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REDUCTION EFFICIENCY OF THE NUMBER OF POLLUTION INDICATOR BACTERIA IN SEWAGE WATERS TREATED IN FISH PONDS***

ABSTRACT: Domestic sewage and that from fruit and vegetable processing which enter the city sewage treatment plant were purified in a system of four fish ponds (1 – facultative, 2 – aerated, 3 and 4 – oxygenated), and in water outflowing from ponds into the river. Most of the bacteria were reduced in 50–93% in pond 1. In the remaining ponds of the technological system any greater reduction was only noted for the total number of bacteria on nutrient agar at 37 °C; for total coliforms; fecal coliforms and the number of fecal streptococci. Most of the bacteria were reduced when sewage was flown through all four ponds. In the water outflow from ponds during the entire study period, the average reduction of the numbers of bacteria on nutrient agar at 20 and 37 °C, of the total coliforms, fecal coliforms and fecal streptococci, amounted to 93–99%.

KEY WORDS: sewage ponds, fish culture, water purification.

1. INTRODUCTION

Ponds are often used for treatment of organic water pollution and of pathogenic micro-organisms incoming with sewage (Mara et al. 1992). This process is related to low exploitation costs and facilitated service (Mac

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Donald and Ernst 1987, Mara and Pearson 1987). High reduction of pathogenic bacteria approaching 100% (Walker and Leclerc 1977), of viruses (Feachem et al. 1985), protozoan cysts (Weaver et al. 1976), and eggs of parasite worms (Mara and Silva 1986) is being required for the water outflows used for irrigation of vegetation cultures and recreation areas (Health aspects [...] 1985, Health guidelines [...] 1989). In this respect, the appropriate construction, quantity, location and depth of ponds (Mayo 1989), as well as hydraulic detention and loading capacities for sewage (Ohgaki et al. 1986, Carre and Baron 1987) are of the primary importance. The stocking of ponds with fry practiced occasionally eliminates the necessity of mechanical removal of suspended matter and algae. The excess of the latter is incorporated into the trophic chain (Smith 1985) which contributes to the improvement of water quality (Schroeder 1977, Carpenter 1978, Henderson 1979). Number of fecal coliforms (*Escherichia coli*) at the water outflow gives a measure of sewage purification efficiency. This provides a good substitute for the numbers of pathogenic organisms difficult to identify, and thus not practical in the continuous control of the sewage treatment plant. Degree of reduction of these bacteria having survival ability close to that of pathogenic bacteria of the genus *Salmonella* enables the estimate of the purification efficiency in ponds (Carrington 1980, Kowal 1982).

The present work shows the results of three years studies on the numbers and reduction degree of chosen bacteria which are considered as indicators of water pollution (total numbers of bacteria on nutrient agar at 20 and 37 °C), and of water sanitary state (total coliforms, fecal coliforms, *Escherichia coli*, fecal streptococci). The study was done on pond water at the domestic sewage treatment plant, fruit and vegetable processing plant, and in inflowing treated sewage, as well as in water outflowing from ponds. The studied ponds are utilized for additional purification treatment of sewage outflow, and for the culture of carp and white fish fry.

2. STUDY SITE

The studies were done in a sewage-agriculture system of four ponds (Fig. 1) having the surface area of 0.94–1.03 ha and average depth of 1.1–2.2 m. The ponds are situated in a lineary fashion in an area of sewage treatment plants receiving domestic, municipal and fruit and vegetable processing wastes, in the town of Olsztynek, Masurian Lakeland, North-East Poland. Following mechanical screening on gratings, sands, and in a preliminary removal and settling container, and after biological purification in two chambers of active removal, and two secondary settling chambers, the ponds are used as the third and last stage of sewage treatment. Separated by dikes they are equipped in 7 transfer outlet boxes.

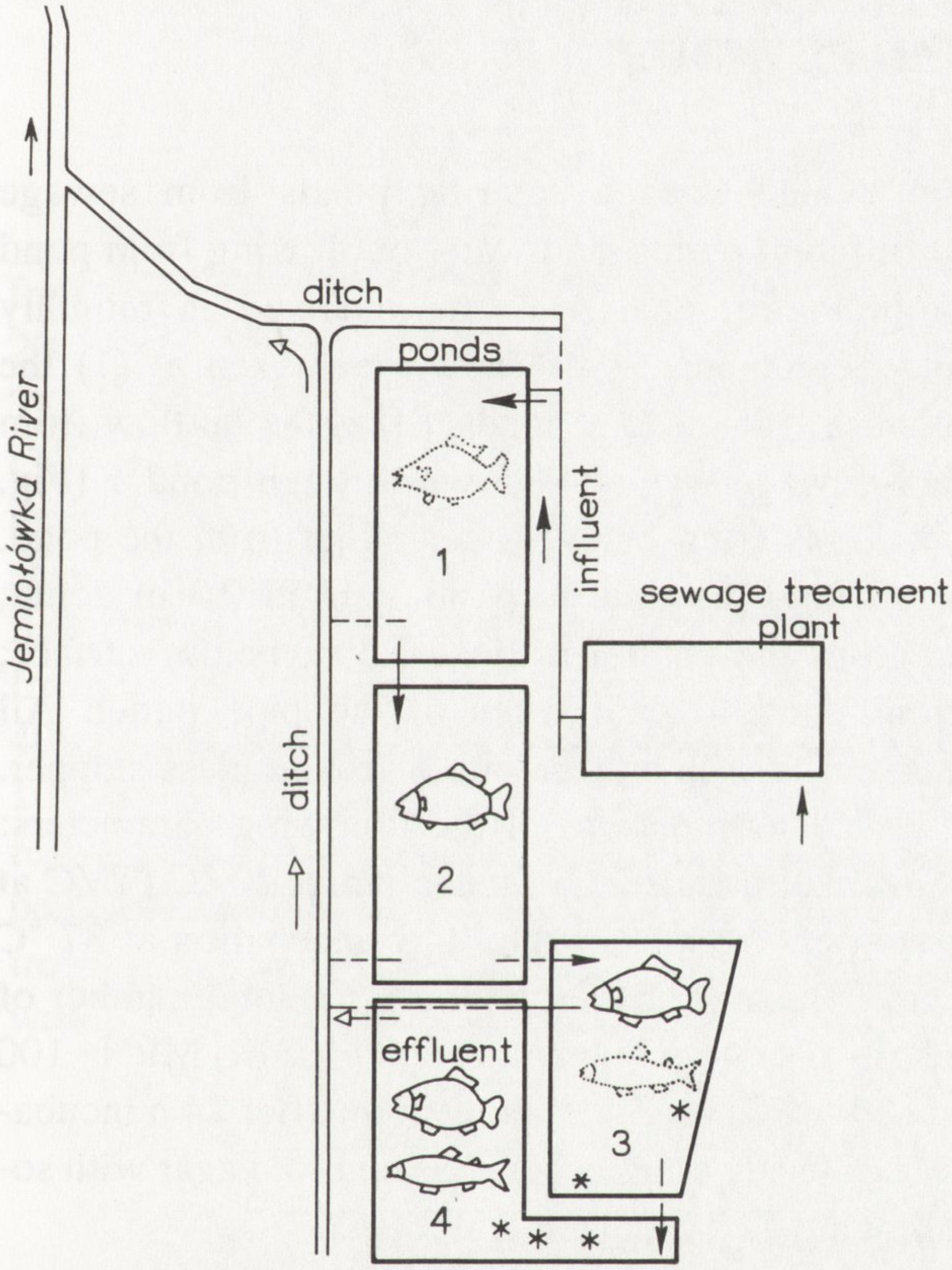

Fig. 1. Scheme of ponds at sewage treatment plant near Olsztynek
1, 2, 3, 4 – ponds. Black arrows indicate direction of treated sewage flow. White arrows indicate occasional flow of treated sewage. Fishes marked by solid line indicate continuos rearing. Fishes marked by broken line indicate periodical rearing

This enables the flow of sewage from one pond to another and directly into a ditch and river. In terms of oxygen conditions the ponds can be classified as 1 – facultative, 2 – aerating, 3 and 4 – oxygenated, reinforced with spring waters. During the various months of the study period, the sewage not always was flown through all four ponds (Fig. 2). This was related to an experimental fish culture in the ponds from 1 July 1985 to 21 December 1988. Hydraulic detention of ponds was variable and approached the quantities of treated sewage outflowing from the treatment plant. It ranged 795–2181 m^3 24 h^{-1}. For the entire pond complex, sewage retention time ranged from 32 to 88 d.

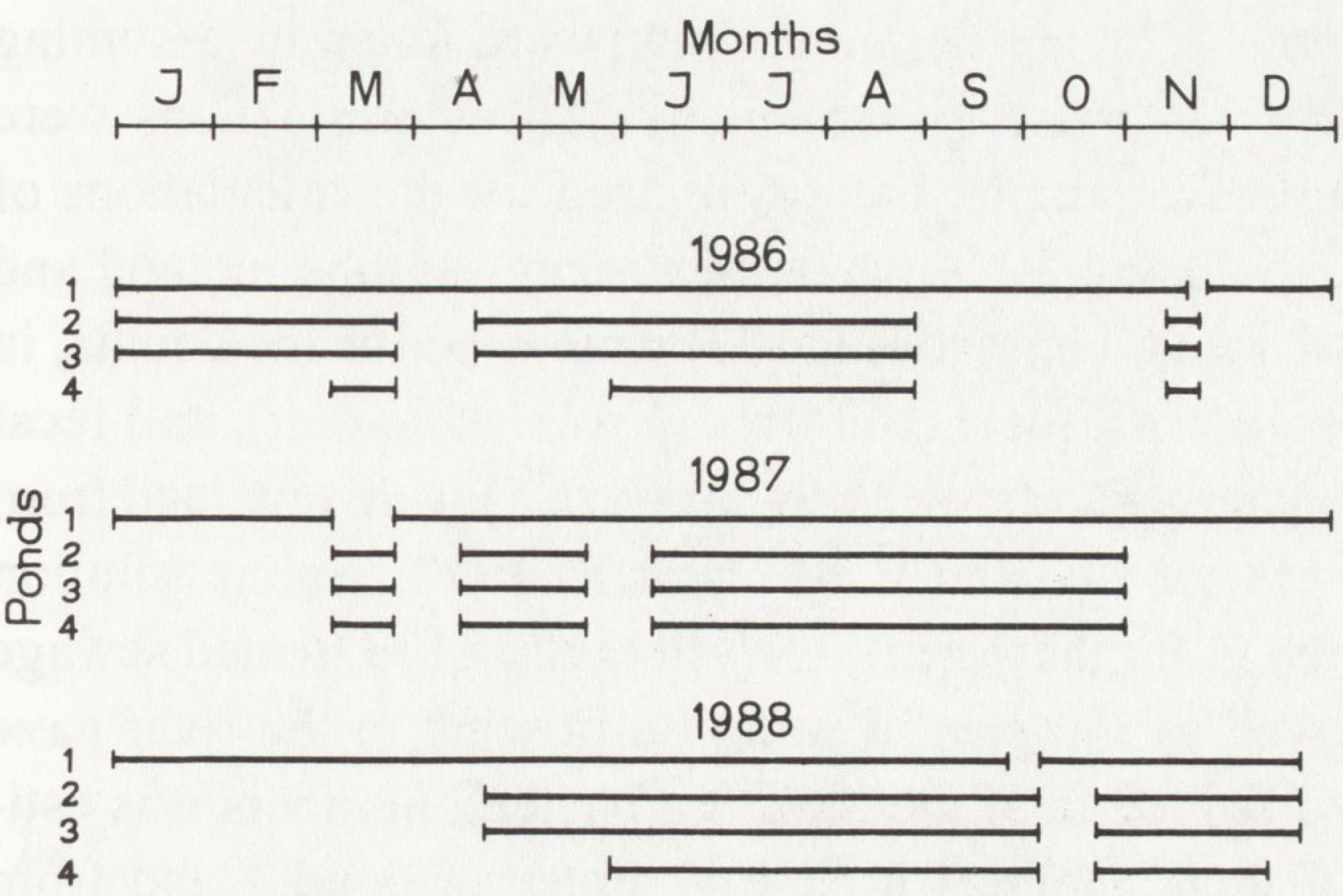

Fig. 2. Flow diagram of the sequentialy treated sewage transported through ponds

3. METHODS

Studies were carried out on treated sewage entering ponds from sewage treatment plants, on waters of the different ponds, and water outflowing from pond No. 4. Water samples for microbiological analyses were obtained in monthly intervals between April 1986 and September 1988. They were taken at (1) the outflow from sewage treatment plant, (2) in all four ponds, (3) at the outflow from pond 4 (Fig. 1). In a period when sewage was only flown through pond 1 (Fig. 2) the outflow sampling point was transferred below water outlet from the pond. Samples of treated sewage were taken at the inlet to pond 1, from 0.3 m depth; in ponds samples were obtained from the central area at 0.3 m below surface; samples of water outflowing from pond 4 were taken at the pipe outlet. All samples were taken directly by 300 cm^3 sterile bottles with a ground glass stopper.

Microbiological studies included determinations of the following parameters: (1) total viable count on nutrient agar following 72 h incubation at 20 °C (TVC at 20 °C); (2) total viable count on nutrient agar following 24 h incubation at 37 °C (TVC at 37 °C); (3) MPN · 100 cm^{-3} (most probable number), i.e. total number of coliforms (TC) on Eijkman's medium after 48 h incubation at 37 °C; (4) MPN · 100 cm^{-3} fecal coliforms – *Escherichia coli* (FC) on Eijkman medium after 24 h incubation at 44.5 °C; (5) MPN · 100 cm^{-3} of fecal streptococci (FS) on broth agar with sodium azide NaN_3 and dextrose following 72 h incubation at 37 °C.

Total number of bacteria on nutrient agar at 20 and 37 °C were determined according to the nationally accepted bacteriological analysis of potable water. Sewage water samples of 1 cm^3, in solutions of 1 : 10; 1 : 100; 1 : 1000; 1 : 10000 were transferred onto Petri dishes to which liquified and cooled nutrient agar was added (broth agar of pH 7.2). After solidification on the medium they were incubated at appropriate temperatures and periods of time. Subsequently the grown colonies were counted. Numbers of CFU · cm^{-3} (Colony Forming Units) were found by assuming that each viable bacteria cell gives a rise to one colony. All determinations were done in three replicates of the same sample; they were used for the calculations of the average numbers of TVC at 20 and 37 °C. A fermentation test tube method and solution method given by Standard methods (1983) were used for the counts, in three replicates, of the total coliforms, fecal coliforms (*Escherichia coli*), and fecal streptococci. Most probable bacteria numbers (MPN · 100 cm^{-3}) were obtained from the MacCrady tables (G. G. Meynell and E. Meynell 1970). Saline solution (0.85% NaCl) was used as solvent for these analyses. Altogether 138 treated sewage and pond water samples, as well as samples of water outflowing to the river have been examined. The degree of reduction of the indicator bacteria numbers was estimated from the difference of their numbers in sewage entering pond 1 and from those found in waters of the different ponds, as well as in water outflowing from pond 4 into the river.

4. RESULTS

In different months of the study period, the treated sewage entering ponds from the sewage treatment plant was characterized by high numbers of TVC 20 °C (3175–11 560 000 CFU · cm^{-3}); TVC 37 °C (1520–600 000 CFU · cm^{-3}); TC (210–1100 000 MPN · cm^{-3}); FC (210–450 000 MPN · 100 cm^{-3}) and FS (39–450 000 MPN · 100 cm^{-3}). During consecutive years, the minimal and maximal numbers of these microorganisms occurred in different months without showing a distinct seasonality (Figs. 3, 4). In the ponds forming the technological line, the numbers decreased gradually depending on the study period. At the outlet from ponds to the river they had reached values of the order of: TVC 20 °C – 40–5500 CFU · cm^{-3}; TVC 37 °C 20–10 750 CFU · cm^{-3}; TC 6–45 000 MPN · 100 cm^{-3}; FC 0–1500 MPN · 100 cm^{-3}, and FS 0–110 000 MPN · 100 cm^{-3}. The reduced numbers of TVC 20 °C and TVC 37 °C ranged widely during the study period. They fluctuated between 0 in April 1986 and January-February 1987 when treated sewage was only flown through pond No. 1, and up to 100% when sewage was transported through all four ponds (Fig. 3). For indicator bacteria of the

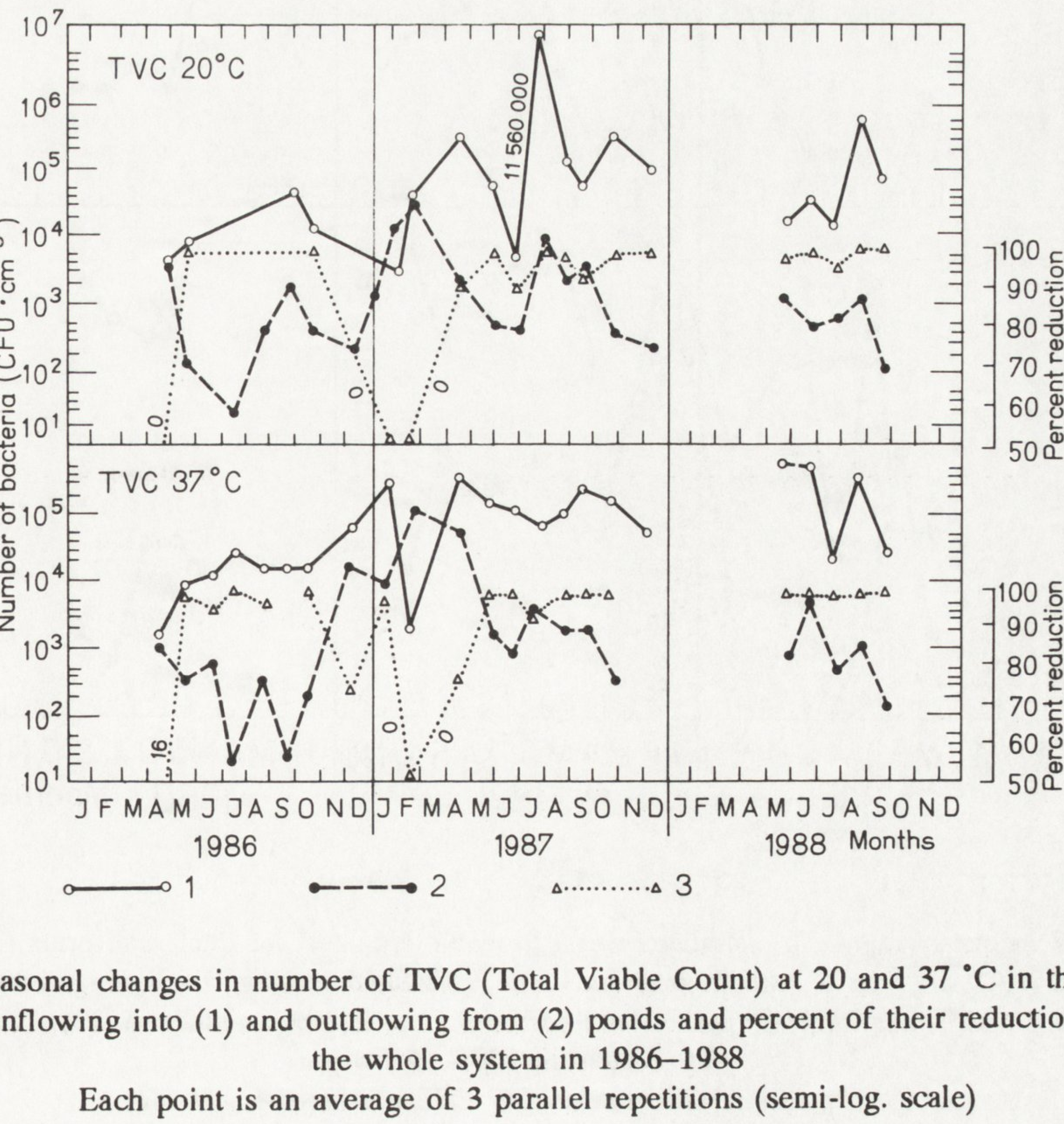

Fig. 3. Seasonal changes in number of TVC (Total Viable Count) at 20 and 37 °C in the treated sewage inflowing into (1) and outflowing from (2) ponds and percent of their reduction (3) in the whole system in 1986–1988

Each point is an average of 3 parallel repetitions (semi-log. scale)

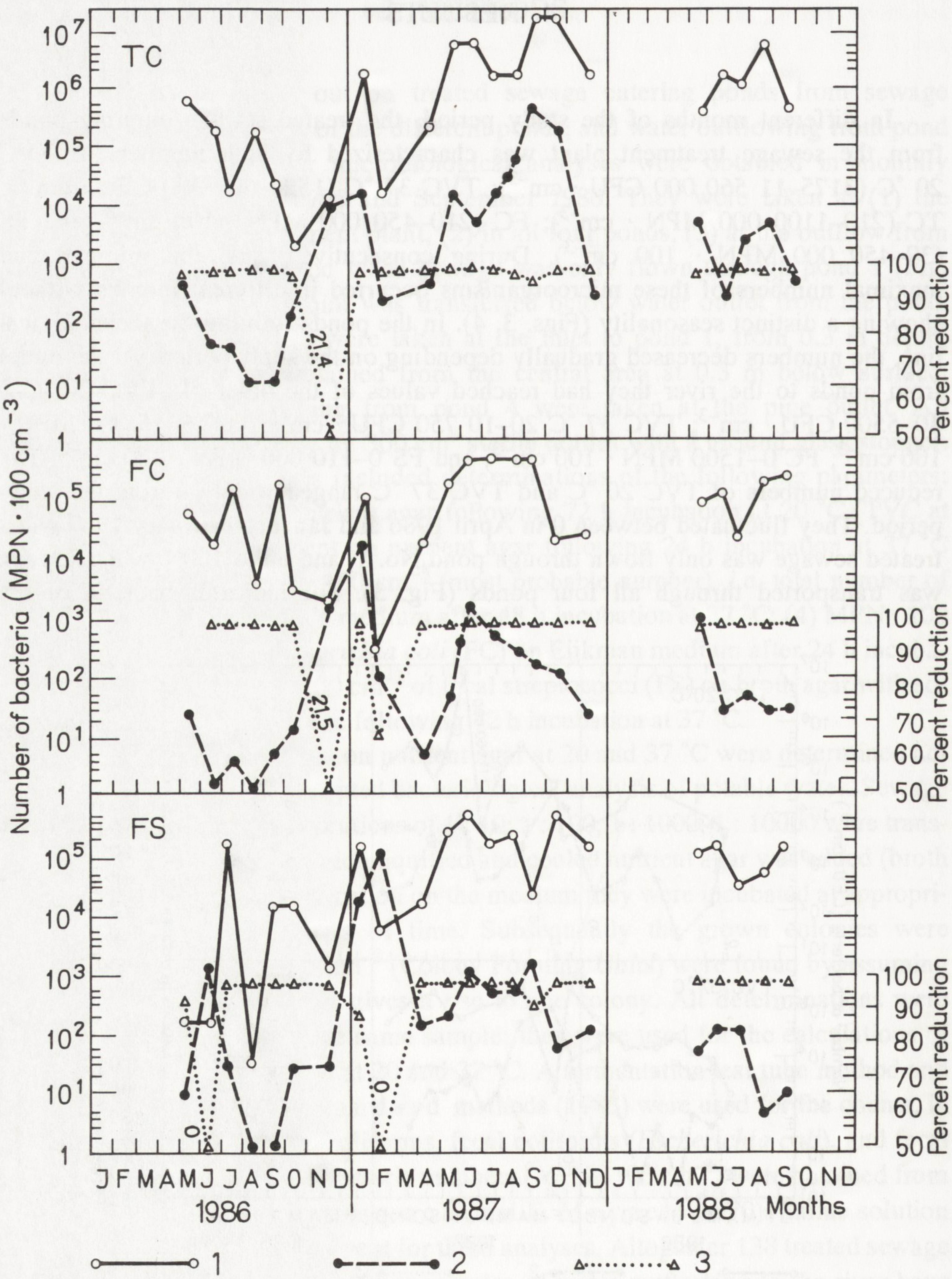

Fig. 4. Seasonal changes in number (Most Probable Number), of total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) in the treated sewage inflowing into (1) and outflowing with water from (2) ponds and percent of the numbers reduction (3) in the whole system in 1986–1988

Each points is an average of 3 replicates (semi-log. scale) sanitary state, the appropriate values are as follows: 0% and more for FS in June 1986 and February 1987; 21.4% in December 1986 up to 100% in different periods for TC and FC (Fig. 4). On the average, in the period 1986–1988 at the outlet from ponds into the river, high reduction numbers were observed for TVC bacteria at 20 °C (99.2%); TVC bacteria at 37 °C (93.3%); indicator bacteria of the TC, FC, and FS groups (94.3–99.9%). These percentages at the sewage outlet corresponded to the numbers of bacteria of the order of 5835 TVC 20 °C and 8920 TVC 37 °C in 1 cm^3, and 3180 TC, 178 FC, and 6150 FS MPN · 100 cm^{-3} (Table 1).

Table 1. Average percent reduction for the number of total viable count (TVC) at 20 and 37° C, total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) in the water of ponds in 1986–1988

Site	Number of samples	TVC 20° C	TVC 37° C	TC	FC	FS
Inflow*	20	100	100	100	100	100
Pond 1	20	93.3**	50.4	91.1	70.6	88.7
Pond 2	20	92.5	85.7	84.8	93.5	98.5
Pond 3	21	96.1	87.2	97.8	99.8	99.5
Pond 4	20	99.7	97.1	98.5	99.8	99.3
Outflow	20	99.2	93.2	98.5	99.9	94.5

*See Fig. 1. **Percent reduction of bacteria numbers calculated from the difference between numbers in the sewage inflowing to pond 1 from the sewage treatment plant, and the densities in waters of particular ponds and those entering river.

5. DISCUSSION

TVC 20 °C number in treated sewage inflowing into ponds from sewage--disposal plant might be related to the contents of organic substances; those of TVC 37 °C, TC, FC and FS to pollution of human and animal origin. Considering the characteristics of TVC group, their minimal and maximal numbers (at 20 °C) usually observed in periods corresponding to respective low or high values of BZT_5 are quite easy to explain (Purzycka and Wojtczak, personal communication). Lack of such relationships between TVC 37 °C, TC, FC, and FS numbers and the content of organic matter expressed by BZT_5 can be explained by the bacterial inhabitance of the alimentary track of humans and warm-blooded animals, thus of an environment rich in specific nutritional constituents, and in anaerobic and/or microaerophilous conditions having the $CO_2 : O_2$ ratio different from that encountered in sewage waters (Legendre et al. 1984, Troussellier et. al. 1986). High TC and FS numbers found in treated sewage in autumn of 1986 and 1987 might have been related to fruit and vegetables processing. Among these indicator bacteria occur genera and species living in soil and on plants (Geldreich 1966). They might enter sewage during the process of fruit and vegetables washing, and may survive the biological purification process by the method of active settlement. They are more resistant to environmental factors than the typical intestine bacteria of the FC (*Escherichia coli*) group.

Relatively higher numbers of indicator bacteria in water of pond No. 1 than in the remaining ponds of the technological system suggest (Legendre et al. 1984, Troussellier et al. 1986). that their density there is being controlled by a continuous sewage inflow having both, high concentration of organic substances and microorganisms, for longer periods of time. This was probably affected by technological conditions of the sewage purification process, as well as by periodical removal of active deposits from the secondary settling containers of the sewage treatment plant. This phenomenon occurred especially in autumn during the intensification of fruit and vegetable processing. The washed out sediment entered with sewage pond 1 and cumulated mainly there. All other environmental factors (temperature, sunlight, pH) could not have significantly affected bacterial density in water of this reservoir. Sedimentation (Gannon et al. 1983. Troussellier et al. 1986) and sewage retention time (Marais 1974) might be the only factors which significantly lower their numbers in water of pond 1, at the point of sewage flow entrance. In water of the remaining three ponds of technology line, where the organic substances content is lower, their "buffering" effect is decreased and modified by other physical-chemical factors. Small dissolution with spring water may also occur in ponds 3 and 4.

Properly planned and exploited ponds (Bartone and Arlosoroff 1987, Mayo and Gondwe 1989) having sufficient sewage retention time should provide 99.99% FC (*Escherichia coli*) reduction. This corresponds to their number not exceeding 1000 MPN 100 cm^{-3} at the outflow point (Meiring et al. 1968). Outflow waters with such densities of these bacteria are acceptable for irrigation purposes of cultivated plants, for sports and recreation areas, and might be directly carried to retention reservoirs (IRCWD 1989, WHO 1989). Reduction of FC (*Escherichia coli*) numbers at the exit from pond 4 had approached nearly 100% in 12 samples out of 20, however, in only one sample the numbers exceeded of 1000 MPN · 100 cm^{-3}. Such effect has been obtained in conditions of 2–3 times longer than reported (Bartone and Arlosoroff 1987) sewage retention time in ponds, at sewage outlet from stabilizing ponds of the Experimental Centre for Biological Treatment of Wastewater (ENTRABES) in Brazil, where the climatic conditions are more favourable. When sewage was transported through only pond 1 (at the retention time of 12 and 9 days in December 1986 and January 1987), the reduction degree of FC (*Escherichia coli*) numbers at the outlet amounted to 21.5 and 99.9%, respectively. Numbers of these bacteria exceeded acceptable values. Degree of reduction numbers of TC, FC, and FS found in the present study was comparable to data of Hoadley et al. (1974) (99.3% for *Escherichia coli*, and 84.4% FS; Ranganathan et al. (1974), (98.3% for *E. coli*, 98.7% bacteria reduction for the group Coli-Aerogenes, and 98.5% for FS) in stabilization ponds in the United States; and Hejkal et al. (1983) (99.7% FC reduction, 99.5% for FS) in stabilization ponds in the U.S. used for culture of commercial fish. On the other hand, the reduction numbers of TVC 20 °C and TVC 37 °C were comparable to data of Post (1970) for ponds in Logan, Utah, the U.S. (0–75% reduction of TVC 20 °C, and 0–80% reduction of TVC at 37 °C.

6. SUMMARY

The present work shows results of three years studies on the numbers and reduction stage of pollution indicator bacteria (total bacteria on nutrient agar at 20 and 37 °C) and of the sanitary state in water of the sewage-aquaculture system of 4 ponds at the sewage treatment plant. The sanitary state indicator bacteria included total coliforms, fecal coliforms, and fecal streptococci. Sewage treatment by the active settlement method included domestic sewage and that incoming from fruit and vegetable processing in the town of Olsztynek, Masurian Lakeland (Fig. 1). The ponds of 1 ha surface area each, and the average depth of 1.1–2.2 m act as the third stage of sewage treatment. In the study period of 1986–1988 they were used for carp and white fish fry cultures. Sewage flow-through ponds was done by outlet boxes having an upper entrance. Additional outlet boxes in ponds enable direct outflow of sewage into the reservoir. For the reason of fish cultivation in ponds, the sewage was not always carried through all four ponds (Fig. 2). Hydraulic detention of ponds varied from 795 to 2181 m^3 24 h^{-1}.

TVC (Total Viable Count) at 20 and 37 °C in treated sewage entering pond 1 from sewage treatment plant was typical for highly polluted surface waters. It decreased gradually in the consecutive ponds of technological system reaching, at the outlet from pond 4. values 1000–10 000 times lower, and characteristic for slightly polluted, or clean waters. Total coliform numbers (TC), fecal coliforms – *Escherichia coli* (FC) and fecal streptococci (FS) in treated sewage entering pond 1 ranged from several tens to several hundred thousands (FC and FS), or 1.1 mln (TC) MPN (Most probable number) 100 cm^{-3} (Fig. 3). In the consecutive ponds of technology system the average number of these bacteria decreased gradually. The average for the study period bacteria reduction numbers of TVC 20 °C, TVC 37 °C, TC, FC and FS in waters of the investigated ponds, observed at the outlet from pond 4 corresponded, respectively, to 99.2, 93.2, 98.5, 99.9 and 99.2% (Table 1). Only in one outflowing water sample from pond 4 (in June 1987), and in two samples of water carried directly from pond 1 into the river (in December 1986 and June 1987) FC numbers exceeded 1000 MPN 100 cm^{-3} (Fig. 4).

7. POLISH SUMMARY

Praca obejmuje wyniki 3-letnich badań liczebności i stopnia redukcji bakterii wskaźnikowych stanu zanieczyszczenia (ogólna liczba bakterii na agarze odżywczym w temp. 20 i 37 °C) i stanu sanitarnego (ogólna liczba bakterii z grupy pałeczki okrężnicy, liczba bakterii kałowych z grupy pałeczki okrężnicy, liczba paciorkowców kałowych) w wodzie systemu ścieki-akwakultura 4 stawów przy oczyszczalni ścieków (metoda osadu czynnego) bytowo-gospodarczych i przetwórstwa owocowo-warzywnego w Olsztynku na Pojezierzu Mazurskim (rys. 1). Stawy te o powierzchni około 1 ha każdy i głębokości 1,1–2,2 m (średnio) funkcjonują jako trzeci stopień oczyszczania ścieków. W okresie badawczym 1986–1988 stawy były wykorzystywane do hodowli karpia i

narybku siei. Przepływ ścieków przez stawy odbywał się za pomocą mnichów przelewem górą. Dodatkowe mnichy w stawach pozwalały na bezpośrednie odprowadzenia ścieków do odbiornika. W związku z rybackim użytkowaniem stawów ścieki nie zawsze przepływały przez wszystkie 4 stawy (rys. 2) Obciążenie hydrauliczne stawów było zmienne, od 795 do 2181 m^3 24 h^{-1}.

Liczby TVC 20 °C i TVC 37 °C w ściekach oczyszczonych, dopływających do stawu 1 z oczyszczalni ścieków były typowe dla silnie zanieczyszczonych wód powierzchniowych. W wodzie kolejnych stawów ciągu technologicznego liczby te zmniejszały się stopniowo, osiągając na odpływie ze stawu 4 wartości 1000–10 000-krotnie niższe, właściwe dla wód nieznacznie zanieczyszczonych lub czystych. Liczby TC, FC i FS w ściekach oczyszczonych dopływających do stawu 1 wahały się od kilkudziesięciu do kilkuset tysięcy (FC i FS) lub 1,1 mln (TC) MPN 100 cm^{-3} (rys. 3). W wodzie kolejnych stawów ciągu technologicznego średnia liczba tych bakterii stopniowo się zmniejszała. Stopień redukcji liczebności TVC 20 °C, TVC 37 °C, TC, FC i FS w wodzie badanych stawów "mierzony" przy odpływie ze stawu 4 (średnio za okres badawczy) i wynosił odpowiednio: 99,2; 93,2; 98,5; 99,9 i 99,2% (tab. 1). Tylko w jednej próbie wody odpływającej ze stawu 4 (w czerwcu 1987 r.) oraz w dwóch próbach wody odprowadzanej do rzeki bezpośrednio ze stawu 1 (w grudniu 1986 r. i w styczniu 1987 r.) liczba FC przekraczła 1000 MPN 100 cm^{-3} (rys. 4).

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