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The Effect of Meadow Irrigation With Biologically Treated Sewage on the Occurrence of Micro-Organisms Indicatory of Pollution and Sanitary State and of Potentially Pathogenic Bacteria in the Grass

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

Olsztyn University of Agriculture and Technology
[1]Department of Ecology, Sanitary Microbiology Unit;
[2]Department of Land Reclamation and Environmental Development,
10-957 Olsztyn-Kortowo, Poland

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Abstract

Studies were carried out to determine numbers of bacteria indicatory of pollution (TVC 20°C, TVC 37°C) and sanitary state (TC, FC, FS, *Clostridium* ***perfringens),*** and of potential pathogens ***(Aeromonas hydrophila,*** *Staphylococcus* sp., *Pseudomonas aeruginosa. Salmonella* sp.) and fungi in meadow grass subject to 8 different variants of irrigation and fertilization in the vicinity of a treatment plant in Olsztynek (Masurian Lake District). Studies were performed in the vegetation season in the course of 2 consecutive annual cycles in 1996 and 1997.

Psychrophilic ammonifiers (TVC 20°C) were the most numerous group of bacteria in all 8 variants of irrigation and fertilization. They were especially numerous in the grass of the third swath (in autumn). As regards the bacteria indicatory of sanitary state, TC and FS were the most numerous, and from among pathogens — *Aeromonas hydrophila* and *Staphylococcus* sp., *Clostridium perfringens, Pseudomonas aeruginosa* and *Salmonella* sp. were rare. Fungi determined on Trichophyton Agar 1 medium were fairly numerous. TC, FS, *Aeromonas hydrophila,* and fungi grown on Trichophyton Agar 1 were usually more numerous in grass from plots irrigated with biologically-treated sewage and treated sewage from a biological pond. Their maximal numbers were found in grass of the 2nd and/or 3rd swath.

There were no statistically significant differences ($\alpha < 0.05$) among amounts of bacteria groups in grass from the 8 experimental fields.

Keywords: grass, sewage, irrigation, fertilization, bacteria, fungi

Introduction

Use of biologically treated sewage to irrigate arable lands, meadows and pastures, with their simultaneous utilization as fertilizers, may be treated as a method of third-degree (chemical) sewage treatment. This method, however, is restricted by a number of medical conditions [16], which inhibit the use of sewage to irrigate crops that are to be consumed fresh and raw [7]. In order to avoid direct consumption of crops irrigated with sewage it seems more appropriate to apply this method to irrigate meadows and pastures. This does not eliminate all problems related to agricultural use of sewage, mostly due to the susceptibility of domestic animals to many pathogenic microorganisms found in biologically treated sewage, such as *Salmonella, Mycobacterium tuberculosis, Mycobacterium bovis* [21] or enteric pathogenic viruses [3]. Dangerous levels of these pathogens necessary to produce clinical symptoms are high in the case of cattle [28], while their survival on plants is low [6, 21], it is rather unlikely that cattle grazing on pastures fertilized with sewage might develop salmonellosis, for example [6]. Strong UV radiation of the sun, high

air temperature, and low moisture are very effective in eliminating pathogenic bacteria from the grass. On the other hand, there may be problems with high numbers of saprophytic bacteria and filamentous fungi, especially in older, ober-ripe grass. These micro-organisms not only lower grass quality (causing its decay), but also produce toxic metabilites, thereby having a negative effect on animal health. More and more attention has been devoted to this problem in recent years [4, 5].

This paper presents the results of studies on the numbers of bacteria indicatory of pollution and sanitary state, and of potentially pathogenic bacteria, in grass from a non-irrigated and unfertilized plot (control), and from plots irrigated and/or fertilized with biologically treated sewage and with mineral fertilizers (NPK) in the vicinity of the municipal sewage treatment plant in Olsztynek. Studies were carried out in two consecutive vegatation seasons: 1996 and 1997.

Materials and Methods

Study Area

Studies were carried out on a meadow belonging to the sewage treatment plant in Olsztynek (Masurian Lake District). The meadow was characterized by low variability of physical and chemical soil properties [27] of classes IVb and Va, with fairly uniform distribution of plants. A field experiment consisting of random blocks began in spring 1996, just prior to the vegetation season. The meadows were divided into experimental plots of unit area 15.57 m^2, out of which 12.0 m^2 were harvested (Fig. 1). Dominating plant species were orchard grass *(Dactylis glomerata),* blue grass *(Poa pratensis),* quack grass *(Agmpyron repens)* and common dandelion *(Taraxacum officinale).*

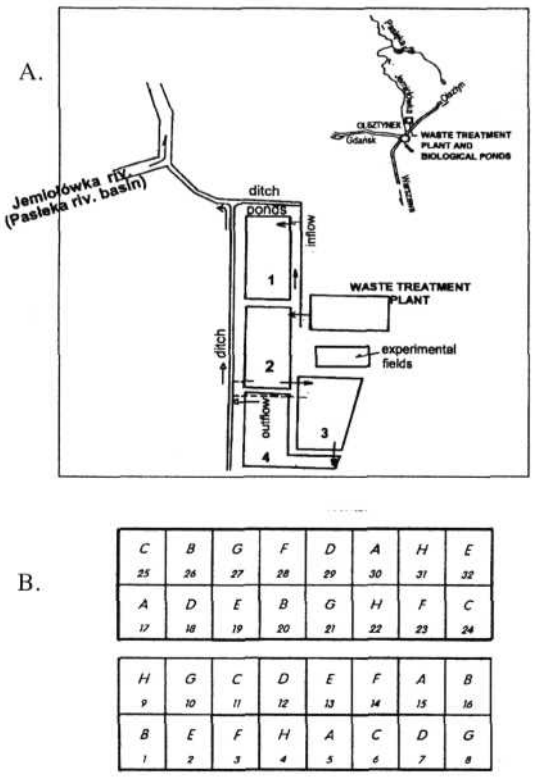

Fig 1, A. Scheme of ponds in waste treatment plant in Olsztynek B. Scheme of experimental fields in the waste treatment plant. For explanation see Table 1.

Sewage

Sewage used to irrigate meadow plots consisted of a mixture of municipal sewage and wastes discharged by a factory processing fruit and vegetables. They were subject to biological treatment and then discharged to ponds. Plots were irrigated with both: biologically treated sewage directly discharged by the treatment plant and sewage taken from the retention ponds.

Experiment

Sanitary and bacteriological studies of meadow vegetation were carried out in 8 experimental variants, each repeated 4 times (Table 1). All experiments began after the first swath on 31 May, 1996.

Sampling Procedure

Samples of grass were collected from 4 plots in each variant on 31 July and 2 October, 1996, and on 6 June, 31 July and 2 October, 1997. About 100 g of grass were collected each time. It was cut in the laboratory into 1 mm piecs, 10 g samples were weighed and homogenized in 300 ml of a sterile physiological salt NaCl. The homogenate was then diluted 1:1, 1:100, 1:1,000 and 1:10,000, and inoculated into appropriate media.

Samples of treated sewage were collected for microbiological examination at the outflow from the secondary sedimentation tank and from biological pond no. 2 (which received biologically treated sewage) on the same days on which the experimental plots were irrigated (from June to August 1996, and from April to August 1997). Samples were collected to sterile 300 ml bottles with ground-in stoppers.

Microbiogical Determinations

Microbiological studies of the grass comprised:

1. Total bacteria counts (CFU/1 g of fresh wt.) in broth agar after 72 h incubation at 20°C (TVC 20°C);
2. Total bacteria counts (CFU/1 g of fresh wt.) in broth agar after 24 h incubation at 37°C (TVC 37°C);
3. Total counts (MPN/1 g fresh wt.) of coliforms (TC) in Eijkman medium after 72 h incubation at 37°C;
4. Counts (MPN/1 g fresh wt.) of faecal coliforms (FC) in Ejkman medium after 24 h incubation at 44.5°C;
5. Counts (MPN/1 g fresh wt.) of faecal streptococci in Slanetz and Bartley medium after 72 h incubation at 37°C;
6. Counts (MPN/1 g fresh wt.) of anaerobic spore-for ming bacteria reducing sulphites *(Clostridium perfringens* — CP) in pasteurised grass homogenates (80°C/10 min) in Wilson-Blair medium after 18 h incubation at 37°C;
7. Counts (CFU/1 g fresh wt.) of *Aeromonas hydrophila* in Rimler-Shotts medium after 24 h incubation at 37°C;

Table. 1 Experimental variants of irrigating and fertilizing grass on plots 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 (dischared by the treatment plant), basic dose (243.7 mm in 1996, 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: N-90 kg/ha, P_2O_5 – 100 kg/ha, K_2O – 135 kg/ha in 1996 (2nd and 3rd swath); N – 120 kg/ha, P_2O_5 – 100 kg/ha, K_2O – 180 kg/ha in 1997
H	Mineral fertilization as in variant G and irrigation as in variant B

8. Counts (CFU/1 g fresh wt.) of *Staphylococcus* sp. in Bacto Staphylococcus Medium 110 according to Champan, after 24 h incubation at 37°C;

9. Presence (or lack) of *Pseudomonas aeruginosa* in mPA Agar medium after 48 h incubation at 41.5°;

10. Presence (or lack) of *Salmonella* sp. in selective Kauffman's medium with sodium tetrathionate after 24 h incubation at 37°C, and then in differentiating medium with xylose, lysine and sodium desoxycholate (XLD), con ditions of the incubation being the same;

11. Numbers (CFU/1 g fresh wt.) of fungi in Tricho phyton Agar 1 medium after 24 h incubation at 37°C.

TVC 20°C and TVC 37°C were determined using the usual procedures of microbiological examination of drinking water. The most probable number of TC, FC and FS was determined according to APHA data [2]. The most probable number of anaerobic sporeforms reducing sulphites *{Clostridium perfingens)* were determined using the dilution method, inoculating 1.0, 0.1 and 0.01 g of the examined grass suspension in test tubes containing Wil-son-BIair medium. Positive results for the presence of *Clostridium perfringens* were checked in skimmed milk. Numbers of *Aeromonas hydrophila* were determined applying the techniques described by Hazen [15] counting typical colonies (yellow) on agar plates. Counts of *Staphylococcus* sp. and fungi were obtained according to Difco Manual [13]. Positive results for the presence of coliforms in fermented samples in Eijkman medium were checked in Endo medium, lauryl-tryptose broth, and in samples stained with the Gram method. Positive results for the presence of faecal streptococci in Slanetz and Bartley medium were checked in m-Enterococcus Agar. Typical colonies of dark red which had developed in this medium were transferred to broth medium and their growth rate was determined in 44.5°C, at pH 9.6, in the presence of 6.5% NaCl, as well as in milk with an addition of 0.01% methylene blue. In the case of *Aeromonas hydrophila,* yellow colonies which had developed after incubation were counted, and selectively confirmed as being *Aeromonas hydrophila* using API-20E (Analytab Products, Plainview, New York) test for oxidasis and a vibiriostatic factor 0/129. As regards *Staphylococcus* sp., orange and yellow colonies were counted and there was no further identification of *Staphylococcus aureus*. The presence of *Pseudomonas aeruginosa* was confirmed in Pseudomonas P. Agar medium observing pyocyanine in Wood's light according to Levin and Cabelli [22]. In determining *Salmonella* sp., typical red colonies with black spots in the middle were confirmed in Kligler medium and broth with urea, and finally during of the slide agglutination test [30] for flagellate antigen HM according to the method given by Burbianka and Pliszka [9]. All quantitative determinations were carried out in 3 parallel repetitions. The results pertaining to TVC 20°C, TVC 37°C, *Aeromonas hydrophila, Staphylococcus* sp. and fungi were obtained based on the colonies which developed in agar media. Counts of TC, FC, FS, and *Clostridium perfringens* were obtained from McCrady's tables; 0,8% NaCl being the diluent used.

Numbers of TVC 20°C, TVC 37°C, TC, FC and FS in water of the biological pond of Olsztynek treatment plant, and in the effluent from the treatment plant were determined using the same media as in the case of grass samples. Numbers of bacteria indicatory of pollution and sanitary state were related to the WHO standards [16], and to regulation by the Council of Ministers on the classification of waters [25] issued on 14 December 1987. The stated amounts of bacteria groups in 1 g of grass wet weight were analyzed statistically using Duncan's Test.

Table 2. Numbers of bacteria and fungi in 1 g of fresh grass collected from control plots (with no irrigation and fertilization) in different vegetation periods of 1996 and 1997.

Micro-organisms	1996		1997		
	swath				
	2	3	1	2	3
TVC 20°C	67×10^6	195×10^6	2.3×10^6	96.8×10^6	269×10^6
TVC 37°C	7×10^6	37×10^6	0.2×10^6	7.7×10^6	5×10^6
CP	4	4	3	3	3
TC	1.4×10^3	48×10^3	1.4×10^3	45×10^3	4.5×10^3
FC	4	750	3	750	40
FS	7.5×10^3	250×10^3	0.2×10^3	14×10^3	1.1×10^3
Ae	–	105×10^3	15×10^3	65×10^3	1600×10^3
Sa	160	3000	80	800	2750
Pa (P/A)	+	+	–	–	+
Sal (P/A)	–	+	–	–	–

TVC 20°C – total viable count at 20°C
TVC 37°C – total viable count at 37°C
CP – *Clostridium perfringens*
TC – total coliforms
FC – faecal coliforms
FS – faecal streptococci
Ae – *Aeromonas hydrophila*
Sa – *Staphylococci*
Pa – *Pseudomonas aeruginosa*
Sal – *Salmonella* sp.
(P/A) – presence/absence

Results

Numbers of bacteria indicatory of pollution and sanitary state in the grass of experimental plots.

Micro-organisms Indicatory of Pollution

From among the groups of indicatory bacteria (TVC 20°C, TVC 37°C) in the grass of experimental plots, less pronounced variations were observed for TVC 20°C (from 290 thousand to 269 million cells in 1 g of fresh grass weight) than for TVC 37°C (from 30 thousand to 256 million cells in 1 g fresh wt.). Psychrophilic bacteria TVC 20°C were equally numerous in grass growing on unfertilized plots and in grass growing on the plots irrigated with clean water, biologically treated sewage, treated sewage collected from the biological pond, and on plots fertilized with mineral fertilizers. They were usually more numerous in grass of the 3rd swath. Mesophilic bacteria TVC 37°C were also numerous in grass of the 2nd swath. Differences in the counts of these two groups of bacteria were usually very small between the plots irrigated with various doses of treated sewage from the biological pond. Higher numbers were observed exceptionally, only in the grass of plots irrigated with higher amounts of sewage (Tables 2-7).

Micro-organisms Indicatory of the Sanitary State

As regards the bacteria which are indicators of the sanitary state (TC, FC, FS, *Clostridium perfringens),* grass collected from the experimental plots contained the lowest numbers of FC and *Clostridium perfringens,* and the highest of TC. *Clostridium perfringens* were noted rarely, their numbers being usually below 10 cells in 1 g of fresh grass weight. TC, FC and FS were usually present in lower numbers in the plots which had neither been irrigated nor fertilized, or those which were irrigated with clean water, more numerously in plots irrigated with biologically treated sewage, treated sewage taken from the biological ponds, and sometimes also in plots fertilized with NPK and irrigated with clean water. Differences in the numbers of these bacteria in grass collected from plots irrigated with the basic dose, 150% and 200% of treated sewage from the biological pond showed no clear trend. Grass collected from plots with no irrigation and no fertilization, and those irrigated with clean water or with biologically-treated sewage, usually contained higher numbers of these bacteria in the 2nd (1997) or 3rd (1996) swath. Grass from plots irrigated with sewage from the biological pond, plots fertilized with mineral fertilizers (NPK), and those with NPK fertilization and irrigated with clean water had higher numbers of these bacteria in different grass swaths (Tables 2-7). There were no statistically significant differences ($a < 0.05$) among amounts of bacteria groups in grass from the 8 experimental fields.

Potentially Pathogenic Micro-organisms

Aeromonas ***hydrophila,*** *Staphylococcus* sp. and fungi determined in Trichophyton Agar 1 were present in grass collected from plots in all 8 variants of the experiment. Other bacteria, such as *Pseudomonas aeruginosa* and *Salmonella* sp. were noted only in rare cases, usually in grass from the 3rd swath. *Aeromonas hydrophila* and *Staphylococcus* sp., and fungi determined in Trichophyton Agar 1 medium were usually more numerous in grass from plots irrigated with biologically treated sewage, or sewage from the biological pond. They were also more numerous in grass from NPK fertilized plots, 3d swath (in 1996 and 1997). Differences in the numbers of these bacteria in grass from plots irrigated with different doses of sewage from the biological pond as well as with pure water showed no clear trend ($a < 0.05$) (Tables 2-8).

Table 3. Numbers of bacteria in 1 g of fresh grass collected from plots irrigated with clean water (basic dose) in different vegetation periods of 1996 and 1997.

Micro-organisms	1996		1997		
	swath				
	2	3	1	2	3
TVC 20°C	81.5×10^6	116×10^6	0.3×10^6	24.4×10^6	43.6×10^6
TVC 37°C	1.5×10^6	5.7×10^6	0.07×10^6	9.4×10^6	3.0×10^6
CP	3	4	3	3	10
TC	45×10^3	110×10^3	45×10^3	110×10^3	4.5×10^3
FC	4	140	3	45	3
FS	3×10^3	2.5×10^3	1.4×10^3	14×10^3	1.5×10^3
Ae	–	27.5×10^3	6×10^3	46×10^3	1325×10^3
Sa	0.6×10^3	4.2×10^3	0.3×10^3	0.9×10^3	1.5×10^3
Pa (P/A)	+	+	+	–	–
Sal (P/A)	–	+	–	–	–

For explanations see Table 2.

Table 4. Numbers of bacteria in 1 g of fresh grass collected from plots irrigated with biologically treated sewage (discharged by the treatment plant) in different vegetation periods of 1996 and 1997.

Micro-organisms	1996		1997		
	swath				
	2	3	1	2	3
TVC 20°C	14.4×10^6	59×10^6	0.56×10^6	69×10^6	115×10^6
TVC 37°C	33×10^6	5.2×10^6	0.03×10^6	18.5×10^6	5.5×10^6
CP	4	3	3	10	10
TC	11000×10^3	140×10^3	11000×10^3	140×10^3	14×10^3
FC	25	450	3	1500	40
FS	25×10^3	200×10^3	1.4×10^3	14×10^3	0.3×10^3
Ae	–	37×10^3	5×10^3	9×10^3	450×10^3
Sa	90	500	60	2000	1300
Pa (P/A)	–	+	+	–	+
Sal (P/A)	–	+	–	–	–

For explanations see Table 2.

Numbers of Bacteria Indicatory of Pollution and Sanitary State in Biologically Treated Sewage, and in Treated Sewage Discharged to Biological Pond

TVC 20°C, TVC 37°C, TC, FC and FS numbers in biologically treated sewage and in treated sewage discharged to the biological pond of the treatment plant in Olsz-

Table 5. Numbers of bacteria in 1 g of fresh grass collected from plots irrigated with treated sewage from the biological pond at a basic dose (a), 150% of the basic dose (b) and 200% of the basic dose in different vegetation periods of 1996 and 1997.

Micro-organisms		1996		1997		
		swath				
		2	3	1	2	3
TVC 20°C	a	37×10^6	37×10^6	1.15×10^6	45.2×10^6	54.4×10^6
	b	54×10^6	23×10^6	1.7×10^6	38.1×10^6	71.6×10^6
	c	41×10^6	12×10^6	3.1×10^6	176.8×10^6	64.0×10^6
TVC 37°C	a	5×10^6	0.5×10^6	1.3×10^6	14.3×10^6	4.2×10^6
	b	7×10^6	6.2×10^6	0.2×10^6	7.2×10^6	21.8×10^6
	c	1×10^6	0.4×10^6	1.1×10^6	32.3×10^6	30.2×10^6
CP	a	4	3	3	3	10
	b	3	4	3	3	3
	c	3	3	3	3	10
TC	a	110×10^3	140×10^3	110×10^3	140×10^3	45×10^3
	b	0.25×10^3	4.5×10^3	0.25×10^3	4.5×10^3	140×10^3
	c	140×10^3	14×10^3	140×10^3	14×10^3	1400×10^3
FC	a	3	110	3	350	3
	b	3	400	3	2500	90
	c	4	2000	3	450	3
FS	a	0.15×10^3	110×10^3	1.5×10^3	11×10^3	0.95×10^3
	b	15×10^3	4×10^3	4.5×10^3	14×10^3	0.75×10^3
	c	2×10^3	4×10^3	4.5×10^3	14×10^3	0.25×10^3
Ae	a	–	26×10^3	30×10^3	16×10^3	550×10^3
	b	–	315×10^3	0.7×10^3	39×10^3	925×10^3
	c	–	21×10^3	3.7×10^3	12×10^3	1100×10^3
Sa	a	575	500	150	1200	7800
	b	530	660	110	3000	700
	c	45	10	85	1850	5200
Pa (P/A)	a	–	+	–	–	–
	b	–	–	+	–	–
	c	–	+	–	–	–
Sal (P/A)	a	–	–	–	–	–
	b	–	+	–	–	–
	c	–	+	–	–	–

For explanations see Table 2.

tynek found from May to August 1996, and from April to August 1997 (Table 9 and 10) ranged within a few orders of value. In biologically treated sewage (effluents from the treatment plant), maximal numbers of indicatory bacteria were found at the beginning of June 1996 and on different days of August 1997, and of TVC 20°C and TVC 37°C, also in April 1997. In treated sewage collected from the biological pond only TC and FS numbers reached higher levels in July 1996. All groups of indicatory bacteria were also a little more numerous at the beginning of August 1997. FC:FS ratio in biologically treated sewage (discharged by the treatment plant) and in sewage from the biological pond ranged from 0.05 to 244.0, being lower than 0.7 in 38-41% of the samples, from 0.7 to 4.0 in 28-47% of the samples, and higher than 4.0 in 12-24%.

Discussion

The results of sanitary and bacteriological examination of grass collected from 8 different variants of the experiment suggest that even the control plots (without irrigation and fertilization) were sometimes characterized by high numbers of heterotrophic ammonifiers (TVC 20°C and TVC 37°C), and of bacteria indicatory of the sanitary state (TC, FC, FS) and potentially pathogenic *(Aeromonas hydrophi-Ia, Staphylococcus* sp. and fungi determined in Trichophyton Agar 1 medium). Exceptionally, also *Pseudomonas aeruginosa* and *Salmonella* sp. attained high numbers in these plots. Numbers of TVC 20°C in the control plots, as well as in plots irrigated with clean water, and with biologically treated sewage and sewage from the biological pond, and fertilized with mineral fertilizers (NPK) were always, however, within the range given by Kaszubiak and Muszynska [20] for grass fertilized with liquid manure. There are no data in available literature on the recommended or maximal permissible numbers of these micro-organisms in cattle pasture lands, so it is very difficult to discuss the results of this study. Schmidt [26] mentioned that numbers of heterotrophic bacteria 1 x 10^6 cells per 1 g of fresh weight represented the upper limit for cattle feeds. This value is lower than the values usually obtained in our

Table 6. Numbers of bacteria in 1 g of fresh grass collected from plots fertilized with mineral fertilizers in different vegetation periods of 1996 and 1997.

Micro-organisms	1996		1997		
	swath				
	2	3	1	2	3
TVC 20°C	103×10^6	7×10^6	0.5×10^6	30×10^6	67×10^6
TVC 37°C	6×10^6	0.4×10^6	0.7×10^6	8.3×10^6	4.2×10^6
CP	45	3	3	3	3
TC	45×10^3	1500×10^3	3	45×10^3	3
FC	25×10^3	3×10^3	3	45×10^3	3
FS	450×10^3	1.1×10^3	4.5×10^3	11×10^3	0.2×10^3
Ae	–	51×10^3	43×10^3	110×10^3	900×10^3
Sa	300	30	215	650	4600
Pa (P/A)	–	+	–	–	+
Sal (P/A)	–	+	–	–	–

For explanations see Table 2.

Table 7. Numbers of bacteria in 1 g of fresh grass collected from plots fertilized with mineral fertilizers and irrigated with clean water (basic dose) in different vegetation periods of 1996 and 1997.

Micro-organisms	1996		1997		
	swath				
	2	3	1	2	3
TVC 20°C	256×10^6	230×10^6	0.8×10^6	17.8×10^6	67.6×10^6
TVC 37°C	18×10^6	11.4×10^6	2.0×10^6	13.2×10^6	7.2×10^6
CP	3	7	3	3	3
TC	11.5×10^3	11×10^3	11.5×10^3	11×10^3	11×10^3
FC	3	950	45	95	3
FS	1100×10^3	20×10^3	4.5×10^3	14×10^3	2.5×10^3
Ae	–	18.5×10^3	32×10^3	47×10^3	1335×10^3
Sa	20	3000	250	400	4200
Pa (P/A)	–	+	–	–	+
Sal (P/A)	–	+	–	–	–

For explanations see Table 2.

study. Grass contamination ith TC, FC, FS and sometimes also *Clostridium perfringens* in all 8 variants of the experiment might have been connected with deposition of pollutants by birds and small rodents in grass grown on plots irrigated with biologically treated sewage, and in the case of sewage collected from the biological ponds - also of micro-organisms contained in this sewage. Also storm water could have been a source of grass contamination with potentially pathogenic bacteria *{Aeromonas hydrophila, Staphylococcus* sp., *Pseudomonas aeruginosa, Salmonella* sp.) and fungi determined in Trichophyton Agar 1 medium. *Aeromonas hydrophila* and *Pseudomonas aeruginosa* are always present in sewage and polluted water, but their numbers are much lower than total coliforms (Niewolak and Opieka - in prep.) The two species are regarded as aquatic organisms because they can be isolated from water even when there are no sources of faecal pollution. Numbers of indicatory bacteria could have been also modified by their content in biologically treated sewage, which frequently exceeded standards adopted for water quality of the 3rd class [25].

Usually lower numbers of the investigated groups of indicatory bacteria (especially TVC 20°C) in grass from the 1st swath (spring) in 1997 might have been related to higher physiologic activity of plants in spring [20]. From a microbiological point of view, lower numbers of heterotrophic bacteria in this grass may suggest its higher quality as animal food [4] compared to grass from the 2nd and/or 3rd swath, which usually contained higher numbers of these bacteria. Higher numbers of these bacteria, as well as of *Enterobacteriaceae* (TC, FC, FS) in grass from the 2nd and 3rd swath may be explained by nutrient (carbohydrates, amino acids) leaking and their washing out from the old, over-ripe grass [8,11]. In addition to this, higher grass densities at the end of the vegetation season may create better thermal conditions for the micro-organisms and improve their survival in lower parts of grass stems [10]. Contrary to this, upper parts of grasses, and even the whole leaf area, can be subject to extremal changes of environmental conditions, especially humidity, temperature, and UV radiation [8,12]. The observed differences in the numbers of bacteria indicatory of pollution and sanitary state in grass from different plots and in different vegetation seasons might also have been caused by mutual relations between the plant and the micro-organism [24]. This can lead not only to seasonal chages of the numbers of bacteria and fungi, but also to diurnal chages [17, 18]. It has been shown [14] that not only the numbers of bacteria and fungi, but also qualitative composition of the microflora which colonises particular grass species can differ depending on environmental conditions, and is often dominated by a limited number of taxons which udergo seasonal succession. Numbers and qualitative composition of bacteria and fungi may significantly effect grass quality. Numbers of these micro-organisms, increasing with grass age [1, 29], and especially of heterotrophic ammonifying bacteria (TVC 20°C) may in turn affect the health of domestic animal fed this grass [4]. Threats to animals health are connected most of all with toxic metabolic products of bacteria (endogenic proteins) and fungi (aflatoxins).

Data on the occurrence of *Salmonella* sp. in grass from the 3rd swath of 1996 do not seem to be alarming, also in view of FC numbers found at the same time. Relatively low numbers of faecal bacteria and coliforms in grass of the control plots (without irrigation and fertilization), as also of the plots irrigated with biologically treated sewage from the treatment plant and biological pond in Olsztynek suggest that if *Salmonella* sp. were present in the grass, their numbers were minimal, not threatening animal health.

Table 8. Numbers of fungi determined in Trichophyton Agar 1 medium in 1 g of fresh grass collected from different experimental plots in autumn 1996 and spring and summer 1997.

Experimental variants	1996	1997	
	swath		
	3	1	2
A	0.6×10^3	0.1×10^3	0
B	2.3×10^3	6.0×10^3	0
C	3.4×10^3	15.6×10^3	1.0×10^3
D	1.5×10^3	5.6×10^3	7.0×10^3
E	0.6×10^3	18.0×10^3	2.7×10^3
F	0.3×10^3	21.5×10^3	0.4×10^3
G	0.4×10^3	5.0×10^3	0.2×10^3
H	28.0×10^3	0.7×10^3	0

A, B, C ... H – For explanation see Table 1.

Table 9. Numbers of bacteria indicatory of pollution (TVC 20°C and TVC 37°C) and sanitary state (TC, FC, FS) in the effluent of the treatment plant in Olsztynek in 1996 and 1997.

Date	TVC 20°C	TVC 37°C	TC	FC	FS	FC : FS ratio
	CFU × 10^3/1 ml		MPN × 10^3/100 ml			
28.05.1996	64.5	31.0	150	140	75	1.86
11.06.	68.1	38.0	450	20	1100	0.18
17.06.	335.0	26.0	140	1100	1100	1.0
5.07.	1060.0	113.1	1400	450	1400	0.32
31.07.	47.2	28.0	1400	110	450	0.24
5.08.	30.3	23.1	1100	15	25	0.6
19.08.	27.8	11.2	250	250	1100	0.22
29.04.1997	660	247.0	140	140	110	1.2
30.04.	656	252.0	140	45	110	0.40
3.05.	285	12.7	140	45	11	4.0
14.05.	105	33.0	140	45	0	–
27.06.	8.5	–	140	45	45	1.0
1.07.	70	362.0	140	110	140	0.78
16.07.	90	18	140	20	110	0.18
17.07.	60	166	140	110	20	5.8
5.08.	280	780	1400	140	140	1.0
13.08.	13.5	113	14000	110	45	2.4
20.08.	40	26.8	140	1400	140	10.0

CFU – colony forming units, MPN – most probable number, TVC 20°C, TVC 37°C, TC, FC, FS – as in Table 2.

Table 10. Numbers of bacteria indicatory of pollution (TVC 20°C and TVC 37°C) and sanitary state (TC, FC, FS) in water of the biological pond of the treatment plant in Olsztynek in 1996 and 1997.

Date	TVC 20°C	TVC 37°C	TC	FC	FS	FC : FS ratio
	CFU × 10^3/1 ml		MPN × 10^3/100 ml			
28.05.1996	17.6	3.3	45	14	7.5	1.86
11.06.	22.0	11.4	9.5	4	9.5	0.42
17.06.	7.4	0.5	110	45	0.95	47.3
5.07.	8.2	–	14000	45	150.0	0.3
31.07.	10.0	6.1	4.5	0.25	0.45	0.55
5.08.	5.8	19.0	14.0	0.95	0.95	1.0
19.08.	21.5	130.0	250.0	25	11.0	2.27
29.04.1997	247.0	25.2	14.0	4.5	0.14	30.2
30.04.	656.0	247.0	140.0	14.0	11.0	1.2
3.05.	48.7	21.5	110.0	14.0	4.5	3.1
14.05.	100.0	50.0	140.0	11.0	45.0	244.0
27.06.	1.2	1.8	110.0	14.0	0.24	58.0
1.07.	293.0	94.0	140.0	14.0	15.0	0.93
16.07.	0.4	0.5	0.5	4.5	28.0	0.16
17.07.	135	31.8	140.0	11.0	25.0	0.44
5.08.	1284.0	772.0	1400.0	140.0	140.0	1.0
13.08.	10.4	9.9	450.0	2.5	45.0	0.05
20.08.	36.0	6.35	45.0	2.5	20.0	0.12

CFU – colony forming units, MPN – most probable number, TVC 20°C, TVC 37°C, TC, FC, FS – as in Table 2.

Taylor and Burrows [28] stated that calves grazing on pastures fertilized with liquid manure containing 10^6 cells of-***Salmonella dublin/ml,*** were infected by this pathogen. They did not observe, however, any cases of salmonellosis in calves grazing on pastures fertilized with manure containig 1000-times fewer *Salmonella* cells. According to Feachem's data of 1978 cited by Kowal [21], there is no proof that cattle grazing on pastures irrigated with sewage were more exposed to salmonellosis than "other cattle", this being so because dangerous *Salmonella* numbers are rather high (10^5 - 10^8 cells). *Salmonella* can pass to cattle in a number of other ways. Based on *Salmonella* sp. counts in sewage and sludge in England, Jones et al. [19] concluded that a 4-week waiting period will prevent salmonellosis in grazing cattle.

Conclusions

1. The results of microbiological examination of grass grown on control plots with no irrigation and fertilization, plots irrigated with clean water, and plots irrigated with biologically treated sewage (effluent from the treatment plant), treated sewage collected from a biological pond, or fertilized with mineral fertilizers showed that it contained different groups of bacteria indicatory of pollution and sanitary state, or even potentially pathogenic.

2. Grass from plots irrigated with biologically treated sewage and treated sewage collected from the biological pond had higher numbers of bacteria indicatory of sanitary state (TC, FC, FS), potentially pathogenic *(Aeromonas hydrophila, Staphylococcus* sp.) and fungi determined in Tri chophyton 1 medium compared to the **control.** However, ther amounts were not statistically significant ($a < 0.05$). The amount of treated sewage collected from the biological pond and used to irrigate the plots had no significant effect on the numbers of these micro-organisms in grass, or **its** effect was not clear.

3. Numbers of heterotrophic bacteria (TVC 20°C, TVC 37°C) as well as of some others (TC, FS, *Aeromonas hydrophila, Staphylococcus* sp.) increased with grass age. The ir maximal numbers found in grass from the 2nd or 3rd swath suggest worse bacteriological quality of grass collec ted later in the season.

4. Sporadic occurrence of *Pseudomonas aeruginosa* and *Salmonella* sp. in all 8 experimental variants may have been caused by their inflow with storm sewage, or else they might have been brought in by birds and small rodents. In view of low FC numbers in the grass, the possible role of these micro-organisms should not be over-rated, as **they** were not likely to become pathogenic to cattle consuming proper feeds containing these grasses (hay).

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