Effectiveness of Sewage Treatment Based on Selected Faecal Bacteria Elimination in Municipal Wastewater Treatment Plant in Toruń

Z. Paluszak*, A.Ligocka, B. Breza-Boruta

Department of Microbiology, University of Technology and Agriculture, Bernardyńska 6/8, 85-029 Bydgoszcz, Poland

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Abstract

The sewage treatment plant was monitored to determine the efficiency of technologies employed to reduce microbiological threats. Every four weeks, for a year, sewage was sampled to investigate the effects of post-mechanical and post-biological treatment. Changes in *Escherichia coli*, D-group streptococci and Salmonella spp. number were analyzed. The reduction of the bacteria in sewage treated mechanically was not significant and did not exceed 1 log. However, a significant reduction in an average number was found. In addition, seasonal variation was found to correlate with the number of each of the selected bacteria groups.

Keywords: sewage, *Escherichia coli*, *Salmonella*, D-group streptococci, sewage treatment

Introduction

Malfunctioning sewage treatment plant operation in the open system poses a hazard to the biological balance of the environment. The greatest danger originates from the microbiological load contained in sewage, especially pathogenic microorganisms and their toxins. Pathogenic microorganisms are most commonly represented and include the *Enterobacteriaceae* family, enterococci, sporulating anaerobic bacteria and others that naturally inhabit human and animal alimentary canals [3]. In addition, however, sewage has certain pathogenic microorganisms from diseased humans and animals, from slaughterhouses and other sources. Pathogenes including *Salmonella, Shigella, Vibrio* and *Clostridium*, enteropathogenic strains of *Eschericha coli* [17,24], *Pasteurella*, *Campylobacter, Brucella* [21], viruses and many others can be isolated from sewage. A number of microorganisms depends on many factors including the type of sewage, their concentration, degree of decay of organic organisms, temperature and pH. One mL of sewage commonly provides from a few to several million bacteria. *Salmonella* are especially dangerous and as such they are used as a sanitary index in Europe [5, 8, 10, 23]. Infections and food poisonings in humans inflicted by *Salmonella* constitute a very important epidemiological concern. In Poland over 94% of mass food poisoning cases and alimentary canal infections are caused by those bacteria [11].

To evaluate the effectiveness of water, waste, and sewage treatment systems, an indirect method is used which identifies bacteria considered to be the so-called sanitary indices. Due to high intensity and survival rate, the number of *E. coli* and faecal streptococci is marked. Bearing in mind an epidemiological threat, utilisation and water treatment are monitored, particularly for the occurrence of *Salmonella*.

*Corresponding author

The aim of the present paper was to provide a microbiological evaluation of municipal wastewater from the Sewage Treatment Plant (STP) in Toruń, to investigate their successive treatment stages and to determine sewage treatment effectiveness based on elimination of selected faecal bacteria.

Materials and Methods

The municipal sewage treatment plant in Toruń works as mechanical-biological STP and cleaned sewage is released into the Vistula river.

The sewage for investigation was taken at monthly intervals from January to December 2001. The samples for analysis were collected from the following treatment operations: I grate (crude sewage), II storage tank (mechanical pre-treatment) and III output (cleaned sewage) after biological treatment.

Salmonellas were enumerated using pepton water C (24h, 37^O) from which the material (0,1 ml) was taken to Rappapourt medium (24h, 43^O). After incubation 0,1 ml aliquot of each dilution was plated in BPL Agar (24h, 37^O). Finally, the serological test (HM serum triplicate) was made.

E. coli were determined on Mac Conkey broth (24h, 37^O), from which the material was inoculated onto agar with tergitol and 1% TTC (24h, 43^O). To confirm obtained results API test was made (20E, bioMerieux).

The number of D-group streptococci was determined by using azid-glucose bouillon (48h, 37^O), from which the material was inoculated onto agar with kanamycin and esculin (48h, 37^O). For the final identification of these bacteria the serological Phadebact® Strep D test was used. The number of *Salmonella, E. coli*, and D-group streptococci was calculated according to three tube most probable number (MPN) method.

All obtained data were analysed using the statistical package "STATISTICA". The differences between bacterial number were estimated using t-Student test (p=0.05).

Results

The results of microbiological research are presented in Table 1. The sewage inflow to the sewage treatment plant showed a high number of the bacteria tested. *E. coli* ranged from 4.5 x 10^4 to 9.5 x 10^8 cells/mL, an average of 3.75 x 10^6 cells/mL. Maximum number was found in January, but minimum - in December and April. Raw sewage provided slightly less faecal streptococci whose number was found to minimise over the research period ranged from 1.5 x 10^3 to 4.5 x 10^6 cells/mL, 4.5 x 10^5 cells/mL on average. The number was maximum in March and was found to be at its lowest in January. Additional results showed that *Salmonella* spp. was numerous in sewage which had not been mechanically treated. Populations were observed in samples of 7-month research material and their count ranged from 0.4 x 10^2 to 2.0 x 10^3 cells/mL. Results obtained show that mechanical treatment had a varied effect on the populations under study. However, the effectiveness was generally low and insufficient. The number of *E. coli* in sewage once it passed through the initial settling tank ranged from 1.1 x 10^4 to 4.5 x 10^8 cells/

Table 1. The number of respective groups of bacteria table over multiple months of research selective to sampling location.

MONTH	*E. coli*			D-group streptococci			*Salmonella* spp.		
	Sampling location*								
	I	II	III	I	II	III	I	II	III
October	2.0 x 10^6	9.5 x 10^5	3.0 x 10^5	9.5 x 10^5	9.5 x 10^5	4.5 x 10^3	n/d	n/d	n/d
November	2.5 x 10^5	1.1 x 10^4	0.9 x 10^2	4.5 x 10^5	9.5 x 10^3	0.4 x 10^2	0.4 x 10^2	0.9 x 10^2	n/d
December	4.5 x 10^4	4.5 x 10^4	1.1 x 10^2	2.5 x 10^5	1.1 x 10^4	0.9 x 10^2	0.9 x 10^2	2.0 x 10^2	n/d
January	9.5 x 10^8	2.5 x 10^8	2.5 x 10^4	1.5 x 10^3	4.5 x 10^3	n/d	7.5 x 10^2	3.5 x 10^2	n/d
February	4.5 x 10^8	4.5 x 10^8	1.5 x 10^3	4.5 x 10^5	4.5 x 10^5	2.5 x 10^2	0.4 x 10^2	1.5 x 10^2	0.4 x10^2
March	4.5 x 10^6	2.5 x 10^5	4.5 x 10^2	4.5 x 10^6	2.5 x 10^5	4.5 x 10^2	2.0 x 10^3	2.5 x 10^3	n/d
April	4.5 x 10^4	4.5 x 10^4	1.1 x 10^2	2.5 x 10^4	2.5 x 10^3	n/d	n/d	n/d	n/d
May	4.5 x 10^6	4.5 x 10^6	4.5 x 10^4	4.5 x 10^5	4.5 x 10^5	2.5 x 10^4	0.9 x 10^2	n/d	n/d
Juni	2.5 x 10^6	2.5 x 10^6	4.5 x 10^3	1.5 x 10^5	1.5 x 10^5	1.5 x 10^3	n/d	n/d	n/d
July	2.5 x 10^6	2.0 x 10^6	2.5 x 10^3	4.5 x 10^4	4.5 x 10^4	2.0 x 10^2	3.5 x 10^2	0.4 x 10^2	n/d
August	2.0 x 10^6	2.5 x 10^5	4.5 x 10^3	4.5 x 10^5	4.5 x 10^4	9.5 x 10^3	n/d	n/d	n/d
September	1.5 x 10^6	4.5 x 10^5	4.5 x 10^3	9.5 x 10^5	9.5 x 10^4	2.0 x 10^4	n/d	n/d	n/d
Average	3.75x10^6	3.75x10^5	2.9x10^3	4.5x10^5	4.8x10^4	2.5x10^2	1.3x10^1	0.9x10^1	n/d

*Sampling locations: I grate (crude sewage), II storage tank (mechanical pre-treatment), III output (after biological treatment).

mL, 3.75 x 10^5 cells/mL on average. The elimination of streptococci was more effective. Having passed through the initial settling tank, their average number decreased by 1 log and amounted to 4.8 x 10^4 cells/mL. Variation in the number of the bacteria in mechanically treated sewage was found to be considerable. As for *Salmonella* spp., no reduction was observed in their number in mechanically treated sewage in November, December, February and March, while in January and July their population decreased and in May they were absent in all of the samples. A considerable reduction in the index bacteria and *Salmonella spp*. was observed in the further treatment stages. The sewage which was directed to the collector *E. coli* rods isolated ranged from 0.9 x 10^2 to 4.5 x 10^4 cells/mL, 2.9 x 10^3, on average. The number of streptococci decreased by a few log in the collector reaching an average of 2.5 x 10^2 cells/ml. *Salmonella* was isolated once only from outflow sewage.

A statistical analysis showed significant differences in the number of *Escherichia coli* and D- group streptococci at subsequent sewage treatment stages (Table 2). An average number of these bacteria prior to treatment is significantly higher then their number after biological treatment ($p<0.05$), which shows high sewage treatment effectiveness. The present research also shows an effective elimination of *Salmonella* spp. in the process of the sewage treatment.

One shall observe a high deviation from the standard in the number of bacteria, which points to a considerable variability in the count of microorganisms isolated at subsequent research stages. The statistical calculations are given in Table 2. The degree of reduction in the microorganisms studied over sewage treatment seasons are shown in Figs. 1-3. *E. coli* numbers were effectively reduced in all the seasons, especially during winter and spring, where reduction was 99.9% and 99.8% respectively (Fig. 1). A similar trend was observed in faecal streptococci, whose reduction has never been lower than 99%. During spring and summer it amounted to 99.94% (Fig. 2). *Salmonella* spp. were eliminated in sewage in all the research months except for February (Tab.1).

Discussion

Most pathogenic microorganisms remain in sewage sludge; however, some of them together with the resultant effluent can reach the environment. The quality of the treatment methods applied is thus of primary importance here. In general, the effectiveness of mechanical treatment is low. In the present study the reduction of bacteria in the sewage treated mechanically was not significant and did not exceed 1 log. This trend is supported by results reported by other authors. Strauch and Böhm [24] observed a reduction rate in *Salmonella* spp. in sewage treated mechanically by 10-90%, namely not exceeding 1 log. Kawamura and Kaneko [6] found a decrease in *E. coli*, enterococci and sporulating anaerobic bacteria number by 60-70%. The main factor contributing to reduction of pathogens in sewage, however, is biological treatment. Here the degree of bacteria reduction is found to range 75-95% on those sprayed and on those to which active sludge was applied, respectively. Treatment of faecal index *E. coli* bacteria with active sludge can be effective, with reduction exceeding 99% [25]. Bonde [1], analyzing the effectiveness of biological sewage treatment, observed that the number of *E. coli* decreased by 95%. A high number of microorganisms in raw sewage does not allow for the possibility of such inefficiency, however, especially when the resultant effluent shows a high number of index microorganisms due to the risk of pathogen penetration into the waters collecting treated sewage [24]. A danger exists that multiplying pathogenic bacteria may penetrate the environment. This depends on competition with native microorganisms and nutrient availability amongst other factors [2]. Pathogens are also capable of moving large distances from their sewage treatment plant. Popp [15] observed a similar concentration of *Salmonella* spp.10 km away from the sewage discharge to those identified in the treated sludge.

The present microbiological analysis showed that the yearly mean of *E. coli* was much lower in the treated sludge as compared with the raw sludge with a very high (99,9%) *E. coli* reduction for post treatment. Szumilas et

Table 2. Results of statistical analysis of the researched groups of microorganisms in respective sampling locations. X –significant differences for $p<0.05$.

Analysed Group	Sampling location*	Bacterial count logarithm	Standard deviation [%]	Student T test (p<0,05)		
				I	II	III
Escherichia coli	I	6.43	131.5%		X	X
	II	6.01	140.4%	X		X
	III	3.39	110.7%	X	X	
D-group streptococci	I	5.25	91.7%		X	X
	II	4.76	84.8%	X		X
	III	2.53	149.2%	X	X	
Salmonella spp.	I	1.32	127.6%			X
	II	1.16	128.4%			
	III	0.13	46.2%	X		

* Sampling locations: as in Table 1

al. [25] evaluated the effectiveness of reduction in the count of E. *coli* faecal bacteria in the Gdańsk-Wschód and Gdynia Dębogórze Sewage Treatment Plants and showed that treatment reduced levels of *E. coli* by over 99.99%. The fact remains, however, that the number of these bacteria in the treated sludge was found to be considerably higher than in the sludge from the treatment plant, amounting to $2x10^5$ cells/mL to $1.9x10^6$ cells/mL. The effectiveness of elimination of respective microorganisms varies. Ruhle [19] recorded a drop in the count of *Yersinia* sp. by 2.5 log, although he still isolated pathogenic strains of *Yersinia enterocolitica* from treated sludge. Gerhardt, Teitge [4] and Popp [16] showed that the number of *E. coli* in the treated sludge decreased by 1-2 log. All that suggests that it is not only the extent of the reduction in the number of bacteria which reflects the efficiency of the treatment but also the real number of index microorganisms discharged to the collector that leads to potential environmental threat.

The present research shows that the average number of faecal streptococci was considerably lower than that of *Escherichia coli.* The ratio of *E. coli* to *Streptococcus faecalis* in the untreated sludge is 15:1, while 7:1 in the treated sludge, which corresponds with reports of other authors. Ziemińska et al. [32] show that the bacteria number of *E. coli* in the Warsaw sludge exceeded that of the *Streptococcus* genus, by an average of 8-10 times, depending on the identification method used. An average ratio of *E. coli* to faecal streptococci is typical for municipal sewage.

The reduction in the faecal streptococci in the treated sludge amounted to over 99.8%. Similar results were obtained by Szumilas et al. [25] who investigated the degree of eliminating faecal streptococci from the Gdańsk and Gdynia sewage treatment plants. Research carried out by Staroniewicz [22] however, shows only a low reduction in the biologically treated sludge which did not exceed 84%.

A special threat is posed by *Salmonella* spp. present in sludge [31]. They are often isolated both from basin waters and from rivers, which is confirmed by examining 1549 samples from stream water used for swimming in southern Bavaria from 1986-1990 which identified 195 positive cases. Since 1988, *Salmonella enteritidis* has been isolated more frequently than *S. typhimurium*, but 10^2 cells/mL is enough to cause disease symptoms [20].

Olszewska [14] reports on the number of *Salmonella* spp. in the waters of the Brda River. She recorded concentrations ranging from $4.0x10^0$ to $9.5x10^2$ cells/100ml, which shows that sewage discharged into waters or soil can result in infection by these pathogens. The research of waters of the Bays of Gdańsk and Puck, being the catchment area of considerable number of Polish rivers, showed the occurrence of these bacteria in about 30% of samples [10]. Different results were recorded by Kohl [10], who was researching the sanitary status of the Gruk and Glon Rivers, and did not isolate *Salmonella* spp. in any of the samples. Zalewski [24], investigating the sewage discharged by the meat-processing plant, observed the occurrence of *Salmonella* spp. rods in 80% of the samples analyzed.

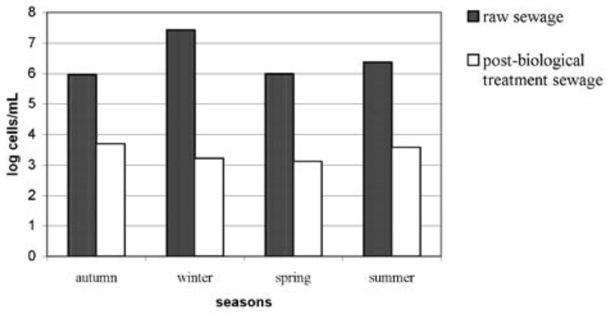

Fig. 1. Reduction in the number of *Escherichia coli* after sewage treatment over respective seasons (average).

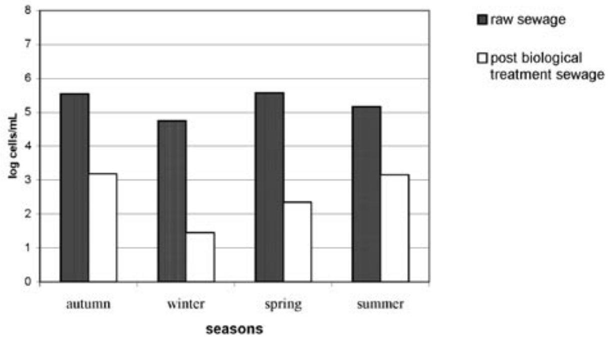

Fig. 2. Reduction in the number of D-group streptococci after sewage treatment over respective seasons (average).

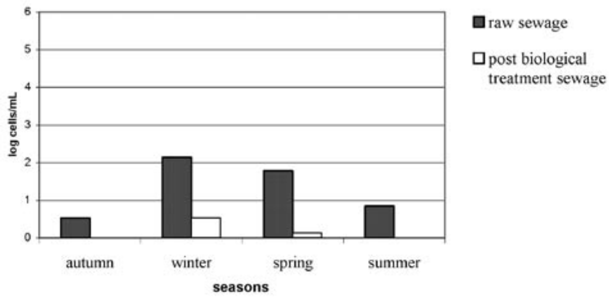

Fig. 3. Reduction in the number of *Salmonella* spp. after sewage treatment over respective seasons (average).

The present research showed the relationship between the degree of reduction of the microbiological contamination and subsequent seasons, and results suggest that despite treatment, effectiveness of bacterial elimination is largely dependent on the weather. The research carried out to investigate the effects of environmental factors on the *E. coli* survival rate in water has shown that temperature has one of the greatest impacts [11]. Rheinheimer [16] noticed that the number of bacteria is higher during the winter period. This is confirmed by the present research, which recorded an increase in the number of *E. coli* bacteria in January and February 2001 by 2 log as well as that despite their high number in winter, they were most considerably reduced over the total research period. Reduction of the number of faecal streptococci group D was highest, like in *E. coli,* in winter. A greater survival rate of this group of microorganisms in low temperature, a high elimination can suggest an effective sewage treatment. Literature reports on *Salmonella* spp. survival rate getting longer at lower temperatures and higher share of solids in the sewage. The greatest survival rate is recorded below 10°C and at the share of over 5% of solids [21]. The present research shows the high effectiveness of the sewage treatment plant studied, which is of special importance as the resultant treated sewage does not cause considerable contamination of the Vistula River near Toruń.

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