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SANITARY AND BACTERIOLOGICAL EVALUATION OF COMMON CARP, TENCH AND CRUCIAN CARP REARED IN A POND SUPPLIED WITH BIOLOGICALLY TREATED SEWAGE

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ABSTRACT. Bacteriological contamination of muscles, skin, and digestive tract content of common carp (1+), tench (1+, 2+), and crucian carp (1+) reared in a pond supplied with biologically treated sewage (sewage treatment plant in Jedwabno, Masurian Lakeland) was studied in 1995 and 1996.

Common carp, tench, and crucian carp at the age 1+, examined in 1995, lived in the pond for one season (about 6 months). Tench 2+, and crucian carp 1+, examined in autumn 1996, were obtained from spawning of 1995 and their entire development took place in the pond. Muscles of all 3 fish species contained high numbers of bacteria determined on broth agar, at 20 and 37°C, coliforms, fecal streptococci, and *Aeromonas* sp. In 1996, also fecal coliforms, and sporadically *Pseudomonas aeruginosa* and *Salmonella* sp. were found.

In the muscles, skin, and digestive tract content of common carp reared in 1995 and 1996 numbers of coliforms, fecal coliforms, and fecal streptococci were similar. The muscles, skin and digestive tract content of tench and crucian carp contained higher numbers of bacteria in 1996 compared to 1995.

Numbers of bacteria determined on broth agar at 20 and 37°C, coliforms, fecal coliforms, and fecal streptococci in the muscles, skin and digestive tract content of fish reared in a sewage-supplied pond reflected their densities in water, often exceeding critical concentration of 5×10^4 CFU per 1 ml of water for TVC 20°C and TVC 37°C.

Keywords: SEWAGE, POND, FRY, FISH CULTURE, MICROORGANISMS, PATHOGENS

INTRODUCTION

Production of large amounts of sewage, deficiency of land and water, increasing demand for protein, and most of all – high costs of fish pond construction, brought about a concept of fish rearing in sewage treatment ponds. Such ponds are used for biological removal of nutrients, nitrogen and phosphorus, from water, and fish may be introduced as top consumers.

Domestic sewage, although nutrient-rich, may be dangerous due to possible presence of toxins which may accumulate in fish muscles (McIntyre and Mills 1975). Use of sewage in fish culture may also involve danger of human infections with pathogenic microorganisms (Geldreich and Clarke 1966). For these reasons, health care institutions in certain European Union countries and in the United States do not

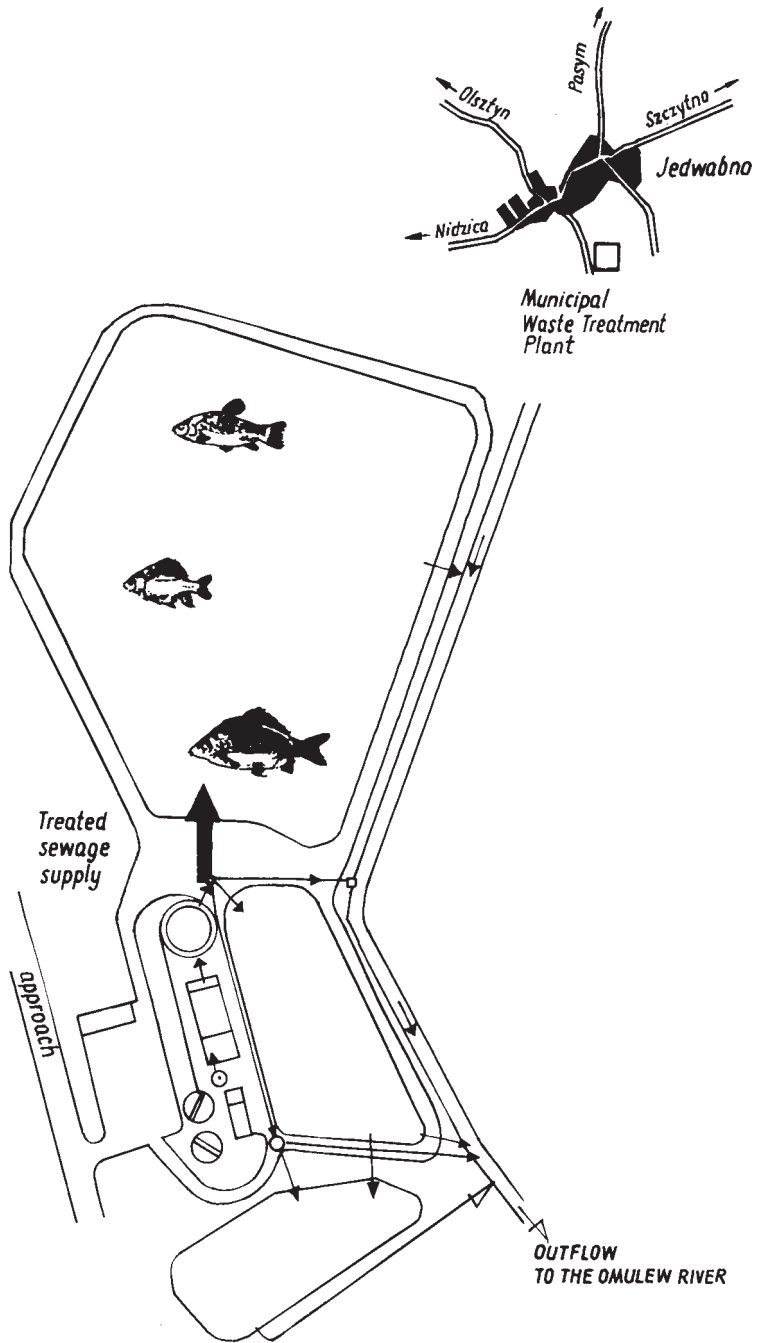


Fig. 1. Location of the experimental pond.

recommended fish culture in sewage-supplied ponds (Bryan 1977, Hejkal et al. 1983). Also, the consumers often reject such food. Taking that into consideration, fish should be reared in purified sewage, or their flesh used for feeding domestic animals, and not consumed directly by humans.

Another way of sewage use is rearing the stocking material and then transferring it into the usual fish ponds. All these procedures eliminate the danger of pathogen transfer to humans, and should be taken into consideration in sewage recirculation systems. Even if sewage-reared fish contain pathogens, they will purify following the transfer to fish ponds, and number of bacteria will be reduced to very low levels.

Another issue is the susceptibility of fish to pathogens which may be present in biologically purified sewage. In the present paper the results of the study on bacteriological contamination of sewage-reared common carp, tench and crucian carp fingerlings are discussed.

MATERIAL AND METHODS

EXPERIMENTAL POND

Common carp, tench, and crucian carp fingerlings were reared in 1995 and 1996 in a pond supplied exclusively with biologically purified domestic sewage from the local sewage treatment plant in Jedwabno (Masurian Lakeland). The pond was stocked in spring 1995 with common carp, tench and crucian carp fry, and crucian carp spawners. The latter spawned in the pond in the same year. In autumn 1995 common carp fingerlings were harvested, and some tench and crucian carp. The remaining tench and crucian carp overwintered in the pond. In spring 1996 the pond was stocked again with common carp fry. All the fish were harvested in autumn 1996.

FISH

Bacteriological examinations of common carp, tench and crucian carp fingerlings at the age 1+, harvested in autumn 1995, and of 1+ common carp and crucian carp, and 2+ tench harvested in autumn 1996, were carried out. Common carp examined in 1995 and 1996, and tench and crucian carp examined in 1995, were kept in the sewage-supplied pond for one rearing season (about 6 months). Tench 2+, and crucian carp 1+ examined in autumn 1996 – about 18 months. Crucian carp investigated in autumn 1996 originated from the spawning in 1995. Their entire

development, from egg till fertilisation, took place in the sewage-supplied pond. Body mass of the fish in 1995 was (g ind.⁻¹):

common carp	594.3 – 716.5
tench	49.5 – 80.5
crucian carp	47.4 – 77.5
and in 1996:	
common carp	348.7 – 422.3
tench (2+)	65.0 – 86.8
crucian carp	34.5 – 45.8

SAMPLING

The fish for microbiological analyses were harvested in autumn 1995 and 1996. Each sample consisted of 5 individuals of each species. The fish were immediately transferred to the laboratory, in the pond water, and dissected according to Buras et al. (1987). Samples of skin were taken (above the lateral line, under the dorsal fin) of muscle, and digestive tract was isolated. The samples were placed in sterile Petri dishes, weighed under sterile conditions, ground in a mortar with sea sand, and suspended in physiological NaCl solution (1 g of tissue or digestive tract content in 10 ml of the solution). The suspensions were homogenized at 1000 rpm for 10 minutes, in the homogenizer Universal Laboratory Aid Type MPW-309, made in Poland. The homogenates were diluted 1:10 – 1:10000 and inoculated into the culture media. Microbiological analyses were completed within 6 hours from fish harvest.

MICROBIOLOGICAL ANALYSES

Microbiological studies on the tissues and digestive tract contents involved evaluation of:

1. total number (CFU g⁻¹ wet weight) of bacteria on broth agar, after 72-hour incubation at 20°C (TVC 20°C);
2. total number (CFU g⁻¹ wet weight) of bacteria on broth agar, after 24-hour incubation at 37°C (TVC 37°C);
3. number (CFU g⁻¹ wet weight) of coliform bacteria (TC) on Endo medium, after 48-hour incubation at 37°C;
4. number (CFU g⁻¹ wet weight) of fecal coliform bacteria (FC) on Endo medium, after 24-hour incubation at 44.5°C;

5. number (CFU g⁻¹ wet weight) of fecal streptococci (FS) on m-Enterococcus Agar, after 72-hour incubation at 37°C;
6. number (CFU g⁻¹ wet weight) of anaerobic sulfite-reducing bacteria (*Clostridium perfringens*) on Wilson-Blair medium, after 18-hour incubation at 37°C (in the samples pasteurized at 80°C for 10 min.);
7. number (CFU g⁻¹ wet weight) of *Pseudomonas aeruginosa* (Pa) on mPa Agar, after 48-hour incubation at 37°C;
8. number (CFU g⁻¹ wet weight) of *Aeromonas* sp. (Ae) on Rimler-Shotts medium, after 24-hour incubation at 37°C;
9. presence or lack of *Salmonella* sp. on selective Kauffman's medium with sodium tetrathionate, after 24-hour incubation at 37°C, and then on separating medium with xylose, lysine, and sodium desoxycholate (XLD), under the same incubation conditions (Bordner and Winter 1978).

Total numbers of TVC 20°C and TVC 37°C were estimated using the method of culture in Petri dishes, and counting the colonies at 5 x magnification. Typical colonies on Endo medium (red, with metallic shine) were confirmed by random transfer into lauryl-tryptose broth, and in Gram-stained preparations. Typical FS colonies on m-Enterococcus medium were randomly confirmed by evaluation of growth in broth at 44.5°C, and pH 9.6, in the presence of 6.5% NaCl, and additionally, in milk with 0.01% methylene blue. Presence of *Clostridium perfringens* was randomly confirmed in milk fermentation test. *Pseudomonas aeruginosa* was identified by pyocyanin detection in Wood lamp light (Levin and Cabelli 1972). Yellow colonies of *Aeromonas* sp. on Rimley-Shotts Agar were randomly checked using API-20E test (Analytab Product, Plainview New York), oxidase test, and in the presence of vibriostatic agent 0/129. Presence of typical red, black-spotted *Salmonella* sp. colonies on XLD medium was confirmed using Kligler's medium, urea broth, and finally – in glass agglutination test for HM flagellar antigen, according to Burbianka et al. (1983). Simultaneously with the evaluation of the number or the presence of microorganisms in fish tissues, numbers of TVC 20°C, TVC 37°C, TC, FC, and FS were estimated in purified sewage discharged from the sewage treatment plant in Jedwabno, and in the experimental pond. The results on bacteriological water quality indices were compared with the values recommended in the Decree of the Minister of Environment Protection, Natural Resources and Forestry of Nov. 5, 1991, concerning classification of waters, and with the WHO data (1989) on the use of sewage in agriculture and aquaculture.

Totally 90 samples of muscles, skin, and digestive tract contents of 30 fish (10 individuals of each species), were analysed. Water analyses involved 26 samples of purified sewage and of water from experimental pond.

RESULTS

FISH

Muscles, skin and digestive tract contents of common carp fingerlings aged 1+ reared in 1995 in the experimental sewage-supplied pond contained exceptionally high numbers of bacteria detected on broth agar at 20 and 37°C. Coliform bacteria (TC) were commonly found in all analysed fish tissues. No thermotolerant *Escherichia coli* (FC), or dormant anaerobic *Clostridium perfringens* (CP) were found in carp muscles or skin. Neither pathogenic *Salmonella*, nor possibly pathogenic *Pseudomonas aeruginosa* occurred in the tissues.

Numbers of TVC 20°C and TVC 37°C in the tissues and digestive tract contents of common carp fingerlings (K1) in 1996 were similar to those found in 1995. Additionally, however, thermotolerant *Escherichia coli* (FC) were present in the tissues, and their number in digestive tract contents was 100 fold higher than in 1995. Also, density of *Aeromonas* sp. increased 1000 fold. Potentially pathogenic *Pseudomonas aeruginosa* were observed in the muscles of some individuals, and *Salmonella* sp. in skin (Fig. 2).

Numbers of TVC 20°C and TVC 37°C in the muscles and skin of tench (K1) in 1995 were similar to those found in common carp (K1) and crucian carp (K1). Tench digestive tract contents contained similar densities of bacteria as in common carp (K1). No *Clostridium perfringens*, *Pseudomonas aeruginosa*, or *Salmonella* sp. were found. The fish harvested in 1996 (K2) contained 1000-10000 fold higher numbers of TVC 20°C, TVC 37°C, TC, FC, and *Aeromonas* sp. in the muscles and skin compared to younger individuals (K1) examined in 1995. The muscles and skin of these fish contained also thermotolerant *Escherichia coli* (FC), and in some cases pathogenic *Salmonella* sp. were observed (Fig. 3).

Values of TVC 20°C, TVC 37°C, TC, FS, and *Aeromonas* sp. in the muscles and skin of crucian carp (K1) in 1995 were similar to those found at the same time in common carp (K1). Digestive tract contents, however, contained less TVC 20°C, TVC 37°C, and TC, and more *Aeromonas* sp. No fecal coliforms (*Escherichia coli*), *Clostridium perfringens*, *Pseudomonas aeruginosa* or *Salmonella* sp. were noted.

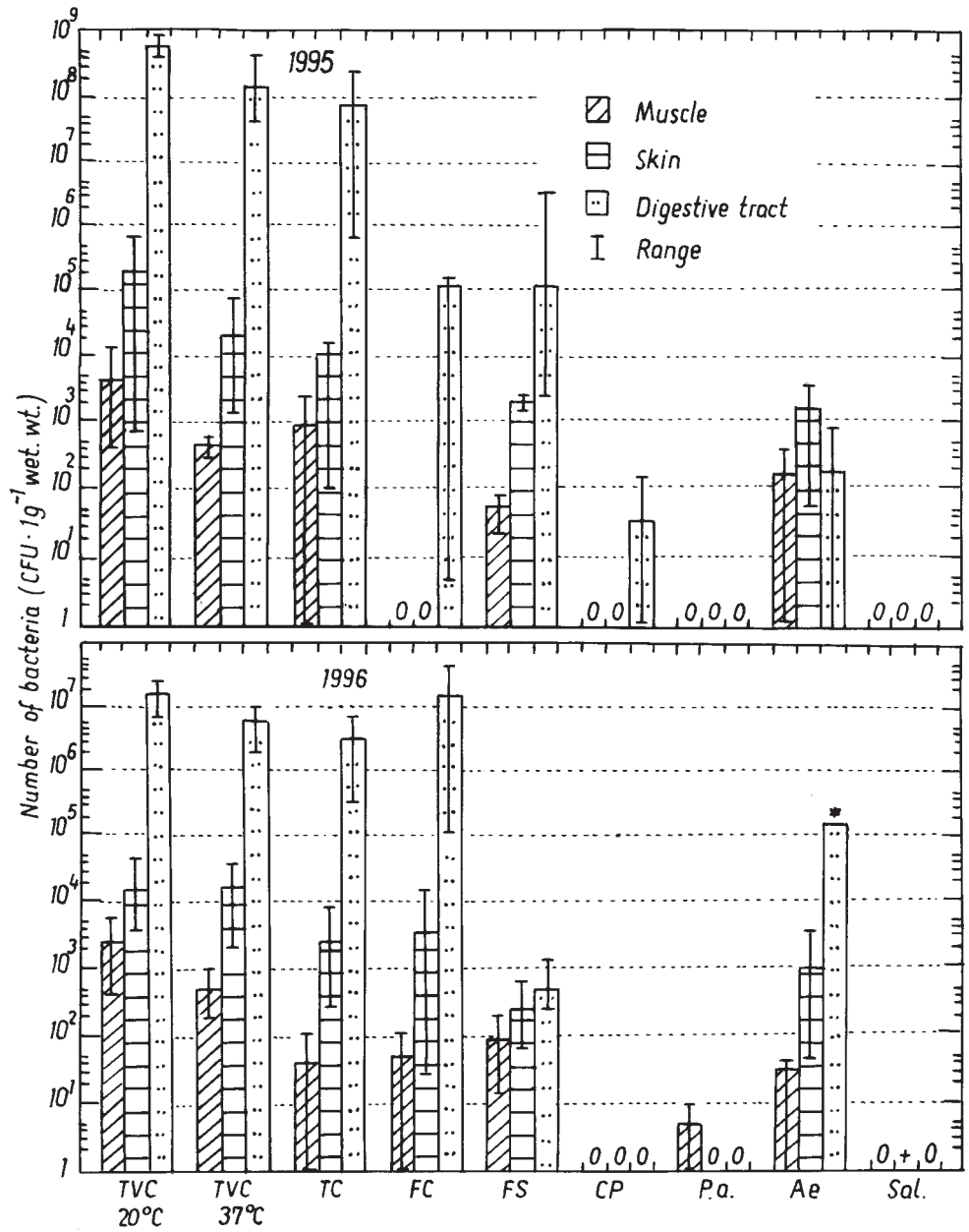


Fig. 2. Number of indicatory and potentially pathogenic bacteria in the muscles, skin, and digestive tract contents of common carp (1+) in 1995 and 1996. * - tested in 1 fish

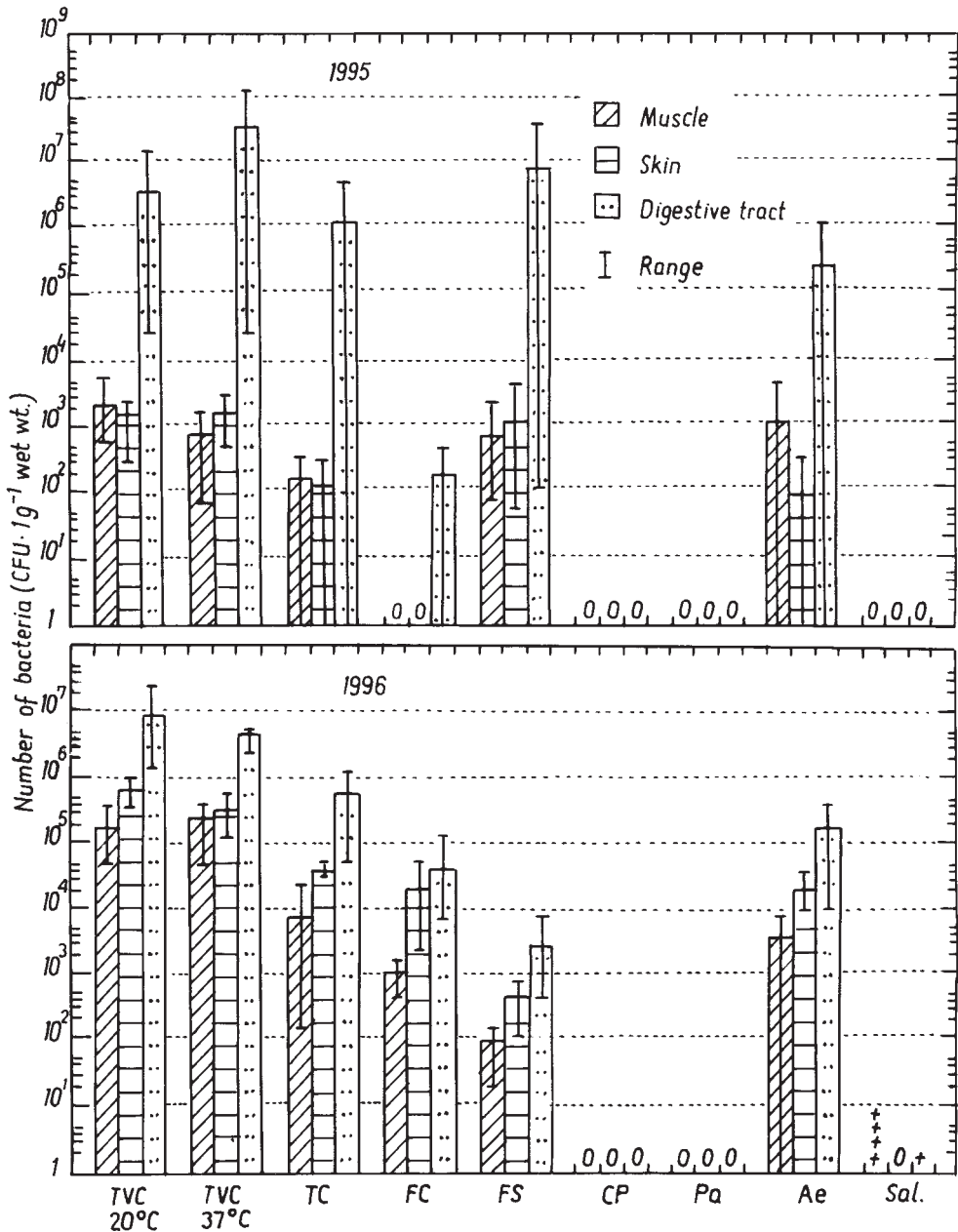


Fig. 3. Number of indicatory and potentially pathogenic bacteria in the muscles, skin, and digestive tract contents of tench 1+ in 1995 and 2+ in 1996.

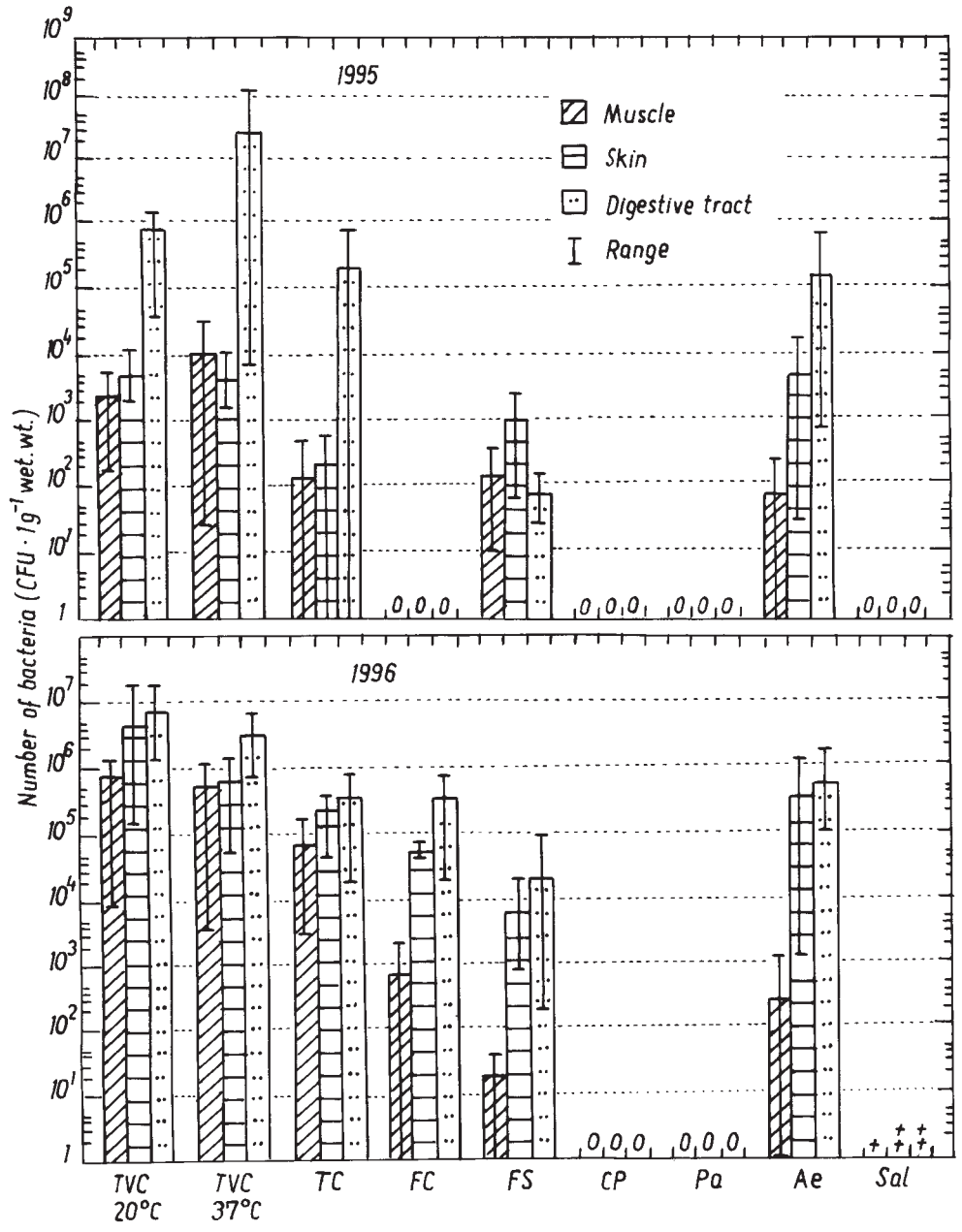


Fig. 4. Number of indicatory and potentially pathogenic bacteria in the muscles, skin, and digestive tract contents of crucian carp (1+) in 1995 and 1996. + present in 1 fish, ++ present in 2 fish

Escherichia coli and *Salmonella* sp., however, appeared in the muscles, skin, and digestive tract contents of K1 crucian carp from 1996. These fish contained also considerably higher numbers of TVC 20°C, TVC 37°C, and TC compared to those harvested in 1995 (Fig. 4).

WATER

Effluent from the sewage treatment plant in Jedwabno contained from 1.4×10^3 to 16.8×10^6 CFU ml⁻¹ TVC 20°C. TVC 37°C ranged from 150 to 18.5×10^6 CFU ml⁻¹, number of coliforms (TC) from 1.4×10^3 to 14×10^6 MPN 100 ml⁻¹, fecal coliforms (*Escherichia coli*) reached from 1.1×10^3 to 450×10^3 MPN 100 ml⁻¹, fecal streptococci (FS) – from 460 to 1.1×10^6 MPN 100 ml⁻¹. FC/FS ratio ranged from 0.04 to 100, being over 0.7 in most samples, which is typical for domestic sewage. FC/FS ratio under 0.7, typical for wastes from animal farms was noted only in several water samples (Table 1).

TABLE 1

Number of indicatory bacteria in water outflowing from sewage treatment plant (purified sewage) in Jedwabno in 1995 and 1996 years

Date	TVC 20°C	TVC 37°C	TC	FC	FS	Ratio FC:FS
	CFU x 10 ³ ml ⁻¹		MPN x 10 ³ 100 ml ⁻¹			
19.04.1995	95.0	6.15	1400.0	20.0	450.0	0.04
26.05	9.3	0.15	11.0	14.0	1.1	12.7
19.06	31.5	3.65	14.0	14.0	14.0	1.0
20.07	1.4	1.4	1.4	1.1	0.46	2.4
29.09	66.5	4.5	110.0	140.0	110.0	1.3
16.10	1.4	0.5	4.5	4.5	1.4	3.2
26.03.1996	74.0	5.5	140.0	11.0	11.0	1.0
22.04	14.2	10.6	110.0	140.0	1.4	100.0
28.05	37.2	6.0	14000.0	240.0	7.5	32.0
20.06	8.5	0.2	150.0	15.0	11.0	1.36
22.07	16800.0	18500.0	4.5	1.1	4.0	0.4
20.08	50.5	15.0	1400.0	450.0	1100.0	0.41
2.10	231.0	198.0	1400.0	450.0	250.0	1.8

CFU – colony forming unit

MPN – most probable number

Pond water contained $3.8 \times 10^3 - 60.8 \times 10^3$ CFU ml⁻¹ of TVC 20°C, and 100- 287 x 10³ CFU ml⁻¹ of TVC 37°C. Number of coliforms (TC) ranged from 15 to 25 x 10³ MPN 100 ml, of fecal coliforms (*Escherichia coli*) – from 0.3 to 140 x 10³ MPN 100 ml⁻¹, and of fecal streptococci – from 0.3 to 140 x 10³ MPN 100 ml⁻¹. FC/FS ratio was within 0.1-32.5, usually over 0.7, typical for domestic sewage (Table 2).

TABLE 2

Number of indicatory bacteria in water from pond at sewage treatment plant in Jedwabno in 1995 and 1996 years

Date	TVC 20°C	TVC 37°C	TC	FC	FS	Ratio FC:FS
	CFU x 10 ³ ml ⁻¹		MPN x 10 ³ 100 ml ⁻¹			
19.04.1995	4.0	0.37	40	0.3	0.3	–
26.05	11.3	0.62	15	93	23	4.0
19.06	13.2	1.5	11000	2000	400	5.0
20.07	3.8	3.4	20000	93	240	0.38
29.09	60.8	5.4	15000	11500	1100	10.4
16.10	35.2	2.1	93	460	20	23.0
26.03.1996	12.5	1.5	14000	14000	450	31.1
22.04	37.5	223.0	460	1400	43	32.5
28.05	30.0	2.9	93	93	9	10.3
20.06	6.4	0.1	7500	4300	2300	1.8
22.07	36.0	287.0	4500	900	900	1.0
20.08	10.0	230.0	25000	25000	9000	2.8
2.10	10.5	7.0	14000	14000	140000	0.1

CFU – colony forming unit

MPN – most probable number

DISCUSSION

Total numbers of bacteria evaluated on broth agar at 20 and 37°C (TVC 20°C, TVC 37°C), of coliforms (TC), and of fecal streptococci (FS) in flesh of common carp fingerlings (K1) reared in the pond supplied with biologically purified domestic sewage, were high. They were usually 10-100-fold higher than in carp reared in traditional fish ponds. They were also higher compared to the values obtained for carps reared in 1986-1989 in sewage-supplied ponds in Olsztynek (Niewolak and

Tucholski 1995). Some common carp individuals harvested in 1996 contained fecal coliforms (*Escherichia coli*) in the muscles and skin. No such bacteria were observed in the carps reared in Olsztynek sewage treatment plant. Muscles of all carps (K1) examined in 1995, and of some individuals analysed in 1996 contained also *Aeromonas* sp. These bacteria were also found in skin and digestive tract contents of some fish. Some of them (*Aeromonas hydrophila*) may cause hemorrhagic septicemia (Haley et al. 1967) and ulcerative disease in fish (Karunasagar et al. 1995). Fortunately, dangerous densities of these bacteria, high enough to cause fish death, are much higher. According to Rahman et al. (1997), for ougon carp, kouhaku carp, and goldfish they reached 10^6 - 10^8 CFU after intraperitoneal injection. *Aeromonas* sp. in the muscles, skin and digestive tract contents of common carp fingerlings and other fish species were observed also by other authors. Fattal et al. (1992) reported that these microorganisms were common in the fish exposed to diluted raw sewage.

Higher numbers of TVC 20°C, TVC 37°C, TC and *Aeromonas* sp. in the muscles, skin and digestive tract contents of tench (2+) and crucian carp (1+) harvested in autumn 1996, compared to values noted in 1995, abundant fecal coliforms and *Salmonella* sp.- which were not observed in 1995 - must have been related to deterioration of bacteriological water quality in the pond in 1996. These conditions were dangerous to fish (WHO 1989). Fecal coliform numbers usually exceeded the maximum level for class III waters (Decree...1991). TVC 20°C, TVC 37°C, TC, FC and FC values observed in the muscles, skin and digestive tract contents of tench (2+) and crucian carp (1+) in 1996 may reflect their content in the pond water. Numbers of bacteria in the pond sometimes exceeded the threshold level (Heever and Frey 1994). Considerable fluctuations of TVC 20°C, TVC 37°C, TC, FC, FS in pond water and in biologically purified sewage might have affected quality of fish flesh and content of bacteria in their muscles, skin, and digestive tract contents (Buras et al. 1987). Fluctuations of the number of indicatory bacteria in sewage might have resulted from disturbances in sewage treatment process. Changes of bacteria density in the pond might have been caused by solar radiation and bactericidal action of UV rays, or activity of algae. Algal development and die-off often involve changes of water pH and build-up of bacteriostatic and/or bactericidal substances (Bahloui et al. 1997) which may result in reduction of allochthonic bacteria.

CONCLUSIONS

1. Numbers of indicatory bacteria in pre-treated sewage, and in water of the pond in which common carp (1+), tench (2+), and crucian carp (1+) were reared, were typical for strongly polluted waters (class III or worse).
2. Poor bacteriological water quality in the pond (caused by the supply of polluted water from the sewage treatment plant) resulted in microbiological contamination of fish flesh (also with fecal bacteria), and presence of bacteria pathogenic to fish and humans.
3. Numbers of bacteria evaluated on broth agar at 20 and 37°C, coliforms, fecal coliforms, and fecal Streptococci in the muscles and skin of tench (2+) and crucian carp (1+) reared in sewage-supplied pond depended on the duration of rearing in polluted water.

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STRESZCZENIE

OCENA SANITARNO-BAKTERIOLOGICZNA KARPIA, LINA I KARASIA HODOWANYCH W STAWIE ZASILANYM BIOLOGICZNIE OCZYSZCZONYMI ŚCIEKAMI

Badano stopień zanieczyszczenia bakteriologicznego mięśni, skóry i treści przewodu pokarmowego krocza karpia (1+), lina (1+ i 2+) i krocza karasia (1+), podchowywanych w stawie zasilanym biologicznie oczyszczonymi ściekami przy oczyszczalni w Jedwabnie, w latach 1995 i 1996.

Kroczyki karpia w wieku 1+, liny w wieku 1+ i kroczyki karasia w wieku 1+ badane w 1995 r. przebywały w stawie 1 sezon hodowlany (około pół roku); kroczyki karpia w wieku 1+ badane w 1996 r. przebywały w stawie 1 sezon hodowlany (około pół roku), natomiast liny w wieku 2+ i kroczyki karasia w wieku 1+ badane w 1996 r. – około pół roku. Kroczyki karasia badane jesienią 1996 r. pochodziły z tarła odbytego w 1995 i cały ich rozwój osobniczy odbywał się w tym stawie. W mięśniach wszystkich 3 gatunków ryb stwierdzono wysokie liczebności bakterii oznaczanych na agarze bulionowym w temperaturze 20 i 37°C, bakterii z grupy pałeczki okrężnicy, paciorkowców kałowych i *Aeromonas* sp., w 1996 r. również kałowych bakterii z grupy pałeczki okrężnicy, wyjątkowo zaś *Pseudomonas aeruginosa* i *Salmonella* sp. W mięśniach, skórze i treści przewodu pokarmowego kroczków karpia hodowanych w 1995 i 1996 r. liczby badanych bakterii wskaźnikowych, w szczególności bakterii oznaczanych na agarze bulionowym w temperaturze 20 i 37°C, liczby bakterii z grupy pałeczki okrężnicy, liczby kałowych bakterii z grupy pałeczki okrężnicy i paciorkowców kałowych były zbliżone. W mięśniach, skórze i treści przewodu pokarmowego lina (2+) i kroczków karasia (1+) – z reguły mniejsze w 1995 r., większe zaś w 1996 r. Różnice te były uwarunkowane pogorszeniem się jakości wody w stawie zasilanym biologicznie oczyszczonymi ściekami. Liczebności bakterii oznaczanych na agarze bulionowym w temperaturze 20 i 37°C częstokroć przekraczały 5×10^4 komórek w 1 ml, powyżej których ryby mogą akumulować w mięśniach również bakterie chorobotwórcze.

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