Sanitary-Bacteriological Evaluation of Meadow Soils Irrigated with Biologically Treated Sewage

S. Niewolak[1], S. Tucholski[2], E. Radziejewska[1]

[1] Department of Environmental Microbiology
[2] Department of Land Reclamation and Environmental Management
University of Warmia and Mazury in Olsztyn, 10-957 Olsztyn-Kortowo, Poland

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Abstract

Studies were carried out to determine numbers of bacteria indicatory of pollution (total number of bacteria on broth-agar at 20 and 37°C) and sanitary state (Total coliforms, fecal coliforms, fecal streptococci and *Clostridium perfringens)* and of potential pathogens *(Aeromonas hydrophila, Staphylococcus sp., Pseudomonas aeruginosa, Salmonella sp.)* and fungi on Trichophyton Agar 1 in soils from meadow subject to 8 different variants of irrigation and fertilization in the vicinity of a treatment plant in Olsztynek. Studies were performed in 1996 and 1997. Experimental variants comprised (A) non-irrigated plots (control); (B) irrigated with fresh water; (C) irrigated with biologically-treated sewage (outflow from a waste treatment plant); (D,E, F) irrigated with treated sewage stored in a biological pond with a basic dose, the increased dose up to 150% and 200%; (G) NPK minerally fertilized; (H) NPK minerally fertilized and irrigated with fresh water. Bacteria determined on broth-agar at 20°C were more numerous in the soils of plots irrigated with effluents from a treatment plant and stored in a biological pond. The differences in the number of bacteria determined on broth-agar at 37°C in the soils of different variants were not significant. Fecal coliforms, fecal streptococci (enterococci), *Clostridium perfringens, Aeromonas hydrophila* and *Staphylococcus sp.* were generally more numerous in the soils of different irrigated-fertilized variants, less numerous in non-irrigated soils (control). The differences in the number of fecal coliforms in the soils of particular variants were ambiguous. All groups of microorganism were more numerous in the surface layer of the soil. Irrigated dose of treated sewage stored in a biological pond did not influence their number in the soil. Fungi determined on Trichophyton Agar 1 were a constant component of microflora of the examined soils whereas *Salmonella sp.* was determined sporadically.

Keywords: soil, sewage, irrigation, fertilization, bacteria of sanitary significance, potentially pathogenic fungi

Introduction

Technical difficulties and high cost of abounded sewage utilization as well as requirements for sewage outflows let out into surface waters tend to use soil as a natural biological filter eliminating compounds of nitrogen and phosphorus. Phosphorus compound can be used by crop plants preventing them from penetrating into surface waters (eutrophication) and underground waters (toxic nitrates). The methods of agricultural utilization of sewage outflow require constant hygienic-sanitary control in order not to contaminate plants, especially those which are eaten in raw state, by heavy metals (Cd, Hg, Pb, Cu, Ni, Zn and others) and pathogenic microorganisms (pathogenic viruses and bacteria, protozoans, eggs of parasitic worms). A proper utilization of sewage outflows in agriculture cannot lead to an increase of soil

Correspondence to: Prof. S. Niewolak

salinity and the decrease of their productive value [6]. This paper presents the results of the examination of the degree of contamination and sanitary-bacteriological state of meadow soils being irrigated with effluents from mechanical-biological waste treatment plant in Olsztynek or being minerally fertilized.

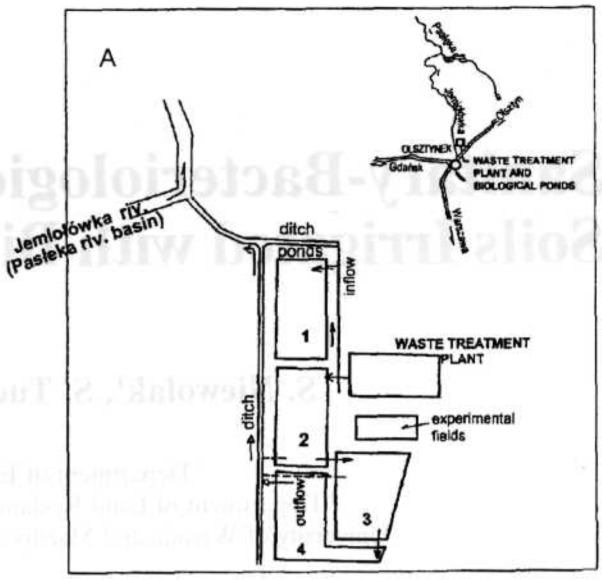
Materials and Methods

The area of research. Studies were carried out in 1996 and 1997 in the area belonging to the municipal waste treatment plant in Olsztynek (Mazurian Lake District). The meadow was characterized by light soil made from light clay sand with some clay layers. Soil quality corresponded to IVb class, it is a rye complex. The distribution of plant species was uniform. *Dactylis glomerata, Poa pratensis, Elymus repens* and *Taraxacum officinale* dominated [13]. The meadow was divided into 32 plots of unit 15.75 m^2 (Fig. 1).

Sewage. 1) Effluents from a mechanical-biological waste treatment plant (screens chamber, grit chamber, primary settling tank combined with primary aeration chamber, activated sludge chamber, secondary chamber) discharged by houses and a factory processing fruit and vegetables in Olsztynek, and 2) the same effluents from a waste treatment plant stored in a biological pond were used in the experiment. Detailed data concerning physico-chemical and bacteriological parameters of these waters were given in earlier papers [13, 17].

Experiment. Sanitary and bacteriological studies of meadow soils were carried out in 8 irrigated-fertilized variants, each repeated 4 times (Table 1).

Sampling procedure. Samples of soils were collected from 4 plots in each variant in sunny weather. In 1996 the soil samples were collected on 11 June, 31 July and 18 October; in 1997 on 14 March, 2 June, 30 July and 8 October. In non-irrigated and non-fertilized (control) (A) and irrigated with treated sewage stored in a biological pond (D) soil samples were collected from 0-10 cm; 15-25 cm and 30-50 cm layers. In the plots of the remaining experimental variants the soil samples were collected only from 0-10 cm layer. 12 soil samples were collected from each plot (48 samples in a given variant) to a sterile cuvette made of enameled metal, mixed in situ and in sterile glass dishes were carried to a laboratory. Before microbiological analysis the soil was mixed again, 10 g samples were weighed and put into Erlenmeyer's flasks



B

C 25	B 26	G 27	F 28	D 29	A 30	H 31	E 32
A 17	D 18	E 19	B 20	G 21	H 22	F 23	C 24

H 9	G 10	C 11	D 12	E 13	F 14	A 15	B 16
B 1	E 2	F 3	H 4	A 5	C 6	D 7	G 8

Fig. 1. Scheme of ponds (A) and experimental fields (B) in Sewage Treatment Plant in Olsztynek. For explanation see Table 1.

Table 1. Experimental variants of irrigating and fertilizing of soil of meadow near the treatment plant in Olsztynek.

Experimental variants	Irrigation and fertilization combinations
A	Control, without irrigation and fertilization
B	Irrigation with clean water (basic dose)
C	Irrigation with biologically treated sewage (discharged by the treatment plant), basic dose (243.7 mm in 1996 and 258.4 mm in 1997)
D	Irrigation with treated sewage from the biological pond, basic dose
E	Irrigation with treated sewage from the biological pond, 150% of the basic dose
F	Irrigation with treated sewage from the biological pond, 200% of the basic dose
G	Mineral fertilization: Dose in 1996: N – 90 kg/ha; P_2O_5 – 100 kg/ha; K_2O – 135 kg/ha. Dose in 1997: N – 120 kg/ha; P_2O_5 – 100 kg/ha; K_2O – 180 kg/ha
H	Mineral fertilization as in variant G and irrigation as in variant B

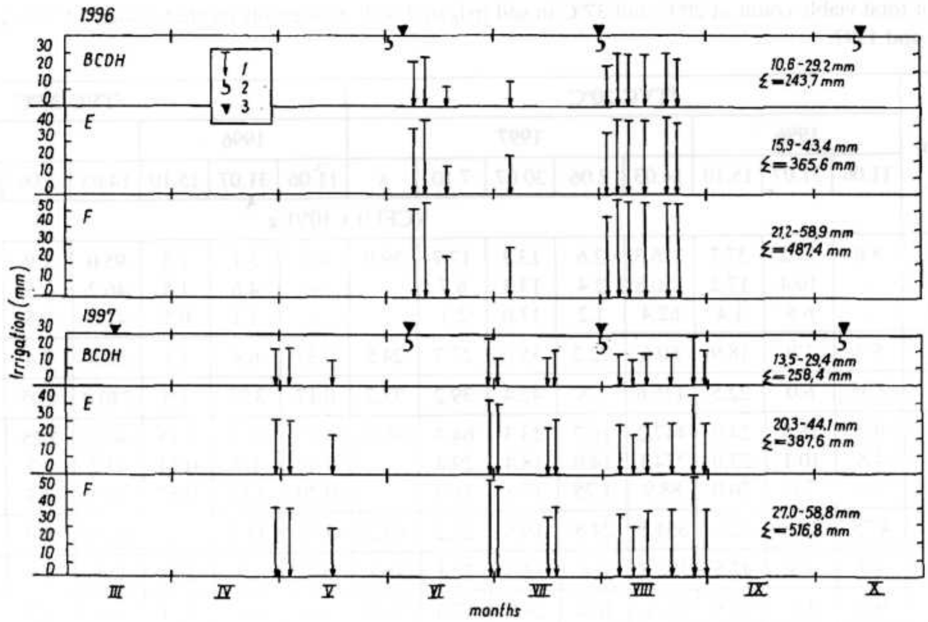


Fig. 2. Scheme of meadow soil irrigation with biologically treated sewage (sewage effluent) (I) and with water from biological pond at Sewage Treatment Plant in Olsztynek (II)
A, B, C...H experimental variants; 1 - irrigation; 2 - hay-making; 3 - soil sampling for studies

with 90 ml sterile physiological solution NaCl. They were homogenized for 15 min on a magnetic stirrer and "inoculated" on earlier prepared culture media with dilution 1: 10, 1:100, 1:1000, 1:10,000 and 1:100,000 in the same physiological salt NaCl. At the same time additional 10 g soil portions (in 2 repetitions) were heated at 105°C for 24 h to determine dry mass.

Microbiological determinations. Microbiological studies comprised the determination of total number of heterotrophic bacteria in broth-agar at 20 and 37°C, total coliforms, fecal coliforms, fecal streptococci (enterococci), *Clostridium perfringens, Aeromonas hydrophila, Staphylococcus sp., Pseudomonas aeruginosa, Salmonella sp.* and fungi on Trichophyton Agar 1 - according to the methodology presented in Table 2. The determinations were carried out in 3 parallel repetitions of the same soil sample counting all or only typical colonies according to the groups and kinds (species)of bacteria and fungi. Positive results for the presence of coliforms, fecal streptococci (enterococci), *Clostridium per-*

Table 2. The media and incubation regime in the determination of the number of indicator of pollution and sanitary state and of potentially pathogenic microorganisms in soil.

Microorganisms	Nutrient medium	Incubation	
		Temp. °C	Time hours
1. Total viable count at 20°C	Broth-Agar	22	72
2. Total viable count at 37°C	Broth-Agar	37	24
3. Total coliforms	Eijkman Medium	37	48
4. Fecal coliforms	Eijkman Medium	44.5	24
5. Fecal streptococci/enterococci	Slanetz-Bartley Medium	37	72
6. Clostridium perfringens[1]	Wilson-Blair Medium	37	18
7. Aeromonas hydrophila	Rimley-Shotts Medium	37	24
8. Staphylococcus sp.	Bacto Staphylococcus Medium 10 Difco	37	24
9. Pseudomonas aeruginosa	mPA Medium	37	24
10. Salmonella sp.	Selective medium of Kauffman	37	24
	Differentiation on XLD (Difco)	37	24
11. Potentially pathogenic fungi	Trichophyton Agar 1 (Difco)	37	24

[1] after heating of soil samples at 80°C/10 min.

Table 3. Number of total viable count at 20°C and 37°C in soil irrigated with biologically treated sewage at Sewage Treatment Plant in Olsztynek, in 1996 and 1997.

Variant	Depth (cm)	[1]TVC 20°C								[2]TVC 37°C							
		1996			1997					1996			1997				
		11.06	31.07	15.10	14.03	2.06	30.07	7.10	x	11.06	31.07	15.10	14.03	2.06	30.07	7.10	x
		[3](CFU) x 10^6/1 g															
A	0-10	8.6	17.2	37.7	316.3	2.6	13.3	17.7	59.0	4.0	5.1	1.5	95.0	1.9	1.9	1.7	15.8
	15-25	–	10.4	17.2	150.8	2.4	13.3	6.7		–	4.6	1.5	46.2	1.5	0.6	1.1	
	30-50	–	6.8	1.4	62.4	1.2	17.0	2.1		–	1.1	0.5	41.8	0.5	0.5	0.6	
B	0-10	5.7	1.9	18.9	60.6	22.2	35.0	27.7	24.5	0.37	6.4	1.1	52.6	7.4	2.4	3.0	10.1
C	0-10	2.35	6.0	22.5	426.6	1.8	42.4	39.2	77.2	0.17	3.75	1.6	240.0	1.05	3.4	2.95	36.1
D	0-10	9.2	15.8	24.0	447.2	16.7	23.3	64.3	85.7	0.47	3.35	2.15	46.2	3.25	1.8	4.45	8.8
	15-25	4.8	10.1	23.0	274.1	14.0	18.4	29.4		0.40	1.7	0.54	43.7	2.3	0.66	1.55	
	30-50	3.6	7.0	20.0	88.9	1.75	17.8	11.0		0.20	1.55	0.52	40.3	2.3	2.2	1.0	
E	0-10	4.75	28.6	22.6	351.8	24.5	19.8	25.2	68.2	3.95	11.3	2.1	51.9	6.05	2.65	2.6	11.5
F	0-10	4.9	3.9	42.5	178.3	4.4	12.3	22.3	38.4	10.2	1.3	2.75	3.95	4.5	1.55	3.55	3.4
G	0-10	9.3	4.8	86.8	16.4	16.4	29.9	47.0	30.0	1.1	8.1	32.1	5.0	1.3	3.7	3.0	7.1
H	0-10	11.3	5.9	11.7	28.4	27.4	21.4	35.6	20.2	0.24	2.65	38.3	42.3	5.05	3.55	3.5	13.6

[1,2] - Total viable count on nutrient medium at 20 and 37°C

[3] - CFU - Colony Forming Units

fringens, Aeromonas hydrophila, Staphylococcus sp., Pseudomonas aeruginosa, Salmonella sp. were proved according to the methodology given in the paper concerning the influence of irrigation on grass bacteriological contamination [13]. The obtained results of the determination of bacteria counts determined on broth-agar at 20°C, fecal coliforms and *Clostridium perfringens* were compared to the criteria of the determination of the degree of soil contamination given in literature [14].

Results

The number of indicatory bacteria of the contamination degree and sanitary state and potentially pathogenic in the soils of experimental plots.

Indicatory bacteria of the contamination degree (Table 3). Total counts of bacteria determined on broth-agar at 20°C (called TVC 20°C) is an indicator of soil contamination degree, an organic substance easily decayed and at 37°C (called TVC 37°C) - domestic sewage. In the soil layer 1-10 cm of different experimental variants TVC 20°C ranged from 1.9 • 10^6 to 426 • 10^6 CFU/1 g dry mass. On average their number was higher in the soil plots irrigated with the effluents from a waste treatment plant and the same effluent stored in a biological pond. Generally they were fewer in spring and summer in 1997, more were found in autumn and winter the same year. The number of TVC 37°C ranged from 0.17 • 10^6 to 240 • 10^6 CFU/1 g dry mass. On the average the lowest number was found in the soils irrigated with fresh water and effluents from a waste treatment plant stored in a biological pond; however, 2-10 times more were observed in the soils directly irrigated with the effluents from a waste treatment plant. The lowest number was generally observed in summer 1996 (in the soils irrigated with fresh water, effluents from a waste treatment plant, the same effluents stored in a biological pond and NPK fertilized), the highest number was in autumn 1996 and winter 1997 (in the soils NPK fertilized and irrigated with fresh water). In non-irrigated and non-fertilized (control) and irrigated soils with waste treated sewage stored in a biological pond TVC 20°C and TVC 37°C decreased in the soil profile. The ratio TVC 20°C:TVC 37°C in 28% non-irrigated and non-fertilized soil samples (control), irrigated with waste treated sewage stored in a pond (150 and 200%) and NPK fertilized was higher than 10; in 42% soil samples irrigated with fresh water and/or NPK fertilized and in 57% soil samples irrigated with sewage effluents directly from the waste treatment plant was lower than 10.

Indicatory bacteria of sanitary state (Table 4 and 5). Clostridium perfringens (0.8-2.0 • 10^3 CFU/1 g dry mass) were the least numerous among the examined indicatory bacteria of sanitary state in the experimental soil variants whereas fecal streptococci (enterococci) called FS were the most numerous (24.9-96.0 • 10^3 MPN/1 g dry mass). The number of total coliforms and fecal coliforms (called TC and FC) were found as 0.03-19.2 • 10^3 MPN/1 g dry mass and 0.03-16.8 • 10^3 MPN/1 g dry mass, respectively. The ratio FC:FS in 61% of the examined soil samples was lower than 0.7 (contamination mainly from animals), in 12% of the soil samples ranged from 0.7 to 4.0 (contamination from people and animals) and in 27% of soil samples was higher than 4 (contamination from people). TC, FS and *Clostridium perfringens* were generally in smaller quantities in non-irrigated and non-fertilized soils (control); however, FC - in the soil irrigated directly with effluents from a waste treatment plant or the same effluents stored in a biological pond and in the soils from

Table 4. The number of total and fecal coliforms in soil irrigated with biologically treated sewage at Sewage Treatment Plant in Olsztynek, in 1996 and 1997.

Variant	Depth (cm)	[1]TC								[2]FC							
		1996			1997					1996			1997				
		11.06	31.07	15.10	14.03	2.06	30.07	7.10	x	11.06	31.07	15.10	14.03	2.06	30.07	7.10	x
		[3]MPN x 10^3/1 g															
A	0-10	4.7	0.5	1.7	0.97	15.7	0.28	5.4	4.17	0.26	0.28	12.3	1.12	0.28	0.28	0.54	2.15
	15-25	–	0.3	0.5	0.26	0.27	0.28	1.2		–	0.27	0.28	0.26	0.27	0.22	0.10	
	30-50	–	0.02	0.27	0.09	0.05	0.15	0.28		–	0.10	1.00	0.05	0.10	0.10	0.03	
B	0-10	1.5	13.0	1.5	0.04	16.8	12.9	1.7	8.7	0.05	0.53	1.5	0.05	13.2	12.9	1.75	4.3
C	0-10	0.47	0.24	5.0	0.03	5.3	13.7	13.3	5.4	0.03	0.3	1.25	0.03	0.3	5.6	0.9	1.2
D	0-10	3.1	4.8	2.5	0.5	13.6	1.15	5.85	4.5	0.26	0.54	1.85	0.04	1.17	0.27	5.85	1.4
	15-25	0.26	4.75	2.1	0.46	0.27	0.5	0.27		0.10	0.48	0.49	0.04	0.27	0.12	0.10	
	30-50	2.4	0.48	1.7	0.25	0.27	0.5	0.11		0.04	0.27	0.31	0.04	0.27	0.12	0.03	
E	0-10	15.6	4.9	12.4	0.26	1.8	5.55	1.3	5.9	0.5	0.5	0.5	0.04	1.9	2.45	0.54	0.91
F	0-10	15.1	17.0	11.6	0.44	1.9	5.5	1.75	7.6	0.5	5.4	14.8	0.05	1.9	2.4	0.54	3.6
G	0-10	1.2	11.3	13.6	2.85	1.75	14.1	1.75	6.6	0.27	1.45	2.45	0.5	0.57	2.55	0.3	1.1
H	0-10	1.2	15.1	16.8	0.20	19.2	16.9	5.5	10.7	0.5	4.85	16.8	0.2	0.62	16.5	0.5	5.7

[1] - Total coliforms;[2] - Fecal coliforms; [3] - MPN - Most Probable Number.

Table 5. The number of fecal streptococci and Clostridium perfringens in soil irrigated with biologically treated sewage at Sewage Treatment Plant in Olsztynek, in 1996 and 1997.

Variant	Depth (cm)	[1]FS								Clostridium perfringens							
		1996			1997					1996			1997				
		11.06	31.07	15.10	14.03	2.06	30.07	7.10	x	11.06	31.07	15.10	14.03	2.06	30.07	7.10	x
		[2]MPN x 10^3/1 g								[3]CFU x 10^3/1 g							
A	0-10	1.15	0.10	2.22	3.0	168.5	29.0	5.4	30.0	0.08	3.4	0.45	0.64	0.02	0	1.2	0.8
	15-25	–	0.10	0.28	1.55	117.6	27.1	2.1	24.8	–	0.32	0.11	0.01	0.003	0	0.06	0.08
	30-50	–	0.01	0.003	1.05	33.7	26.5	0.1	10.5	–	0.2	0.11	0	0.003	0.09	0.005	0.06
B	0-10	0.01	0.36	0.02	20.2	167.7	29.4	5.6	32.4	0.06	4.7	1.0	0.56	7.55	0.002	0.36	2.0
C	0-10	1.15	0.12	0.17	3.3	165.7	3.1	1.15	24.9	0.05	1.6	7.1	0.02	0.38	0.015	0.47	1.37
D	0-10	0.05	0.12	1.4	3.1	308.6	127.6	18.2	65.5	0.24	2.4	1.25	0.03	4.2	0.005	1.0	1.3
	15-25	0.01	0.10	0.025	2.55	150.5	127.5	4.9	39.1	0.34	1.5	1.25	0.005	0.8	0	0.06	0.56
	30-50	0.003	0.01	0.02	2.0	150.5	120.9	0.05	39.0	0.01	0.16	1.25	0	0.15	0	0	0.36
E	0-10	1.55	12.0	1.7	113.4	57.6	5.5	1.15	27.5	0.56	4.65	0.3	0.83	2.8	0.01	0.44	1.37
F	0-10	0.02	0.12	12.2	1.15	190.5	13.5	138.4	50.6	0.22	1.95	0.015	0.56	3.3	0.045	0.20	1.39
G	0-10	1.2	0.45	0.5	0.04	340.0	14.0	127.2	69.0	0.13	0.45	2.7	0.25	5.4	0.06	0.80	1.39
H	0-10	0.02	0.22	1.4	46.4	192.0	1.1	18.4	37.0	0.65	2.15	2.63	0.02	1.9	0.01	0.23	1.08

[1] - Fecal streptococci;[2] - Most Probable Number; [3] - Colony Forming Units.

plots NPK fertilized, in larger quantities they were found in irrigated soils with fresh water and in soils NPK fertilized (FC and FS also in soils irrigated with treated waste stored in a biological pond). Their minimum and maximum soil contamination was observed in a different period depending on the group of these bacteria and an experimental variant. The number of indicatory bacteria of sanitary state decreased in a soil profile sometimes to zero as in case of *Clostridium perfringens* in non-irrigated and non-fertilized (control) soils and in the soils being irrigated with treated sewage stored in a biological pond.

Bacteria and potentially pathogenic fungi (Table 6 and 7). *Aeromonas hydrophila* and *Staphylococcus sp.* were found in soils of all experimental variants during the

Table 6. The number of Aeromonas hydrophila and Staphylococcus sp. in soil irrigated with biologically treated sewage at Sewage Treatment Plant in Olsztynek, in 1996 and 1997.

Variant	Depth (cm)	Aeromonas hydrophila								Staphylococcus sp							
		1996			1997					1996			1997				
		11.06	31.07	15.10	14.03	2.06	30.07	7.10	x	11.06	31.07	15.10	14.03	2.06	30.07	7.10	x
		¹(CFU) x 10^3/1 g															
A	0-10	–	8.5	24.0	0	0	20.0	22.5	12.5	–	9.6	14.5	8.6	5.2	2.1	22.0	10.3
	15-25	–	143.0	2.2	0.3	0	3.0	0.15	24.7	–	5.2	4.7	3.8	0.04	1.0	6.0	3.5
	30-50	–	10.0	0.2	0.6	0	13.5	0	4.05	–	3.2	0.5	5.5	0.62	9.5	2.6	3.6
B	0-10	–	1.0	55.0	6.0	1.1	0	0	10.5	–	6.9	9.0	8.8	9.7	13.5	6.5	9.0
C	0-10	–	4.75	42.0	0.04	0.6	0	2.0	8.2	–	2.3	10.4	0.5	41.0	3.7	13.6	12.0
D	0-10	–	4.25	60.0	0.25	0.9	1.3	5.0	12.0	–	2.7	30.0	1.2	43.0	3.8	9.6	15.0
	15-25	–	0.05	5.0	0.64	0	0.12	2.6	1.4	–	4.2	9.4	9.3	4.1	1.5	2.6	1.4
	30-50	–	75.0	19.0	0.68	0	0.2	0.2	15.8	–	0.2	7.5	3.8	2.9	3.7	1.0	3.2
E	0-10	–	13.6	4.8	0.04	0.8	5.0	7.0	4.8	–	12.6	40.0	9.6	7.2	2.8	12.0	14.0
F	0-10	–	1.9	44.0	0.04	0.15	10.0	1.2	9.5	–	6.0	3.0	6.5	6.5	44.0	8.2	12.3
G	0-10	–	0.04	55.0	0.23	0.1	23.0	1.0	13.2	–	18.0	11.0	12.0	6.0	82.0	11.0	23.3
H	0-10	–	4.7	0.1	0	4.8	0	0	1.6	–	21.0	10.0	7.0	12.0	120.0	35.0	34.2

¹ – Colony Forming Units

entire research period. In surface soil (0-10 cm layer) their number did not exceed $60 \cdot 10^3$ and $120 \cdot 10^3$ CFU/1 g dry mass, respectively. *Pseudomonas aeruginosa* was found sporadically, *Salmonella sp* .was observed only in autumn in 1996 in soils being irrigated with treated sewage stored in a biological pond (in a layer 15-25 cm and 30-50 cm deep). Fungi determined on Trichophyton Agar 1 were only examined in summer and autumn 1996 and in spring 1997. Their number ranged from $11.4 \cdot 10^3$ to 230 . 103 CFU/1 g dry mass. More of them were found in soils irrigated with fresh water or treated sewage stored in a biological pond. The number of *Aeromonas hydrophila, Staphylococcus sp.* and fungi determined on Trichophyton Agar 1 decreased in a soil profile in soils non-irrigated and non-fertilized (control) and in soils irrigated with treated sewage stored in a biological pond. They were exceptionally numerous in a layer at 15-25 cm and 30-50 cm deep.

Number of Indicatory Bacteria and Degree of Bacteriological Contamination of Soils Irrigated with Biologically Treated Sewage

The presentation of the results of TVC 20°C, TC and *Clostridium perfringens* counts in soils of different irrigated-fertilized experimental variants at a waste treatment plant in Olsztynek with criteria of the estimation of soil purity suggested by Parrakova and Mayer [14] show a lack of greater differences among them. As far as the number of TVC 20°C is concerned all the examined soil samples irrigated with fresh water and/or NPK fertilized and 86% examined soil samples non-irrigated (control) or irrigated with effluent from a waste treatment plant or with the same effluents but stored in a biological pond (independent from irrigation doses) would be regarded as unpolluted. As far as the number of TC is concerned most examined soil samples non-irrigated (control) showed "weak" or "average" contamination; as far as the number of *Clostridium perfringens* is concerned the soil samples were treated as "pure". Soil samples irrigated with effluents from a waste treatment plant or the same effluents stored in a biological pond snowed most often "average" *(Clostridium perfringens)* or "strong" contamination. The majority of the examined soil samples NPK fertilized and both NPK fertilized and irrigated with fresh water could be regarded as "average" or "strongly" contaminated (TC) or "weakly" or "average" contaminated *(Clostridium perfringens)* (Table 8).

Discussion

More counts of indicatory bacteria of the degree of contamination (TVC 20°C and TVC 37°C) in soils irrigated directly with effluents from a waste treatment plant than in non-irrigated soils (control) can be partly explained by a great number of these bacteria brought to soils during irrigation (up to few hundred thousand, sometimes 1 million cells in 1 ml). It also concerns the number of TVC 20°C in soils irrigated with treated waste additionally stored in a biological pond also containing high numbers of these microorganisms [13]. The lack of significant differences in FC number in non-irrigated soils (control) and irrigated with effluents from a waste treatment plant or the same effluents but stored in a biological pond can be explained by the quick death of these bacteria in soil, especially in spring and summer at higher temperature and in sunny weather. It also concerns pathogenic bacteria as *Salmonella.* These bacteria were found only in autumn 1996 in soils irrigated earlier (sum-

Table 7. Number (presence) of Pseudomonas aeruginosa and fungi determined on Trichophyton Agar 1 in soil irrigated with biologically treated sewage at sewage Treatment Plant in Olsztynek in 1996 and 1997.

Variant	Depth (cm)	Pseudomonas aeruginosa						Microfungi		
		1996		1997				1996		1977
		31.07	15.10	14.03	2.06	30.07	7.10	31.07	15.10	3.06
		presence/absence						[1]CFU x 10^3/1 g		
A	0-10	+	–	–	+	–	–	70	230	35
	15-25	+	+	–	–	–	–	152	43	0
	30-50	+	–	+	–	–	–	30	1.5	2
B	0-10	+	–	–	+	–	–	214	138	38
C	0-10	+	–	+	+	–	–	38.5	106.5	82
D	0-10	+	+	–	–	–	–	11.4	212	154
	15-25	+	–	–	+	–	–	80	218	32
	30-50	+	+	–	–	–	–	26	36	15
E	0-10	+	–	–	–	–	–	115	104	68
F	0–10	+	+	–	+	–	–	132	160	122
G	0–10	+	+	+	–	–	–	80	223	14
H	0–10	+	–	–	–	–	–	88	22	102

[1] – Colony Forming Units

mer) with treated sewage stored in a biological pond. It is interesting that at the same time these bacteria were also found in the same plots in grass [13]. Their presence may be connected with fecal contamination of soils by migrating birds or sea-gulls feeding on biological ponds near the waste treatment plant in Olsztynek. It is unlikely that they could survive since the period of last irrigation in summer. Baubinas and Vlodavets [1] found fast dying of *Salmonella eastbourne, Salmonella* Heidelberg and *Shigella sonnei* within the first hours after soil irrigation with sewage. However, Gerba et al. [9] observed a longer survival rate of *Salmonella* in winter. In the vegetation period the factor modifying bactericidal influence of UV rays on *Escherichia coli* and *Salmonella* spp. may be shading by plants. Chandler and Craven [5] and Gudding and Krogstad [10] show that survival rates of *Escherichia coli* and *Salmonella typhimurium* in soil depends on the content of moisture. Fast dying out of these bacteria was found in soil containing less than 10% humidity. The presence of TC and FS in soils of different experimental irrigated-fertilized variants is not an exceptional phenomenon. These bacteria are common in soil, on plants and show a higher survival rate in disadvantageus environmental conditions than FC and *Salmonella* [16]. Their presence in non-irrigated (control) soils and others may be connected with pollutants deposited by fowl, domestic animals (dogs, cats) and wild rodents [7, 8]. In soils irrigated with effluents from a waste treatment plant or the same effluents but stored in a biological pond their source could be sewage [13]. *Aeromonas hydrophila* was a constant component of bacterial microflora of the examined soils. These potential pathogenic bacteria are strictly connected with the presence of assimilative sources of carbon and energy. Generally they survive longer than *Escherichia coli* [4]. Their few counts in winter 1997 were associated with a maximum number of TVC 20°C. It is thought that below 15°C they cannot stand competition for carbon and energy sources with autochtonic psychrophilic bacteria. Sporadic occurrence of *Pseudomonas aeruginosa* and the presence of potential pathogenic bacteria *Staphylococcus* and fungi determined on Trichophyton Agar 1 may be connected with an occasional soil contamination by gulls feeding on a biological pond at the waste treatment plant in Olsztynek. Generally greater counts of the examined indicatory bacteria of the degree of contamination, sanitary state and potentially pathogenic and fungi (determined on Trichophyton Agar 1) in a soil layer 0-10 cm deep non-irrigated (control) and irrigated with waste treated sewage stored in a biological pond and smaller ones in layers 15-25 cm and 30-50 cm correspond to the data in literature concerning the influence of irrigation with sewage on the number of these microorganisms in a soil profile [2, 3, 6, 7, 9, 11, 12, 15].

Conclusions

1. Soil irrigation directly with effluents from waste treatment plant or the same effluent but stored in a bio logical pond in the waste treatment plant in Olsztynek did not cause an increase in the degree of their contami nation. The amount of irrigating dose of treated waste taken from a biological pond for the irrigation of these soils did not infuence the degree of their contamination significantly.

2. Differences in the number of the examined indi catory bacteria groups of the degree of contamination and sanitary state and potentially pathogenic in non-ir-

Table 8. The analysis of soil quality using criteria given by Parrakova and Mayer [14]. Percent of samples relevant to the given class.

Variant	Depth (cm)	[1]TVC 20°C				[2]TC				Clostridium perfringens			
		[3]CFU x 10^6/1 g				Titre				Titre			
		[4]I	II	III	IV	I	II	III	IV	I	II	III	IV
		$0\text{-}10^5$	$10^5\text{-}5.\ 10^5$	$5.\ 10^5\text{-}10^6$	$10^6\text{-}5.\ 10^7$	$0\text{-}10^0$	$10^0\text{-}10^{-2}$	$10^{-2}\text{-}10^{-4}$	$10^{-4}\text{-}10^{-6}$	$0\text{-}10^{-1}$	$10^{-1}\text{-}10^{-2}$	$10^{-2}\text{-}10^{-3}$	$10^{-3}\text{-}10^{-5}$
A	0-10	86	14	0	0	0	43	43	16	44	28	28	0
	15-25	86	14	0	0	0	83	17	0	68	32	0	0
	30-50	100	0	0	0	0	100	0	0	80	20	0	0
B	0-10	100	0	0	0	0	16	44	44	28	28	44	0
C	0-10	86	14	0	0	16	28	28	28	44	28	28	0
D	0-10	86	14	0	0	0	14	72	14	28	28	44	0
	15-25	86	14	0	0	0	72	28	0	44	28	29	0
	30-50	100	0	0	0	0	72	28	0	59	29	15	0
E	0-10	86	14	0	0	14	58	28	0	14	58	28	0
F	0-10	86	14	0	0	0	12	44	44	29	42	29	0
G	0-10	100	0	0	0	0	14	28	58	28	28	44	0

[1] - Total viable count of heterotrophic (saprophytic) bacteria on nutrient medium (Broth - Agar)
[2] - Total coliforms
[3] - Colony Forming Units
[4] - I - unpolluted, II - little polluted, III - average polluted, IV - strong polluted

rigated and non-fertilized (control) soils and in NPK fertilized and/or irrigated soils with fresh water and effluents from the waste treatment plant or the same effluents but stored in a biological pond were not significant; however, they were more often found in soils irrigated with effluents from a waste treatment plant, seldom in case of the same effluents stored on a biological pond.

3. Aeromonas hydrophila and Staphylococcus sp. were only more numerous from among the examined microorganisms being potentially pathogenic in soils of all irrigated-fertilized variants. Their number generally exceeded the number of fecal coliforms. Fungi determined on Trichophyton Agar 1 were less numerous, *Pseudomonas aeruginosa* were seldom found and *Salmonella* sp. was found exceptionally.

4. Majority of the examined groups of indicatory bacteria of the degree of contamination and sanitary state both in non-irrigated and non-fertilized soils (control) and irrigated with effluents from a waste treatment plant stored in a biological pond were more numerous near the surface (in a layer 0-10 cm); in deeper layers of soil (15-25 cm and 30-50 cm) their number usually decreased - in case of the TVC 20°C and TVC 37°C several times.

5. The amount of doses irrigated with biologically treated waste additionally stored in a biological pond and then used for soil irrigation did not influence the number of the examined indicatory bacteria of the contamination degree (TVC 20°C,TVC 37°C) and potentially pathogenic *{Aeromonas hydrophila, Staphylococcus sp.)* and fungi determined on Trichophyton Agar 1. Only the number of indicatory bacteria of sanitary state (TC, FC, FS) reached a slightly higher values in soils irrigated with their maximum dose.

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References

1. BAUBINAS A.K., VLODAVETS V.V. Dynamics of the pu rification of domestic fecal sewage farms. Gig. Sanit. **39,**100, **1974.**
2. BELL R.G., BOLE J.S. Elimination of fecal coliforms bac teria from soil irrigated with municipal sewage lagoon efflu ent. J. Environ. Qual., 7,193, 1978.
3. BITTON G., HARVEY E. W. Transport of pathogens through soils and aquifers. In: Environmental Microbiology (Ed. R. Mitchell). Wiley-Liss, New York, N.Y, 103, 1992.
4. BRANDI G.S, SCHIAVANO G.F., SALVAGGIO L., ALBANO A. Survival of Aeromonas hydrophila, Aeromonas caviae and Aeromonas sobria in soil. J. Appl. Bacteriol., **81,** 439, **1996.**
5. CHANDLER D.S., CRAVEN J.A. Environmental factors affecting Escherichia coli and Salmonella typhimurium number on land used for effluent disposal. Aust. J. Agric. Res, **29,** 577, **1978.**
6. EPSTEIN E, CHANEY R.L. Land disposal of toxic sub stances and water related problems. Journ. WPCFed., 50, 2037, **1978.**
7. FAUST M.A. Relationship between land-use practices and fecal bacteria in soil. J. Environ. Qual, **11,** 141, 1982.
8. GELDREICH E.E. Sanitary significance of fecal coliforms in the environment. Water Pollution Control Research Series. US Department of Interior. Federal Water Pollution Control Administration. Publication WP-20-3. November 1966, 1-122, **1966.**

9. GERBA C.P., WALLIS C, MELNICK J.L. Fate of waste-water bacteria and viruses in soil. J. Irrig. Drainage Div., **3,** 157, **1975.**
10. GUDDING R., KROGSTAD O. The persistence of Es cherichia coli and Salmonella typhimurium in fine-grained soil. Acta Agric. Scand., **25,** 285, **1975.**
11. MARCULESCU I, DROCEN N. Investigation using label led bacteria in the study of irrigation with sewage. Stud. Epurarea Apelor, **59,** 66, **1962.**
12. MATHUR R.P, CHANDRAS, BWARDWAY K.A. Two dimensional study of travel of pollution in ROORKEE soil. J. Inst. Eng. (India). Part PH **48,** 197, **1968.**
13. NIEWOLAK S., TUCHOLSKI S. The effect of meadow irri gation with biologically treated sewage on the occurrence of microorganisms indicatory of pollution and sanitary state and of potentially pathogenic bacteria in the grass. Pol. J. Environ. St., 8, 39,1999.
14. PARRAKOVA E., MAYER J. Vergleichende Mik-robiologisctie Untersuchungen verunreinigter Boden. I. Teil. Quantitative Veranderungen der Mikroorganismen. Zbl. Bakt. Parasitk., Infektionskrankheiten und Hygiene. II. Abt. Bd. **126,** 521, **1971.**
15. ROMERO T.G. The movement of bacteria and viruses through porous media. Ground Water, **8,** 37, **1970.**
16. STODDARD C.S., COYNE M.S., GROVE J.H. Fecal bac teria survival and infiltration through a shallow agricultural soil: timing and tillage effects. J. Environ. Qual., **27,** 1516,1998.
17. TUCHOLSKI S, MARKIEWICZ K., KOC J., MAR-KIEWICZ E. Zawartosc niektorych mikroelementow w sciekach oczyszczonych i wodach stawow nimi zasilanych. Zeszyty Problemowe Postepow Nauk Rolniczych, Z. **434,** 961, **1996.**