Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2003
Volume 6
Issue 2
Series
ENVIRONMENTAL DEVELOPMENT

CZESZEJKO K., BOGUSŁAWSKA-WĄS E., DĄBROWSKI W., KABAN S., UMAŃSKI R. 2003. PREVALENCE OF *LISTERIA MONOCYTOGENES* IN MUNICIPAL AND INDUSTRIAL SEWAGE **Electronic Journal of Polish Agricultural Universities**, Environmental Development, Volume 6, Issue 2.
Available Online http://www.ejpau.media.pl

PREVALENCE OF *LISTERIA MONOCYTOGENES* IN MUNICIPAL AND INDUSTRIAL SEWAGE

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ABSTRACT

An overall number of sixty samples of treated and untreated sewage was tested in our studies. The samples represented both municipal and industrial sewage (30 samples each type) and were collected respectively at the point of sewage discharge to a river, and at a poultry processing plant, namely from a secondary settler and an aeration chamber, as well as at the discharge of the treated sewage to the environment. Industrial sewage was tested with both chemically treated and untreated samples. The samples were analysed microbiologically for the presence of *Listeria* sp with a modified MPN method using membrane filtration, fecal coliforms by means of MPN method based on the PN-77 C-04615 standard, including the ISO 7251:1993 standard, and tested for the total microbial count on agar plates at 20°C. Results revealed lack of correlation between an indicator of fecal contamination and the presence of *Listeria* sp. Species analysis of isolated *Listeria* spp. proved *Listeria monocytogenes* to be the dominant species.

Key words: *Listeria* monocytogenes, sewage, sewage treatment plants

INTRODUCTION

Being a good solvent water is particularly prone to contamination. Not only humans, animals, birds but also the environment itself provides a reservoir for microorganisms found in water. Water cycling in nature may cause undesirable pathogens to spread. Municipal sewage, i.e. domestic, alike the industrial one and rainfalls, is commonly delivered to surface waters.

Both domestic and industrial sewage have been a serious problem in Poland. In 1996, Szczecin alone produced 29 hm^3 of municipal sewage. 57.9% of industrial plants have no sewage treatment facilities, 11.1% of which discharge their sewage to surface waters, and 46.8% to sewers or directly to the ground. The number of sewage plants is not sufficient when compared to the needs. Unfortunately, sewage treatment plants may also contribute to exposing their employees and neighbours to a potential health hazard. Microbial aerosols created there may pose a health threat. Numerous studies pointed out to possible transmitting *Salmonella enteritidis* and *Salmonella boydii*, reoviruses, enteroviruses [8] as well as hemolytic bacteria [26] by air.

In a multistage process of complete sewage purification sewage silts are formed in many stages. Silts may be used in agriculture for ‘irrigation and fertilisation of arable lands and ponds for fish raising and breeding (Act Register 2001.115.1229). According to the EC regulations, the only bacteriological agent which restricts such application of sewage is *Salmonella* sp. [14]. Studies carried out on sewage silts used for plant fertilisation displayed various results both confirming [21], and denying [3,29] the occurrence of *Salmonella* sp. The increase in infections caused by ‘new and emerging pathogens’ is observed [13,24]. Studies confirm prevalence of bacteria of *Campylobacter* sp. [32], *Vibrio cholerae* (despite purification in a stabilising pond) [30], *Salmonella* [31,2], and *Listeria monocytogenes* [14,1] in waters. Water contaminated with waterborne pathogens is the most frequent cause of reclaiming water that is intended for drinking. However, there are no requirements for testing water for the presence of pathogens such as *Listeria monocytogenes*. That microorganism is resistant to temperature fluctuations - its growth within the temperature range from 3°C to 44°C was recorded, it tolerates wide pH ranges (4.3-9.6), high concentrations of NaCl (10-30%), decreased O_2 concentrations, and CO_2 presence in the environment [11]. *Listeria monocytogenes* related disease is called listeriosis.

Listeriosis is basically a foodborne disease. It may be either sporadic or epidemic; its outbreaks involved from tens to several hundreds people. In the United States *Listeria monocytogenes* is reported to be a causative agent of 1700 sepsis cases per year, with a 25% death rate [33]. Such a high death rate quickly drew both scientific and social interest to the microorganism. *Listeria* rods are commonly present in nature, also in aquatic environments, which represent a potential source of listeriosis infection.

Daily about 100 000 m^3 of untreated, or treated insufficiently, municipal sewage is discharged to the Odra river from the area of Szczecin. The aim of our studies was to evaluate sewage contamination with *Listeria* spp. and efficacy of its elimination in the municipal sewage treatment plant, as well as in the one located in the poultry processing plant.

MATERIALS AND METHODS

60 samples of sewage were analysed; among them 30 municipal sewage samples collected from a mechanical and biological sewage treatment plant (BGN, BGO), and other 30 industrial sewage samples taken from the poultry processing plant (PN, PCH, PKN, POW, PO) (Table 1).

Table 1. Types of sewage and their origin

Abbreviation	Type of sewage	Origin
BGN	household	the point of sewage delivery to the treatment plant
BGO	household	the emission point of treated sewage to the environment
PN	industrial	the point of sewage delivery to the mechanical treatment plant
PCH	industrial	the point of sewage discharge from chemical treatment plant
PKN	industrial	the aeration chamber
POW	industrial	secondary settler
PO	industrial	a discharge point of treated sewage to the environment

Listeria spp. were isolated from the tested samples on Fraser Broth Base supplemented with an antibiotic (Oxoid) by a membranous filtration. A 10-mL sample of water was filtered via membranous filters (0.45µl) (Millipore) in three repetitions and placed in a selective medium. 1 mL and 0.1 mL quantities of water were poured directly into the tubes. All samples were vortexed for 1 minute, and then incubated at 30°C for 72 hours. Positive cultures were reinoculated on Listeria Selective Agar Base supplemented with antibiotic (Oxoid) and incubated at 30°C for 72 h. Characteristic colonies were identified by API®LISTERIA (BioMerieux).

Selected samples underwent microbiological analysis according to the PN-77 C-04615 standard - *Indication of coliforms by a tube fermentation method* – including the ISO 7251:1993 standard – *The general rules of indication of potential Escherichia coli- the most probable number method* – on LPB medium (BTL).

The total microbial count was also performed on a nutrient agar (Difco) incubated at 20°C for 72 hours.

Statistical analysis, i.e. calculations of means values, standard deviations, as well as significance and correlation tests were performed with Statistica PL software.

RESULTS

Quantitative analysis revealed statistically insignificant ($p<0.05$) elimination of all groups of microorganisms tested during municipal sewage purification.

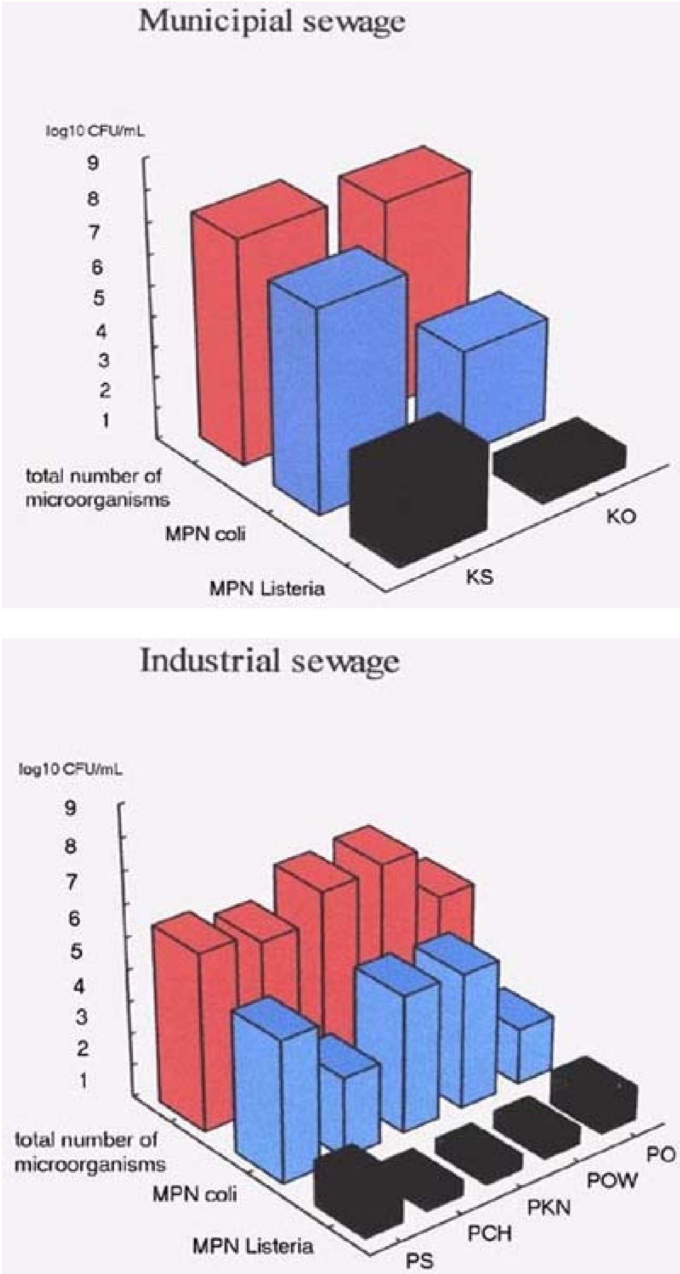
With reference to the untreated sewage a significant decrease ($p<0.05$) in the total number of microorganisms was observed for the sewage collected at the point of discharge to the river. At further stages of purification no significant differences in the total number of microorganisms were recorded (Table 2 and Figure 1).

Table 2. Significant differences in the total number of microorganisms/ coli index/ *Listeria* spp. index for particular sewage types

	BGN	BGO	PN	PCH	PKN	POW	PO
BGN		-/-/-					
BGO	-/-/-						
PN				-/X/X	-/-/X	-/-/X	X/X/-
PCH			-/X/X		-/-/-	-/-/X	-/-/X
PKN			-/-/X	-/-/-		-/-/X	-/X/X
POW			-/-/X	-/-/X	-/-/X		-/X/-
PO			X/X/-	-/-/X	-/X/X	-/X/-	

X – statistically significant differences ($p<0.05$)
- statistically insignificant differences

Fig. 1. Concentration of microflora in the tested samples of sewage



An analysis aimed at detecting coliform presence displayed significant decrease ($p<0.05$) in their number in relation to the untreated sewage: two logarithms down in the industrial sewage after chemical purification, and three logarithms down in the treated one. No significant differences in the number of coliforms between the untreated sewage and the one at its further biological purification stages (Table 2 and Figure 1) were found.

A quantitative analysis of *Listeria* sp. in the industrial sewage showed significant decrease ($p<0.05$) in the MPN of *Listeria* after chemical treatment and in the aeration chamber. Sewage analyses performed in the secondary settler and at the point of treated sewage discharge to the environment revealed significant increase in the number of *Listeria* in comparison to the previous stages of the sewage treatment. At these stages of purification the level of *Listeria* spp. was similar to the one in the untreated sewage ($p<0.05$) (Table 2 and Figure 1).

Table 3. Species differentiation of *Listeria* spp.

Sewage	Species	[%]
BGN	*L. monocytogenes* *L. seeligeri* *L. grayi*	90.0 5.0 5.0
BGO	*L. monocytogenes*	100.0
PN	*L. monocytogenes*	100.0
PCH	*L. monocytogenes*	100.0
POW	*L. monocytogenes*	100.0
PKN	*L. monocytogenes*	100.0
PO	*L. monocytogenes*	100.0

Species analysis of *Listeria* spp. displayed *Listeria monocytogenes* as dominant (90%) in the untreated municipal sewage. Moreover, the presence of *Listeria seeligeri* and *Listeria grayi* was detected, 5% of each, whereas in the treated municipal sewage and in the industrial sewage at all the stages of its treatment (Table 3) solely the presence of *Listeria monocytogenes* was disclosed.

DISCUSSION

Industrialisation and urbanisation have negatively affected the quality of surface waters that provide drinking water to majority of Polish population. It has been reported that in Poland about 20% of sewage is discharged directly into the surface waters. Moreover, no analysis are officially required on sewage intended for agricultural utilisation, though it may be a cause of introducing to water not only allochtonic microflora but also pathogenic microorganisms. According to the regulation of the Minister for the Environmental Protection, Natural Resources and Forestry dated November 5th, 1991, sewage introduced to surface waters cannot affect adversely the natural biocoenosis typical for such waters. Therefore, it should not contain pathogenic microorganisms and its coliform index should correspond to the quality class of water into which the sewage is discharged.

The index of sanitary contamination in the tested samples reveals that coliforms are mainly eliminated during chemical treatment of industrial sewage. The increase in the amount of coliforms observed during biological treatment may result from contamination of the secondary settler by birds or other animals. However, a significant decrease in the coliforms? index in the treated sewage may suggest utilisation of *Escherichia* sp. in an active sediment. Neither during mechanical nor biological treatment of domestic sewage a significant decrease in the number of coliforms was observed, and the quality of the treated sewage met the requirements for waters of the third class quality.

The studies revealed that in the process of mechanical and biological treatment a reduction in the number of classic microbiological indices fell to the level that compliant with the official standards. Nevertheless, the decrease was not correlated with the reduction of isolated *Listeria* spp.

Our work proved that though a significant decrease in the number of *Listeria sp.* was observed already during chemical treatment, the treatment of the municipal sewage was not efficient enough to eliminate pathogenic microorganisms. Even pH of 11 only reduced the number of the bacteria but not eliminated them completely. Hence, reports on efficient elimination of microorganisms due to high pH values have been disproved in this case [17]. Additionally, in the industrial sewage collected at the treated sewage discharge to the environment, a rise in the amount of *Listeria monocytogenes* was observed. It may result from the outdoor location of both the aeration chamber and the secondary settler, since birds are known to be a reservoir of this pathogen [35,18,34]. It is then very likely that contamination caused by their faeces increased the number of *Listeria* sp. Earlier studies also highlighted inefficiency of biological treatment in eliminating *Listeria monocytogenes* from sewage [22].

Usually research is focused on the presence of *Listeria monocytogenes* in domestic [6,28] and industrial [14,1,23] sewage. Occurrence of different pathogenic microorganisms in sewage, such as *Candida albicans* [16], *Salmonella typhimurium* [30], neuroviruses [25] and even parasite eggs *Ascaris suum* [17] have been also reported.

It is well known that waters quality depends on the quality of the sewage discharged into. Ferguson et al. [19] found the increased number of fecal bacteria, fecal coliforms, spores of *Clostridium perfringens* and *Aeromonas* correlated with the amount of sewage introduced into a river estuary. Similar results were presented by Contreras-Coll et al. [10] who confirmed occurrence of different groups of pathogenic sewage-borne microorganisms in various European recreational waters. We find it alarming that in the samples collected at the sewage discharge to the environment, *Listeria monocytogenes* constituted a 100% of all *Listeria* spp. Most likely it is the reason for a highly frequent (54,5%) contamination of Odra waters with this pathogen [12].

Still, even more alarming is the occurrence of *Listeria monocytogenes* detected not only in rivers [27,4], lakes [7,20], sea waters, but also in mountain surface and ground waters that very often serve as drinking water reservoirs for people and animals [32,4].

Quality evaluation of water meant for consumption is based mainly on application of fecal coliform and fecal streptococci counts. Nevertheless, it turns out that even water that meets the requirements defined in the official standards, may contain other, not specified in the regulations, dangerous pathogens. The supposition was confirmed by the results presented by El-Taweel and Shaban [16] who studied samples intended for consumption that were collected from sewage treatment plants. Studies of samples that underwent the entire treatment process showed that despite maintaining adequate levels of standard microbiological contamination indices, such as total number of bacteria, fecal coliforms, fecal streptococci, etc., the admissible limits for so-called ‘new indices’ were well exceeded. Namely, a total number of yeasts, *Candida albicans*, *Aeromonas hydrophila*, and streptococci were exceeded. *Salmonella* sp., *Vibrio* sp., *Listeria* sp. were also isolated from those samples.

Both the quoted and obtained in the presented study results highlight the problem of pathogenic *Listeria* distribution via waters. Our studies indicate inefficiency of sewage treatment plants with respect to eliminating the microorganisms in question. Even a multistage treatment process applied to municipal sewage cannot entirely eliminate this pathogen. Necessity and significance of introducing additional indices for correct and reliable evaluation of microbiological quality of waters is underlined.

CONCLUSIONS

1. Combined mechanical and biological treatment of domestic sewage does not eliminate *Listeria monocytogenes*.
2. Mechanical and biological treatment of sewage from a poultry processing industry does not eliminate *Listeria monocytogenes*.
3. A lack of correlation between *Listeria* sp. and MPN of fecal coliforms in municipal sewage was observed.
4. Irrespectively of the level of contamination evaluated in municipal sewage, *Listeria monocytogenes* was found to be dominant species of *Listeria* sp.

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