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Transmission of Specific Groups of Bacteria through Water Distribution System

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Abstract

Microbial contamination of a water distribution system was examined. The number and the taxonomy of non-pigmented and pigmented heterotrophic bacteria (HB), number of bacteria (*Pseudomonas* sp., *Enterococcus* sp., *Campylobacter* sp., *Yersinia* sp., representatives of the *Enterobacteriaceae*, coagulase-positive staphylococci, and *C. perfringens*) in the bulk water phase, biomass of zoogloeal aggregates of bacteria, fungi, algae, protozoa and rotifers (ZABFAPR) (separated from the above on 5 μm pore size filters) and in pipe sediments was determined. An increased number of HB occurred at the sampling sites situated as close as 4.2 km to the Water Treatment Plant (WTP), and was especially significant at 10.3 km. It was shown that the main reservoir of hygienically relevant bacteria did not occur in the water phase which is monitored in routine control analyses carried out by the WTP laboratories, but in the ZABFAPR biomass not monitored so far.

Key words: *Campylobacter* sp., *Yersinia* sp., bacterial regrowth, opportunistic pathogenic bacteria, water distribution system

Introduction

Risk assessment of the transmission of potentially pathogenic microorganisms in drinking water distribution systems has been investigated with special commitment by epidemiologists and sanitary engineers over the last few years. This interest is prompted by the number of epidemic diseases of the digestive tract caused by unidentified microorganisms, greatly exceeding that caused by typical pathogenic microorganisms such as *Salmonella* sp., *Shigella* sp., *Vibrio* sp., (Anderson *et al.*, 1997) as well as the recent rapid increase in the population of the immunosuppressed particularly susceptible to infection. Those include, for instance, cancer and AIDS patients undergoing chemotherapy, individuals suffering from diabetes and people with various implants. Numerous bacterial, cyanobacterial and fungal species described as opportunistic microorganisms are causative agents of these infections. Their role in transmitting epidemic diseases through water distribution systems was described by Grabińska-Łoniewska (2005).

As the review of literature on the subject provided in it shows, the scope of relevant examinations conducted so far does not include the influence of the quality of intake waters delivered to the Water Treatment Plant (WTP), treatment effectiveness, distance from the WTP and the number of microorganisms occurring both in the bulk water phase as well as in the biomass of zoogloeal aggregates of bacteria, fungi, algae, protozoa and rotifers (ZABFAPR) "suspended" in it, in the pipe wall biofilm and in pipe sediments on the contamination degree of water distribution systems with potentially pathogenic microorganisms.

Therefore, the aim of this study was to determine the occurrence of selected groups of pathogenic and opportunistic pathogenic bacteria in intake waters and after various stages of their treatment as well as in sections of the water distribution system located at different distances from the WTP, in samples collected from the water phase, the biomass of ZABFAPR and pipe sediments.

For the purposes of the study, numbers of pigmented and non-pigmented heterotrophic bacteria (HB),

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bacteria of the genus *Pseudomonas* growing at 7°C, 30°C and 42°C, bacteria of the family *Enterobacteriaceae*, of the genera *Yersinia* and *Campylobacter*, faecal streptococci, *Enterococcus* sp. coagulase-positive staphylococci, *C. perfringens* were determined in the samples. The quantitative composition of ZABFAPR was determined in the biomass separated from the water phase on 5 μm pore size filters.

Experimental

Material and Methods

Collection of samples. Water samples were collected between December (2000) and November (2002) from different sites of municipal water supply of the city of Warsaw. These included intake waters supplying the Water Treatment Plant (WTP), water treated in technological lines I and II of WTP, a mixture of the above waters, disinfected with a mixture of ClO_2 and Cl_2 – transferred into the distribution system and waters from 7 sites within the distribution system located on 4.2; 4.3; 5.4; 5.7; 8,7; 10.0 and 10.3 km from the WTP. Description of water treatment in technological lines I and II and methods of sample collection are given elsewhere (Grabińska-Łoniewska *et. al.*, 2007).

Samples preparation. Water samples freed from chlorine (using 10% solution of sodium thiosulphate) were used for examinations. The biomass of ZABFAPR was separated from 20 l water samples collected from the system using the filtration method through a cellulose nitrate membrane filter (Whatman Schleider and Schuell), pore size 5 μm, with a vacuum pump (Merck ME 2). The biomass retained on the filter was eluted for 30 min in a shaker (Elpan, type 357), with the amplitude 6 and 200 cpm/min, to 50 ml of 0.28% solution of sodium pyrophosphate. The prepared suspension was used for microscopic examinations. The suspension (as above), disrupted in an ultrasound disintegrator UD 20, vibration amplitude A = 20 μm for 40 s, was used for microbiological examinations. Using this method of sample preparation, the obtained suspension contained both microorganisms occurring inside zoogloeal aggregates of bacteria, as well as inside and on the surface of cells of algae and protozoa colonising the water phase in the water distribution system.

Samples from which the biomass of ZABFAPR was removed using the method described above were used to prepare the suspension of microorganisms collected from the water phase in the water distribution system. They were concentrated using the filtration method (as above) through a membrane filter, pore size 0.22 μm (*Campylobacter* sp.) and 0.45 μm (other bacteria). Depending on degree of water contamination, filtration was performed from: 5.0; 1.0; 0.5; 0.1 litre as well as 10 and 1 ml. The filter was then placed in 10 ml of a 0.28% solution of sodium pyrophosphate and shaken for 30 min in a shaker (as above) to wash the microorganisms off the filter. The suspension prepared was used to inoculate culture media.

Sediments were scraped from 62.8–80 cm^2 of the inner surface of sample sections, weighed, ground in a mortar, suspended in 0.28% solution of sodium pyrophosphate and shaken in shaker (as above) to elute microorganisms from the mineral fraction of the sediments. Non-concentrated samples were used for inoculation.

Microbiological determinations. The number of pigmented and non-pigmented heterotrophic bacteria (HB) was determined in culture on broth agar medium (MPA) at 26°C after 7 days of incubation. Bacteria of the genus *Pseudomonas* were incubated on King B medium at 7°C for 10 days, 30°C – 4 days and 42°C – 2 days. VRBG agar medium was used to enumerate bacteria of the family *Enterobacteriaceae*, and the Enteroplast – EPL 21 test (temp. 37°C) was used for confirmatory re-examination (Burbianka *et al*., 1983). *Yersinia* sp. bacteria were first enumerated on Endo MLCe agar medium after 2–5 days of incubation at 30°C. Colonies characterised by typical morphology (round, 1–2 mm in diameter, dark red), non-cytochrome oxidase producing were re-examined for confirmation using Enteroplast EPL 21 (Krogulska and Maleszewska, 1992).

The occurrence of *Campylobacter* sp. was initially determined on Oxoid Brucella liquid enrichment medium supplemented with compounds lowering E_h and after incubation at 37°C for 4 h, also with a mixture of antibiotics. After 20 h of incubation at 42°C, the culture was transferred to Oxoid Brucella agar supplemented with 5% of blood and incubated for 2 days at 42°C in anoxic conditions. Characteristic colonies (pink point, beige coloured) were re-examined for confirmation using the PCR method (Or and Jordan, 2003).

The density of faecal streptococci was determined according to PN-82/C-04615/25, coagulase-positive staphylococci – PN-75/A-04024, *C. perfringens* – PN-77/C-04615. The determination (*Enterococcus* sp.) results of the number of HB, bacteria of the genus *Pseudomonas* growing at 7, 30 and 42°C, bacteria of the family *Enterobacteriaceae*, bacteria of the genera *Yersinia* and *Enterococcus* sp. are reported as cfu/l, cogulase-positive staphylococci as MPN per 100 ml, and bacteria of the genus *Campylobacter* as a titre.

The biomass of ZABFAPR of the water distribution system was observed under a phase contrast microscope (Opton). Determinations results are reported as the density of individual groups of organisms expressed per litre of the sample.

Microbiological examinations of pipe sediments collected from the inner surface of sample sections of water distribution pipes were conducted at the same time. Their scope was as above; however, the density of bacteria of the family *Enterobacteriaceae* and *Cl. perfringens* was not determined due to technical reasons. The results are reported as cfu/100 g.

Results and Discussion

The examinations conducted show different degrees of microbiological contamination of waters supplying the WTP. Infiltration waters supplying technological line I were characterised by low density of non-pigmented and pigmented HB, bacteria *Pseudomonas* sp. growing at 7°C, 30°C and 42°C (11.7×10^4; 2.8×10^4; 350; 13.8×10^3 and 86 cfu/l, respectively) and *C. perfringens* (33 cfu/l). Bacteria of the family *Enterobacteriaceae*, *Yersinia* sp., *Campylobacter* sp. and *Enterococcus* sp. were not found, and the MPN of coagulase-positive staphylococci was <0.3 (Table I). Taxonomic examinations showed that *Pseudomonas pseudomallei* and *Ochrobactrum anthropi* constituted non-pigment-producing bacteria, *Pseudomonas mendocina* and *Micrococcus varians* – pigment-producing bacteria, and *Ochrobactrum anthropi* and *Stentrophomonas maltophilia* – pigmented varieties (Table II). The river open sedimentation basin supplying water for technological line II showed high contamination with non-pigmented and pigmented HB, bacteria *Pseudomonas* sp. growing at 7°C, 30°C and 42°C, bacteria of the family *Enterobacteriaceae* and coagulase-positive streptococci (99×10^6; 0.8×10^6; 84.5×10^6; 74; 67×10^6; 1.6×10^6; 1.2×10^6 cfu/l and MPN = 960, respectively). The presence of *Campylobacter* sp. was also recorded in the basin (titre 10). *P. aureofaciens*, *Flavimonas oryzihabitans*, *Chryseomonas luteola*, *Photobacterium damsela*, *Listonella damsela* and a non-pigmented variety of *Micrococcus varians* constituted the microflora of non-pigment-producing bacteria. Three species of opportunistic pathogens to humans and warm-blooded animals, including *P. fluorescens* and *P. putida*, were identified in the group of pigmented HB. Strong microbiological contamination of this reservoir should be associated with the presence of numerous waterfowl in it.

As the findings given in Table I show, a significant reduction in the density of HB occurred in the infiltration water treatment process in technological line I (aeration and filtration) while the density of bacteria *Pseudomonas* sp. and coagulase-positive staphylococci increased. Second technological line, comprising such unit processes as sorption, coagulation

Table I
Quantity of different groups of bacteria in intake river waters, water after different treatment stages in WTP and pomped to water supply net (mean values)

Determination			I technological line		II technological line		Pure water pomped to water supply net
			Infiltration river intake	After different stages of treatment (range)	River sedimentation basin	After different stages of treatment (range)	
Total number of cfu, temperature of incubation 26°C (cfu/l)		pigmented	2.8×10^4	$88.4 \times 10^2 – 214 \times 10^3$	0.8×10^6	$7 \times 10^3 – 21 \times 10^3$	8.8×10^3
		non-pigmented	11.7×10^4	$4.6 \times 10^3 – 163 \times 10^3$	99×10^6	$9.5 \times 10^3 – 134 \times 10^3$	2.2×10^3
		total	13.3×10^4	$34.6 \times 10^3 – 253 \times 10^3$	99.8×10^6	$16.5 \times 10^3 – 155 \times 10^3$	11×10^3
Pseudomonas sp. (cfu/l) temperature of incubation	7°C	fluorescing	25	nf – 273	2.8×10^6	300 – 1470	nf
		non-fluorescing	300	293 – 2130	81.7×10^6	$4.2 \times 10^3 – 8.4 \times 10^3$	nf
		total	350	310 – 2340	84.5×10^6	$4.5 \times 10^3 – 9.5 \times 10^3$	nf
	30°C	fluorescing	nf	nf – 486	2.7×10^6	1200 – 1330	nf
		non-fluorescing	13.8×10^3	41.2×10^3—207×10^3	72×10^6	$34.2 \times 10^3 – 100 \times 10^3$	13.6×10^3
		total	13.8×10^3	$41.2 \times 10^3 – 207 \times 10^3$	74.6×10^6	$35.4 \times 10^3 – 101.5 \times 10^3$	13.6×10^3
	42°C	fluorescing	nf	nf	nf	nf	nf
		non-fluorescing	86	256 – 2870	1.6×10^6	296 – 1600	nf
		total	86	256 – 2870	1.6×10^6	296 – 1600	nf
Total *Enterobacteriaceae* (cfu/l)			nf	nf	1.2×10^6	nf	nf
Yersinia sp. (cfu/l)			nf	nf	nf	nf	nf
Campylobacter sp. (titre)			0	0	10	0	nf
Enterococcus sp. (cfu/l)			nf	nf	nf	nf	nf
Coagulase-positive staphylococci (MPN)			<0.3	1 – 8	960	0.3 – 2	nf
Clostridium perfringens (cfu/l)			33	nf	nf	nf	nf

Abbrevations: nf – not found

Table II
Comparison of the selected bacteria species occurring in intake river water, water treated in WTP and pomped to water distribution system

Sampling location		Group of bacteria	
		non pigmented bacteria	pigmented bacteria
I technological line	infiltration intake of river water	*Pseudomonas pseudomallei*	*Pseudomonas mendocina*
		Ochrobactrum anthropi	*Ochrobactrum anthropi*
			Micrococcus varians
			Stentrophomonas maltophilia
	after different stages of treatment	*Pseudomonas pseudomallei*	*Micrococcus varians/roseus*
		Aeromonas hydrophila	*Micrococcus varians*
		Ochrobactrum anthropi	*Stentrophomonas maltophilia*
		Pantoea ssp. 4	
II technological line	river water sedimentation basin	*Pseudomonas aureofaciens*	*Pseudomonas fluorescens*
		Flavimonas oryzihabitans	*Pseudomonas putida*
		Chryseomonas luteola	*Flavimonas oryzihabitans*
		Photobacterium damsela	
		Listonella damsela	
		Acinetobacter baumannii/calcoaceticus	
		Micrococcus varians	
	after different stages of treatment	*Listonella damsela*	*Pseudomonas putida*
		Ochrobactrum anthropi	*Flavimonas oryzihabitans*
		Chryseomonas luteola	
Pure water pomped to water distribution system		*Pseudomonas pseudomallei*	*Pseudomonas putida*
		Photobacterium damsela	*Flavimonas oryzihabitans*

and filtration, was characterised by a high effectiveness of removal of all the groups of bacteria examined even though the quality of the water supplied to it was much lower. Consequently, the water pumped to the water distribution system, being a mixture of waters treated in I and II technological lines, disinfected with a mixture of ClO_2 and Cl_2, met the microbiologically relevant recommendations specified in the legal regulation in force in Poland (Regulation of the Minister of Health of Nov. 19, 2002, Item 1718).

The examinations of the occurrence of HB the water distribution system showed that their quantity in the water phase increased together with the distance from the WTP. Similar observations were made by Payment e*t al.* (1988), Gibbs *et al.* (1993) and Hirata *et al.* (1993). The increase was particularly significant in the sites located 10.0 and 10.3 km from WTP. An increased number of non-fluorescing *Pseudomonas* spp. growing in 7, 30 and 42°C on the sampling site located 10.3 km from WTP was also determined (Table III). Studies by Gibbs *et al.* (1993) have shown that HB counts increased through the distribution system until 30–40 hour period of water retention in the system, and consequently became stable. The number of HB (26°C) in the system studied by us was 10–100 fold lower than found by Payment *et al*. (1988) (20°C – 10^6–10^7 cfu/l). It was probably caused by the usage by these authors more complex agar medium (R2A-Difco 1825-17-1) for enumeration of HB. According to Norton and Chevallier (2000) this medium favoured the growth of the water distribution system bacteria belonging to the genera *Acinetobacter*, *Alcaligenes*, *Pseudomonas*, *Klebsiella* and *Hydrogenophaga*, whereas after Martiny *et al.* (2005) from the genera *Brevundimonas*, *Hydrogenophaga*, *Aquabacterium*, *Luteimonas*, *Legionella*, *Methylomonas*, *Pseudomonas*, *Thiobacillus*, *Acidobacterium* and *Planctomyces*.

Our studies have shown occurrence of small quantities of bacteria of the family *Enterobacteriaceae* at some sampling sites of the distribution system. However, bacteria of the genera *Yersinia*, *Campylobacter* and *Enterobacter*, *C. perfringens* and coagulase-positive staphylococci were not detected (Table III). These findings are similar to the conclusions of Payment *et al.* (1988) who found that the water distribution system in Canada was free of total and faecal coliforms, *Aeromonas hydrophila*, *Enterococci*, *Pseudomonas aeruginosa* and *C. perfringens*.

Biodiversity of bacterial species also increased together with the distance from WTP. Two species of non-pigmented HB were found in the pure water pumped to the water supply system, *i.e. P. pseudomallei* and *Photobacterium damsela*, and 2 species of pigmented HB: *P. putida* and *Flavimonas oryzihabitans*, while

Table III
Quantity of different groups of bacteria in water and biomass of ZABFAPR separated from it on 5 μm pore size filtres in water distribution net depending on the distance from WTP (mean values)

Determination			Distance from WTP									
			4.2 km		5.4 km		5.7 km		8.7 km		10.3 km	
			water	biomass	water	biomass	water	biomass	water	biomass	water	biomass
Total number of cfu, temperature of incubation 26°C (cfu/l)		pigmented	14.1×10^3	14.1×10^3	5.3×10^3	4.7×10^3	35.3×10^3	87.9×10^3	15.3×10^3	1.9×10^3	10.9×10^3	5×10^3
		non-pigmented	60.4×10^3	71.8×10^3	6.3×10^3	12.4×10^3	5.2×10^3	8.9×10^3	22.5×10^3	15.2×10^3	109.1×10^3	7.6×10^3
		total	74.5×10^3	85.9×10^3	11.6×10^3	17.1×10^3	40.5×10^3	96.8×10^3	37.8×10^3	17.1×10^3	120×10^3	12.6×10^3
Pseudomonas sp. (cfu/l) temperature of incubation	7°C	fluorescing	nf	nf	nf	nf	nf	nf	nf	nf	nf	nf
		non-fluorescing	nf	2.7×10^3	1.7×10^3	3.5×10^3	2.2×10^3	10.2×10^3	4.1×10^3	4.3×10^3	19.4×10^3	13.3×10^3
		total	nf	2.7×10^3	1.7×10^3	3.5×10^3	2.2×10^3	10.2×10^3	4.1×10^3	4.3×10^3	19.4×10^3	13.3×10^3
	30°C	fluorescing	nf	nf	nf	nf	nf	nf	nf	nf	3.3×10^3 *Ps. aeruginosa*	nf
		non-fluorescing	35.2×10^3	55.1×10^3	11.2×10^3	51.7×10^3	41.1×10^3	100.9×10^3	29.5×10^3	$12 \cdot 10^3$	102.3×10^3	14.3×10^3
		total	35.2×10^3	55.1×10^3	11.2×10^3	51.7×10^3	41.1×10^3	100.9×10^3	29.5×10^3	$12 \cdot 10^3$	102.3×10^3	14.3×10^3
	42°C	fluorescing	nf	nf	nf	nf	nf	nf	nf	nf	nf	nf
		non-fluorescing	nf	4 800	457	3 980	100	500	13 400	3 200	2 000	1 000
		total	nf	4 800	457	3 980	100	500	13 400	3 200	2 000	1 000
Total *Enterobacteriaceae* (cfu/l)			nf	2175	320	1050	110	3300	4 790	nf	nf	nf
Yersinia sp. (cfu/l)			nf	nf	nf	nf	nf	nf	nf	nf	nf	nf
Campylobacter sp. (titre)			nf	nf	nf	nf	nf	nf	nf	nf	nf	nf
Enterococcus sp. (cfu/l)			nf	nf	nf	nf	nf	nf	nf	nf	nf	nf
Coagulase-positive staphylococci (MPN)			nf	6	nf	200	nf	2	nf	nf	nf	nf
Clostridium perfringens (cfu/l)			nf	nf	nf	nf	nf	nf	nf	nf	nf	nf

Abbrevations: nf – not found

Table IV
Species of selected bacteria occurring in water and biomass separated from it on 5 µm pore filtres in the water leaving WTP and in distribution system

Location	Water		Biomass	
	non-pigmented bacteria	pigmented bacteria	non-pigmented bacteria	pigmented bacteria
Pure water pomped to water distribution net	*Pseudomonas pseudomallei*	*Pseudomonas putida*		
	Photobacterium damsela	*Flavimonas oryzihabitans*	–	–
Water distribution net (4.2–10.3 km from WTP)	*Pseudomonas pseudomallei*	*Pseudomonas pickettii*	*Pseudomonas pseudomallei*	*Pseudomonas fluorescens*
	Pseudomonas stutzeri	*Enterobacter sakazakii*	*Pseudomonas stutzeri*	*Pseudomonas aeruginosa*
	Aeromonas hydrophila	*Aeromonas sorbia*	*Comanzonas testosteroni*	*Pseudomonas putida*
	Acinetobacter baumanii/calcoaceticus	*Myroides/Chryseomonas indologenes*	*Sphingobacterium paucimobilis*	*Aeromonas sorbia*
	Photobacterium damsela	*Stentrophomonas maltophilia*	*Rahnella aquatilis*	*Stentrophomonas maltophilia*
	Pasteurella aerogenes	*Micrococcus varians*	*Micrococcus varians*	*Micrococcus varians*
	Micrococcus sp.	*Micrococcus varians/roseus*	*Flavimonas oryzihabitans*	*Micrococcus sp.*
	Flavimonas oryzihabitans	*Flavimonas oryzihabitans*		*Flavimonas oryzihabitans*
		Ochrobactrum anthropi		*Ochrobactrum anthropi*

8 species of non-pigmented HB and 9 species of pigmented HB were recorded in the water of the distribution system (Table IV). *P. stutzeri* and *Flavimonas oryzihabitans* were found to be characteristic of water in the distribution system in the group of non-pigmented HB. Their pathogenicity has not been fully explored yet. *P. stutzeri* is regarded to be a saprophytic organism but it was also isolated from clinical material. Non-pigmented varieties of *Micrococcus* sp., *P. pseudomallei*, *Acinetobacter baumannii*/*calcoaceticus* and *Photobacterium damsela* should be considered as accompanying organisms, occurring in water periodically. The occurrence of bacteria of the genus *Pseudomonas*, *Aeromonas* (*A. hydrophila*/*caviae*), *Acinetobacter calcoaceticus*/*baumanii*, *Micrococcus* sp. and *Xanthomonas* sp. in water distribution systems in South Africa was demonstrated by Pavlov *et al.* (2001).

Micrococcus varians was a species of pigmented bacteria characteristic of the water phase in the distribution system studied. Non-pigmented varieties of *Flavimonas oryzihabitans*, *P. pickettii*, *Myroides*/*Chryseomonas indologenes*, *Aeromonas sorbia*, *Enterobacter sakazakii*, *Stentrophomonas maltophilia* and *Ochrobactrum anthropi* can be considered to be accompanying bacteria, occurring periodically. *M. varians* is widespread in aquatic and soil biotopes; it also occurs on the skin in humans. Some strains may have features of opportunistic pathogens. *Ochrobactrum anthropi* is phylogenetically close to the genera *Achromobacter* and *Alcaligenes*, which are saprophytes common in water and soil. The occurrence of these bacteria in the digestive tract of vertebrates and in clinical material (blood, urine, faeces) may provide sufficient grounds to consider it as having the features of opportunistic pathogens.

Our studies have expanded the existing knowledge on bacterial species occurring in water distribution systems. Lahti (1993) found only *Aeromonas* sp., *Serratia fonticola*, *Enterobacter aerogenes, Rahnella aquatilis* and *Moellerella wisconsensis* in the water phase in a distribution system in Finland. Pavlov *et al*. (2001) recorded the occurrence of bacteria belonging to the genera *Aeromonas*, *Acinetobacter*, *Aureobacterium*, *Bacillus*, *Klebsiella*, *Moraxella*, *Pseudomonas*, *Staphylococcus, Tsukamurella* and *Vibrio* in water in a distribution system in South Africa. Rusin *et al*. (1997) identified 10 genera of bacteria (*Pseudomonas*, *Flavobacterium*, *Corynebacterium*, *Nocardia*, *Mycobacterium*, *Erwinia*, *Enterobacter*, *Serratia, Micrococcus* and *Xanthomonas*), and Hirata *et al*. (1993) – 5 genera (*Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Bacillus* and *Methylobacterium*), but did not determine the species to which they belonged.

The degree of microbiological contamination of water distribution systems is defined not only by the density of micro-organisms in the water phase that is

Table V
Composition of biomass of ZABFAPR separated from water on 5 μm pore size filtres on the different sections of water distribution system made of cast iron.

Distance from WTP	Biomass composition, number in liter		
4.2 km	zoogloeal aggregates of bacteria	13 000	plant scraps 3000 mineral contaminations 54 000
	fungal spores	1 000	
	Chlorella sp. dead	19 000	
	Euglena viridis dead	2 000	
	Lionotus sp. dead	2 000	
	Protozoa nd. autolized	3 000	
5.4 km	zoogloeal aggregates of bacteria	53 000	plant scraps 3000 mineral contaminations 20 000
	filamentous bacteria	1 000	
	fungal hyphae	1 000	
	Chlorella sp. dead	7 000	
	Melosira sp. dead	1 000	
	Cyanophyta nd dead	1 000	
	Mastigota nd. dead	2 000	
	Protozoa nd. dead	2 067	
5.7 km	zoogloeal aggregates of bacteria	7 000	plant scraps 3000 mineral contaminations 16 000
	fungal spores	4 000	
	Chlorella sp. dead	2 000	
8.7 km	zoogloeal aggregates of bacteria	27 000	plant scraps 1000 mineral contaminations 40 000
	fungal spores	7 000	
	Chlorella sp. dead	2 000	
	Protozoa nd. dead	333	
10.0 km	zoogloeal aggregates of bacteria	62 800	plant scraps 4000 mineral contaminations 60 900
	fungal hyphae	330	
	fungal spores	9 000	
	Chlorella sp. dead	17 300	
	Melosira sp. dead	670	
	Tabelaria sp. dead	3 000	
	Nitschia sp. dead	600	
	Amoeba nd. dead	330	
	Euglena nd.	2 000	
	Rotatoria nd.	4 000	

monitored in routine control examinations but also by their number in the biomass of ZABFAPR "suspended" in water and in the biofilm and sediments on inner surfaces of water pipes, not monitored so far. According to Fleming *et al*. (2002), the participation of biofilm and water phase in the total number of microorganisms occurring in water distribution systems equals 95 and 5%, respectively. Our studies have shown that the number of living microbial cells determined with the breeding method, "immobilized" in the biomass suspended in water, may be even 10–30-fold greater than that in the water (Table III). Zoogloeal aggregates of bacteria whose density ranged between 1.3×10^4–1.5×10^5/l were the main component of the biomass. Furthermore, the following organisms were identified in variable numbers: filamentous bacteria, fungal hyphae and spores, algae, protozoa and rotifers (Table V). Their density exceeded the number of plankton organisms recorded by Klimowicz (1989) in the water of distribution system of the city of Warsaw by a hundred times or even more than a thousand times. The difference can also be attributed to a more accurate method of detection of plankton organisms in water (membrane filter, pore size 5 mm) than used by this author filtration method with a plankton net. A low taxonomic diversity of these organisms is noteworthy. Algae (*Chlorella* sp*., Melosira* sp., *Tabelaria* sp., *Nitschia* sp.), protozoa belonging to flagellates and amoebae, as well as rotifers occurred in the biomass "suspended" in the water phase. The timing of the studies which coincided with autumn and winter, *i.e.* the time of low species biodiversity in the water of the reservoir supplying the WTP, was most probably one of the contributing factors. Klimowicz (1989) and Płachta *et al.* (1992) stress the close relationship between the number as well as the species composition

Table VI
Quantity of bacterial biomass and different groups of microbes in the water distribution systems sediments depending on the kind of pipes and period of their exploitation.

Determination		Pipe characteristics					
		Cast iron joints ϕ 50 mm		Spheroidal graphite iron pipe ϕ 250 mm		Steel joints ϕ 40 mm	
		Period of exploitation (years)					
		52	42	31	2	46	15
Quantity of sediment (mg/cm^2)		458	1024	616	1291	427	261
Quantity of biomass (ng 100/g)		454	147	147	7.2	2683	nw
Number of heterotrophic bacteria (cfu/100 g)		454×10^3	147×10^3	147×10^3	7189	2683×10^3	nd
Number of pigmented bacteria (cfu/100 g)		nd	nd	2066	7053	nd	nd
Bacteria from the genus *Pseudomonas* on Kings B agar, incubation in the indicated temperature (cfu/100 g)	7°C	195×10^3	nd	60×10^3	7253	915×10^3	nd
	30°C	379×10^3	209×10^3	194×10^3	14023	1006×10^3	nd
	42°C	nd	nd	nd	nd	366×10^3	nd
Number of faecal enterococci, *Enterococcus* sp. (cfu/100 g)		nd	nd	nd	nd	nd	nd
Campylobacter sp., titre		nd	nd	nd	nd	nd	nd
Yersinia sp. (cfu/100 g)		nd	nd	nd	nd	nd	nd
MPN of coagulase-positive staphylococci		0	0	0	0	0	0

of plankton occurring in water in a water distribution system and the quality of water supplying the system.

The fact that the biomass of ZABFAPR "suspended" in the water phase in water distribution systems is a reservoir of microorganisms potentially hazardous to water consumers is of epidemiological importance. This is corroborated by the identification in it of coagulase-positive staphylococci and faecal streptococci, that were not found in water. On the several sampling sites, much more higher number of the bacteria of the family *Enterobacteriaceae* and non-fluorescing *Pseudomonas* spp. growing in 7, 30 and 42°C were accounted in biomass, too. The results of taxonomic examinations of the bacteria isolated from the biomass support the suggestion. As was shown, the majority of these organisms are regarded as opportunistic pathogens to humans and warm-blooded animals. The urgent need to examine the density of microorganisms both in the water phase and in the biomass of ZABFAPR "suspended" in it in water distribution systems is confirmed by the findings specified above, given in Fig. 1. As shown in the figure, while the HB count in water in the examined drinking-water distribution system did not exceed the values permitted by legal regulations, the abundant number of these bacteria in water and in the biomass of ZABFAPR was significantly higher than these values in many sections of the system.

In the biomass of ZABFAPR separated from water *P. stutzeri* and *Flavimonas oryzihabitans* were characteristic species among non-pigmented bacteria, while non-pigmented varieties of *Micrococcus varians*, *P. pseudomallei*, *Comanzomonas testosteronii*, *Sphingobacterium pancimobilis* and *Rahnella aquatilis* were accompanying species. *Ochrobactrum anthropi* occurred most frequently among pigmented bacteria; *Micrococcus varians, Flavimonas oryzihabitans*, *P. fluorescens*, *P. aeruginosa*, *P. putida, Aeromonas sorbia* and *Stentrophomonas maltophilia* were characterised by a less frequent occurrence. The majority of the above bacterial species are considered to be opportunistic pathogens. Only *Stentrophomonas maltophilia* and *Comanzomonas testosteroni* are water-inhabiting saprophytes, while the pathogenicity of *Sphingobacterium paucimobilis* and *Rahnella aquatilis* is unknown.

Our examinations showed that pipe sediments are not, as suggested by Fleming *at al.* (2002), the main place of the accumulation of biomass microorganisms in water distribution systems. Assuming that the mass of one bacterial cell is 10^{-12}/g, the quantity of the biomass accumulated in sediments of cast iron joints over 31–52 years ranged between 147–454 ng/100 g. More biomass accumulated in steel joints that had been in operation for 46 years (2683 ng/100 g). A smaller quantity of the biomass (7.2 ng/100 g$^-$) was recorded in the sediment in a pipe made of spheroid graphite iron used for 2 years, characterised by the highest copper concentration (0.654% dw t) in the mineral fraction of the sediment. As faecal enterococci, coagulase-positive staphylococci, *Campylobacter* sp.,

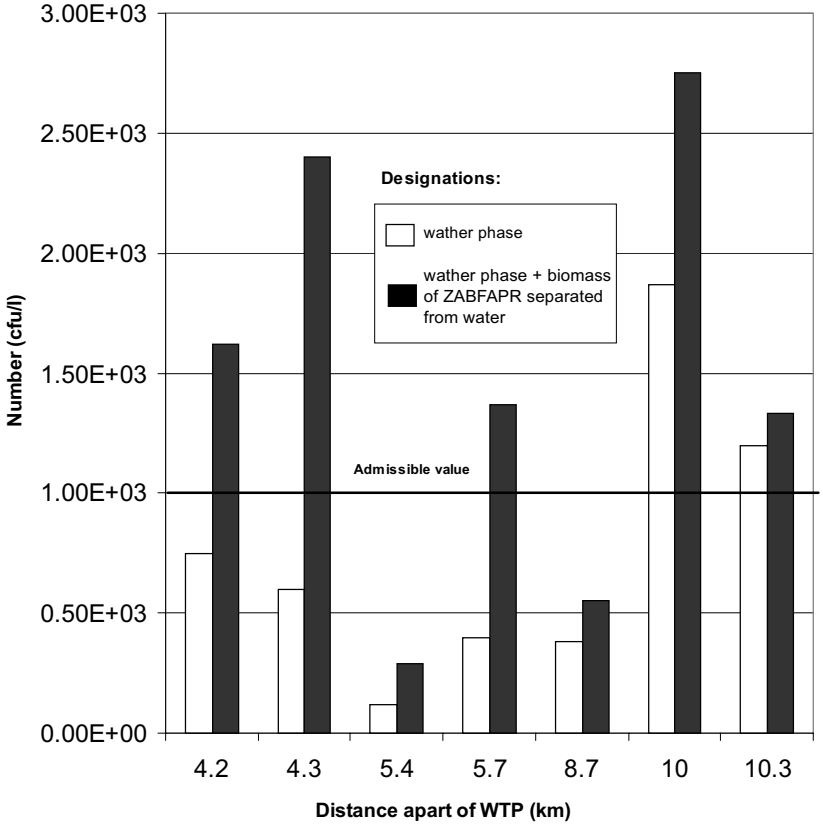

Fig. 1. Comparison of the number of heterotrophic bacteria in water phase and together in water phase and biomass of ZABFAPR separated on 5 ěm pore size filtres on the different sections of distribution system with admissible value for drinkable water in Poland.

Yersinia sp. and *P. fluorescens* did not occur in sediments of water distribution systems, they do not pose a health risk to humans (Table VI).

The biomass of bacteria accumulated in the sediments consisted mostly of heterotrophic bacteria. The number of these bacteria after 31–52 years of exploitation in cast iron joints ranged between 147×10^3 – 454×10^3 cfu/100 g, steel joints was 2683×10^3 cfu/100 g, and in the pipe made of spheroid graphite iron 147×10^3 cfu/100 g. Pigmented bacteria occurred in these biotopes occasionally and in small numbers, possibly indicating that transmission of opportunistic pathogenic bacteria belonging to the genera *Corynebacterium*, *Mycobacterium* and *Serratia* by sediments in water distribution systems is not a health risk.

The results of these observations do not reflect the results of studies by other authors (Joret *et al.*, 1991, Kerr *et al*., 1999, Fleming *et al*., 2002) due to the different scope and manner of presentation. Kerr *et al*. (1999) as well as Fleming *et al*. (2002) assessed the degree of microbiological contamination of pipe sediments exclusively on the basis of the density of heterotrophic bacteria, and the results of the determination were given per cm^2 of the internal surface of the pipes. The density of these bacteria in the sediments in cast iron pipes given by these authors are divergent as they equalled 1×10^7–1.8×10^7 and 10^3 cfu/cm^2, respectively.

The following general conclusions can be reached from this work.

- The degree of microbiological contamination of water distribution systems, determined by the density and the species composition of microorganisms occurring in pipe sediments, is of little importance for the assessment of the contamination degree.
- The scope of bacteriological control examinations of water, accepted in Poland and the world (total number of bacteria on broth agar medium (MPA) in culture at 22 and 37°C, *E. coli* or thermotolerant coliform bacteria, coliform bacteria, faecal streptococci, *C. perfringens*), is insufficient to assess fully the degree of microbiological contamination of water distribution systems.
- The scope of these examinations should be expanded to include the number of heterotrophic bacteria, including pigmented bacteria in culture at 26°C for 7 days in samples collected from the water phase and the biomass of ZABFAPR ”suspended” in it.
- Periodically, especially during high flows immediately after the start up of a water supply system or a break in the flow, the identification of dominating species of heterotrophic bacteria, including pigmented

bacteria, is recommended. These studies allow to assess the degree of the contamination of water distribution systems by opportunistic pathogenic species of bacteria.

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