographic unit in the Mazurian Lake District. It is a source of drinking water for the inhabitants of this region. Sanitary-bacteriological examinations of the water taken from this reservoir carried out in 1989-1997 [18, 31] showed a higher number of index bacteria of contamination (TVC 20°C, TVC 37°C) and sanitary state (TC, FC, FS) in waters of wells and piezometric bore-holes located on breeding farms in comparison to the norms recommended for water used for drinking and farm needs [35]. Many heterotrophic bacteria may have features of pathogenic bacteria similar to index bacteria of contamination degree depending on environmental conditions. This group includes microorganisms which may influence human health, though they are not described as pathogenic ones. Their occurence in larger quantities (above 500 CFU/1 cm^3 water) may cause gastric diseases [33]. Therefore, the subject of the present research comprises the estimation of quantitative and qualitative composition of heterotrophic bacteria in waters of wells and piezometric bore-holes of Omulewski Reservoir in two periods: before the liquidation of most cattle and swine farms (1989-1991) and after their liquidation (1994-1995). The present research concerns the examination of sanitary-bacteriological state [18, 31] and influence of intensification of breeding on microflora of undergroud waters of Omulewski Reservoir being the largest in the Mazurian Lake District without isolation from the surface by an impermeable layer of soil.

Material and Methods

Study Area

Omulewski Reservoir is the subject of bacteriological examinations (Fig. 1). It is the largest reservoir of undergroud waters (without isolation from the surface by an impermeable layer of soil), situated in the western part of the great "Mazurian-Kurpiowski outwash" region, in the river catchment of Omulew and Narew and partly the Orzyc river [41]. According to Szczepkowski [41] a free mirror of these waters is at a depth from 1 to several meters depending on the local morphometric situation. Maximum thickness of water layers is at 120 m and is situated in the middle part of Omulewski Reservoir (near Wielbark, Przezdziek and Baranowo). It shallows towards south-east direction. Shallow underground waters, lack of isolation from the surface point out to infiltration character of supplying this reservoir by atmospheric waters [40, 41].

The total area of this hydrogramphic unit is 820 km^2. Forests comprise 50%, arable soils 24%, grasslands 12%, the rest 14% includes lakes, rivers, and buildings [25], it is quoted according to Szczepkowski [41].

Monitoring Wells

The examination comprised undergroud waters taken from 11 wells and 3 piezometric bore-holes situated in the southern part of Mazurian Lake District with the greatest thickness of water layers [41].

Figure 1 presents the localization of particular stand, whereas their short description is given in Table 1.

The examined wells were divided into 4 groups because of differentiated management of the area and contamination:

I. the wells situated in areas completely forested, the least susceptible to contamination of antrophogenic type in Sedansk and Narty, they are called "control wells";

II. the wells situated in areas utilized by individual farmers in Jedwabno and Kot;

III. the wells situated in large farms: in Janowo (fox farm), Dzierzki and Wielbark (swine farms), in Wesotowo (cattle farm) and few hectares agricultural farms in Przezdziek, Baranowo and Wyzegi;

IV. piezometric bore-holes in Wesolowo:

- no. 1 - situated on the quarter used for straw stor age,
- no. 2 - on the area of the quarter used for running and feeding young cattle,
- no. 3 - situated outside the buildings of the cattle farm, several dozen meters from the place where mineral fertilizers were stored at the edge of a forest.

Sampling

Samples of undergroud waters of Omulewski Reservoir (from 11 wells and 3 piezometric bore-holes) for bacteriological examinations were taken in 2 research periods: before the liquidation of large cattle and swine farms (from October 1989 until December 1991) and

Table 1. Characteristics of wells and bore-holes of Omulewski Aquifer according to Kochańska [24],

No.	Locality	Utilizer	Depth of well (m)	Type of water
1.	Janowo	Fox farm (2 000)*	42.0	CO_3/Ca/Mg[1]
2.	Sedańsk	Fox farm	38.0	CO_3/Ca/Mg
3.	Dzierzki	Swine farm (2 500)	60.0	CO_3/Ca/Mg
4.	Narty	Forester's lodge	40.0	CO_3/Ca/Mg
5.	Jedwabno	Dairy	49.0	–
6.	Kot	Polythene processing plant	20.0	CO_3/Ca[2]
7.	Wesołowo	Cattle farm (700)	38.0	CO_3/Ca
8.	Wesołowo – bore-hole no. 1	Cattle farm	6.8	
9.	Wesołowo – bore-hole no. 2	Cattle farm	23.5	
10.	Wesołowo – bore-hole no. 3	Cattle farm	21.5	
11.	Wielbark	Swine farm (10 000)	60.0	CO_3/Ca/Mg
12.	Przeździęk	Cattle farm (500)	34.0	CO_3/Ca
13.	Baranowo	Cattle farm (500)	64.0	CO_3/Cl/Ca[3]
14.	Wyżegi	Cattle farm (1 000)	25.0	CO_3/Ca/SO_4[4]

* - given in brackets number of animals; [1] - carbonate-calcite-magnezite;[2] - carbonate-calcite;[3] - carbonate-chloride-calcite;[4] - carbon-ate-calcite-sulphate.

after their liguidation (from March 1994 until December 1995).

Water samples from wells in Janowo, Sedarisk, Dzierzki, Narty, Przezdziek, Baranowo and Wyzegi were taken from valves supplying water from a well bore-hole to a water supply system, whereas in Jedwabno, Kot, Wesolowo and Wielbark, the samples were taken behind a water supply system from the user's valve as there were no technical possibilities of collecting samples. Sampling for bacteriological examinations from 11 wells was preceeded by valve disinfection and pumping out water for 10 minutes. In the case of samples from 3 piezometric bore-holes every time 100 dm^3 of stagnating water was pumped out with a hand water pump.

Samples of underground waters were collected into 300 ml sterile glass flasks with corks and they were carried to a laboratory in refrigerators at 4-6°C. The time from the moment of taking the water samples to carrying out analysis did not exceed 12 h.

A total of 241 samples of underground waters from 11 wells and 3 piezometric bore-holes were taken from the above stands in both research periods.

Bacteriological Analysis

Bacteriological analysis concerning the quantitative and qualitative compositions of microflora of underground waters in 2 research periods (in 1989-1991 and 1994-1995) comprised:

1. Determinaton of the number of heterotrophic (sap rophitic) bacteria on TGY medium containing pepton, glucose and yeast extract (in dilution 1:8) after 7 days disseminations incubation at 20°C [36] by the method of agar plates.

2. Identification of grown heterotrophic bacteria. Dif ferentiated morphological colonies were split off into agar slants with the same medium and incubated at 20"C. After several passings and obtaining pure cultures they were identified according to Bergey's key [6] and schemes given by Hendrie et al. [20], LeChevallier et al. [29] and data included in Skerman's book [38].

Results

Number and Qualitative Composition of Heterotrophic Bacteria in Waters of Wells and Piezometric Bore-Holes of Omulewski Reservoir before the Liquidation of Large Cattle and Swine Farms (in 1989-1991)

Total number of heterotrophic bacteria determinated on TGY (1:8) medium ranged up to few numbers depending on the way of utilizing the soil (groups of wells). In the research period on the average the smallest numbers were found in I and II groups of wells (65-250 and 63-160 CFU/lcm^3 water), a little bit higher in III group of wells situated in large farms (140-590 CFU/lcm^3 water),

Table 2. Number and percent of viable morphological forms of heterotrophic bacteria in the water of wells and bore-holes of Omulewski Aquifer in 1989-1991.

Morphological forms	Years											
	1989				1990				1991			
	I[a] (4)[b]	II (4)	III (14)	IV (6)	I (7)	II (8)	III (28)	IV (12)	I (6)	II (8)	III (28)	IV (12)
	Number (CFU/1 cm^3)											
Gram-negative rods	45[c] 31–81[d]	36 10–75	150 21–540	748 400–1270	110 14–380	110 19–380	100 4–530	4 073 44–22 420	145 30–480	51 37–84	278 22–4 200	2 839 500–13 900
Gram-positive cells rod-shaped	6 4–14	10 0–16	15 0–100	72 40–180	10 2–22	15 0–40	11 0–40	1 005 14–9 480	40 0–110	18 10–36	70 0–1 200	1 110 50–5 100
Gram-positive cocci	9 1–26	13 1–27	44 0–190	128 20–330	27 1–90	31 4–90	25 0–130	1 570 12–8 100	56 10–170	32 15–52	189 14–3 700	1 536 190–6 900
Unidentified strains	5 0–15	3 0–8	12 0–40	72 10–230	5 0–10	4 0–11	4 0–20	1 172 0–6030	9 0–40	14 0–20	53 0–900	431 30–1 600
Total	65 40–145	63 10–130	221 26–760	1 020 560–1 800	150 25–500	160 27–380	140 8–590	7 820 70–45 000	250 40–800	115 65–185	590 33–10 000	5 196 890–27 000
	Percent											
Gram-negative rods	71	59	68	73	73	69	70	52	57	44	47	48
Gram-positive cells rod-shaped	9	15	7	7	7	9	9	13	17	16	12	19
Gram-positive cocci	13	21	20	13	18	19	18	20	22	28	32	26
Unidentified strains	7	5	5	7	2	3	3	15	4	12	9	7
Total	100	100	100	100	100	100	100	100	100	100	100	100

[a] - group of wells, [b] - number of samples,[c] - average,[d] - intervals.

the highest in piezometric bore-holes (1020-7820 CFU/lcm^3 water). The least fluctuations of the number of microorganisms were found in all groups of wells in 1989 and the highest in 1991. In all water samples taken from wells Gram-negative rods dominated, they were 44-73% of total number of determined heterotrophic bacteria. Percentage participation of Gram-positive coccus and cells rod-shaped were differentiated depending on soil utilization (groups of wells) and was 13-32% and 7-19%, respectively (Table 2).

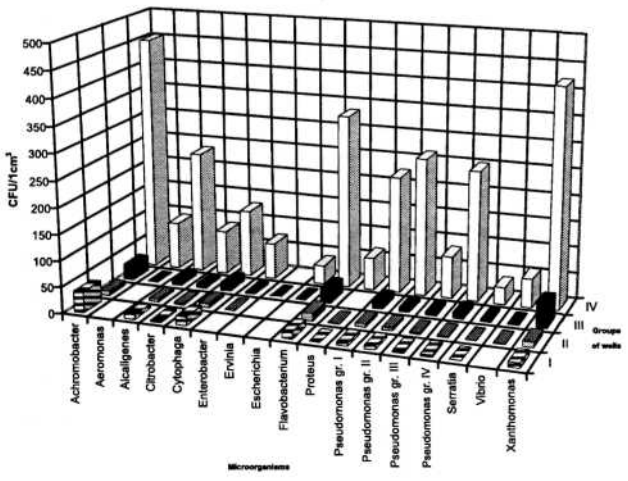

Fig. 2. The average number of Gram-negative bacteria in the underground waters of the particular groups of wells of Omulewski Aquifer in 1989-1991.

In the discussed research period 17 kinds of Gram-negative, 5 kinds of Gram-positive cells rod-shaped and 6 kinds of Gram-positive coccus were isolated and identified in the water of samples taken from wells and piezometric bore-holes of Omulewski Reservoir. Their mean number in 1 cm^3 of water of particular well groups was presented in Figs. 2-3. *Ach-romobacter* dominated in all samples during the whole research period. *Flavobacterium* and *Xanthomonas* were found in smaller quantities. The most numerous bacteria were represented by *Bacillus, Micrococcus* and *Sarcina* among Gram-positive bacteria regardless of the way of utilizing the soil (groups of wells).

Number and Qualitative Composition of Heterotrophic Bacteria in Waters of Wells and Piezometric Bore-Holes of Omulewski Reservoir after the Liquidation of Large Cattle and Swine Farms (in 1994-1995)

In the second research period total number of heterotrophic bacteria ranged from several dozen CFU/1 cm^3 up to few thousand CFU/1 cm^3 depending on area utilization, and it was lower than in the first period of research

Table 3. Number and percent of viable morphological forms of heterotrophic bacteria in the water of wells and bore-holes of Omulewski Aquifer in 1994 - 1995.

Morphological forms	Years							
	1994				1995			
	I[a] (4)[b]	II (8)	III (28)	IV (12)	I (4)	II (8)	III (28)	IV (12)
	Number (CFU/1 cm³)							
Gram-negative rods	158[c] 81–230 d	640 37–3 100	199 7–890	1 052 430–2180	146 63–300	287 24–1 630	183 24–630	1 246 220–2 050
Gram-positive cells rod-shaped	27 0–50	37 0–220	19 0–290	88 0–300	12 0–32	81 0–570	8 0–56	90 0–360
Gram-positive cocci	6 0–10	121 0–600	19 0–220	122 0–810	44 6–80	76 0–470	61 0–410	220 0–630
Unidentified strains	9 4–20	92 4–350	13 0–90	328 30–520	8 0–15	16 0–130	3 0–60	104 20–300
Total	200 105–310	890 38–4 000	250 8–1 200	1 590 480–5 000	210 95–380	460 27–2 800	255 38–310	1 660 420–3 100
	Percent							
Gram-negative rods	79	72	78	66	67	70	72	75
Gram-positive cells rod-shaped	13	4	8	6	8	6	3	6
Gram-positive cocci	3	14	8	8	18	21	24	13
Unidentified strains	5	10	6	20	7	3	1	6
Total	100	100	100	100	100	100	100	100

[a] - group of wells, [b] - number of samples,[c] - average, [d] - intervals.

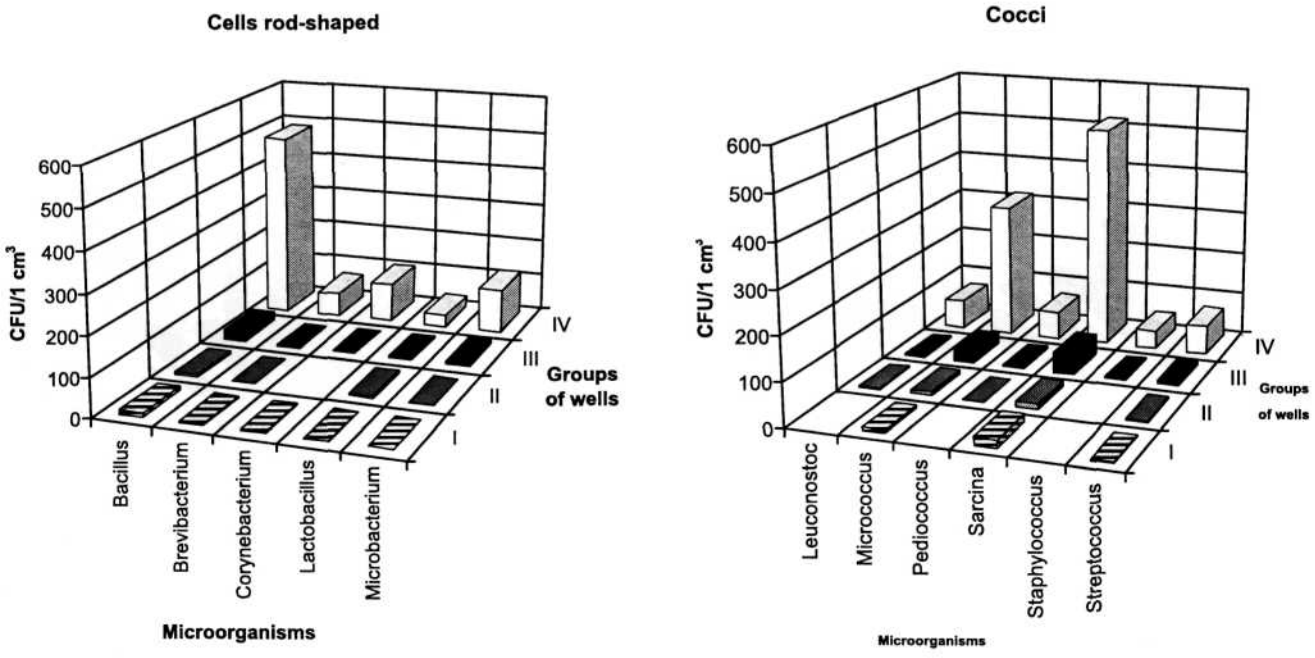

Fig. 3. The average number of Gram-positive bacteria (cells rod-shaped and cocci) in the underground waters of the particular groups of wells of Omulewski Aquifer in 1989-1991.

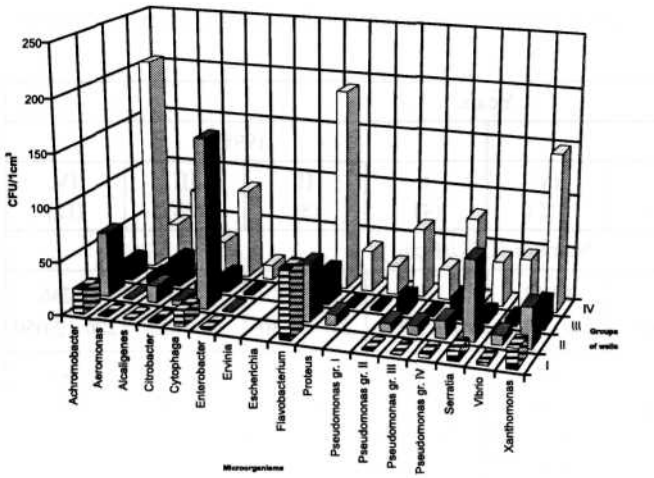

Fig. 4. The average number of Gram-negative bacteria in the underground waters of the particular groups of wells of Omulewski Aquifer in 1994-1995.

only in piezometric bore-holes (IV group of wells). The lowest number was found in I group of wells water samples (200-210 CFU/1 cm^3 water), a little higher one in II and III groups of wells (460-890 and 250-255 CFU/1 cm^3 water, respectively), and the highest one in IV group of wells (1590-1660 CFU/1 cm^3 water). Gram-negative rods dominated in the water of all groups of wells and they constituted 66-79% of microorganisms. Gram-positive coccus and cells rod-shaped were 3-24% and 3-13% of total heterotrophic bacteria (Table 3).

Strains *Flavobacterium* and *Achromobacter* dominated among identified Gram-negative rods. *Serratia, Vibrio* and *Xanthomonas* (Fig. 4) were found in smaller quantities (generally in all water samples). Gram-positive cells rod-shaped were represented mainly by *Bacillus. Brevibacterium* and *Lactobacillus* were found in the samples taken from piezometric bore-holes (IV group), whereas *Microbacterium* were in the water samples taken from control wells (I group) and wells situated on the areas utilized by individual farmers (II group). *Micrococcus* and *Sarcina* dominated in waters I, HI and IV wells group and *Staphylococcus* in the samples II group (Fig. 5).

Discussion

The number of heterotrophic bacteria in wells and piezometric bore-holes of Omulewski Reservoir fluctuated less (in 1994-1995) or more (in 1989-1991) depending on area utilization (wells group) and research period (level of agriculture and breeding). Generally it was similar to the number given in literature [3, 22, 27, 39, 43] for water formations in Germany and the USA. The liquidation of large cattle and swine farms and fox farms in 1992 and 1993 (except the swine farm in Wielbark) did not cause a decrease in the number of the examined bacteria. Significant changes in the number of heterotrophic bacteria were observed only in water taken

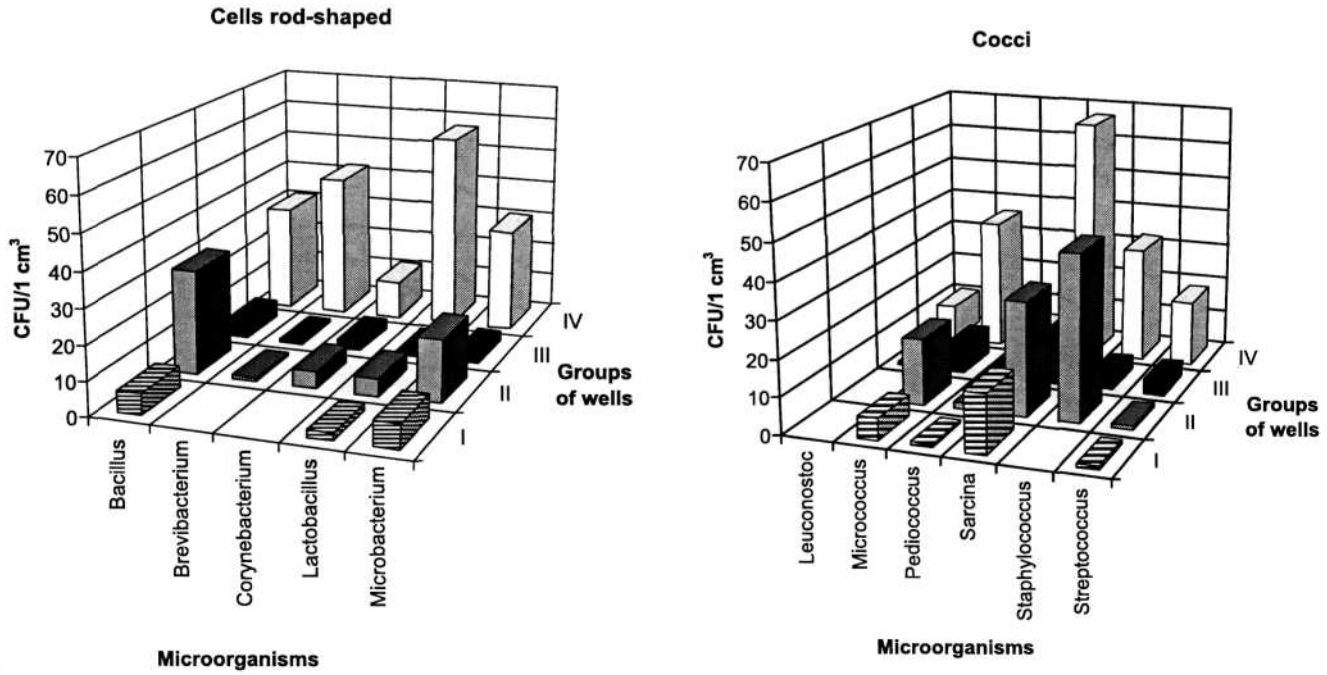

Fig. 5. The average number of Gram-positive bacteria (cells rod-shaped and cocci) in the underground waters of the particular groups of wells of Omulewski Aquifer in 1994-1995.

from shallow piezometric bore-holes in Wesolowo (IV group) situated on the run and feeding place of young cattle which had the highest bacteriological contamination [18, 31]. According to the data presented by Niewolak [31] in 1989-1993 the number of index bacteria contamination (TVC 20°C and TVC 37°C) and sanitary state (TC, FC, FS) in the water of pizometric bore-holes were: 10-122500 and 0-62800 CFU/1 cm^3 and < 1-200, < 1-22 and < 1-2400 MPN/ 100 cm^3, respectively. The results of the above bacteria index of the degree of contamination and sanitary state in these waters comprising the years 1995-1997 showed: 8-21550, 0-5050 CFU / 1 cm^3 and < 1-18, < 1-3 and < 1-2400 MPN/ 100 cm^3, respectively [18]. The number of heterotrophic bacteria in 1994-1995 decreased 3-4 times in the water of this wells group. A significant decrease of these bacteria was observed in Gram-positive group. They were still much higher quantities than in control wells (I group) ranged as [24, 25, 31] typical for unpolluted waters. Higher number of heterotrophic bacteria in waters II group of wells in 1994-1995 and III group in 1989-1991 and in piezometric bore-holes (IV wells group) might be caused by leakage of non-used mineral fertilizers and wastes from large cattle and swine farms where there was litter. In both research periods the differences in the quantities of examined heterotrophic bacteria were caused by the way of utilizing the area situated under the water layers and different chemical composition of particular well [25] connected with different degree of contamination.

In both research periods (before and after liquidation of cattle and swine farms) Gram-negative rods dominated in the water samples taken from wells and piezometric bore-holes of Omulewski Reservoir. They were 44-79% of all determined heterotrophic bacteria. Gram-positive coccus was found in fewer quantities (3-32%). Percentage participation of Gram-positive cells rod-shaped among all the isolated and identified strains was only 3-19% and was significantly lower in 1994-1995 than in 1989-1991. The examinations of underground water reservoirs in Germany and USA [1, 26, 39] showed a domination of Gram-negative bacteria which were found in large quantities because of sandy structure of the bed [10]. Gram-positive bacteria [21, 27], dominated in underground water reservoirs with clay beds as they were more resistant to unfavourable environmental conditions [7].

Achromobacter, Flavobacterium and *Xanthomonas* dominated among isolated and identified Gram-negative rods regardless of the research period (before or after liquidation of most cattle and swine farms) and area utilization (well groups) in underground waters of Omulewski Reservoir. *Cytophaga* and *Alcaligenes* were in smaller quantities. *Bacillus, Micrococcus* and *Sarcina* were the most common and numerous among Gram-positive bacteria. In both research periods the least differentiation as far as qualitative composition of isolated and identified heterotrophic bacteria is concerned showed water samples of control wells situated on forested areas (I group), the highest differentiation was observed in the waters of wells located on breeding farms (III group) and waters from piezometric bore-holes in Weso3owo (IV group) as they were the shallowest and the most endangered by the migration of contaminations.

In literature the differentiation of qualitative composition of heterotrophic bacteria of underground water reservoirs is explained [3, 8, 19, 34] by differences of structure and depth of reservoirs of underground waters, physico-chemical properties of water layers and possibilities of wastes leakage.These factors can increase the growth and develompent of particular kinds and groups of heterotrophic bacteria which constitute microflora of these specific water ecosystems [11, 23].

Conclusions

1. Differences in quantitative and qualitative composi tion of heterotrophic microflora of Omulewski Reservoir depend on the depth of underground waters and the way of utilizing the area and wastes penetration into water layers.

2. In both research periods higher numbers of hetero trophic bacteria correspond to increased numbers of in dex bacteria of contamination and sanitary state degrees especially in shalow waters of piezometric bore-holes on the area of breeding farms.

3*Achromobacter, Flavobacterium* and *Xanthomonas* of Gram-negative rods dominated regardless the level of breeding intensification (research period) and the way of utilizing the area (wells groups) in the waters of wells and piezometric bore-holes. Gram-positive bacteria were represented by *Bacillus, Micrococcus* and *Sarcina.*

4. Liquidation of breeding farms decreased the number of isolated and identified kinds of heterotrophic bacteria but only in the case of piezometric bore-holes.

Acknowledgement

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