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THE SURVIVAL OF SELECTED FECAL BACTERIA IN PEAT SOIL AMENDED WITH SLURRY

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ABSTRACT

Slurry often contains many pathogenic microorganisms, which are dangerous to human and animal health. The aim of study was to estimate the elimination rate of indicators and pathogenic bacteria – *Escherichia coli*, fecal streptococci D-group and *Salmonella senftenberg* W_{775} in peat soil according to soil storage temperature and slurry addition. The elimination rate of fecal bacteria was more effective in 20°C than in 4°C. The slowest reduction of the fecal bacteria was observed in soil with slurry. The salmonellas were eliminated more slowly in comparison to another group of investigated microorganisms. The fastest reduction of streptococci was estimated in 20°C, however *E.coli* in 4°C. To minimize the hazard for environment, slurry should be subjected to monitoring investigation before use on arable land.

Key words: slurry, peat soil, *Escherichia coli*, *Salmonella*, fecal streptococci.

INTRODUCTION

The agricultural utilization of slurry is common in many countries. Slurry rich with nutrients easily available for plants is widely used as an organic fertilizer [10, 18]. In Poland there are few monitoring microbiological investigations concerning hygienic status of slurry spread out on fields. However, it is often not stabilized before using. If slurry is not stored for appropriate time or not subjected to hygienic processes it may contain many pathogenic bacteria, viruses, fungi and ova of parasites [1, 9, 11, 19]. The microorganisms present in slurry used

for fertilizing may migrate to ground waters, contaminate plants and pose hazard for human and animal health. Especially enteropathogenic strains of *E. coli, Salmonella* and streptococci are potentially dangerous from sanitary and epidemiological point of view, due to their high resistance for environmental factors. Contamination of environment is mainly caused by sick animals or animals which do not show any symptoms of disease [6]. The evaluation of risk of environment contamination resulting from using slurry can be achieved by monitoring of the count of selected fecal microorganisms. Fecal bacteria do not remain in soil for a long time and are gradually eliminated. The information about elimination rate of fecal microorganisms in soil is very important for sanitary and epidemiological reasons. Due to much permeability and high level of ground waters, even accidental use of slurry for fertilizing in this type of soils can be extremely dangerous.

The aim of the investigation was to estimate elimination rate of fecal bacteria in peat soil in laboratory conditions and to study the effect of temperature and addition of slurry on their survival rates.

MATERIALS AND METHODS

Experimental procedure

Ten grams of freshly taken and sieved peat soil were placed in plastic tubes. The soil was wetted with 0.5 ml of suspensions of *Salmonella senftenberg, Escherichia coli* and streptococci D-group (density of 10^7 - 10^9 MPN·ml^{-1}). One ml of cattle slurry was additionally given to some of the samples. Water capacity of all samples was brought to 60%. The study was carried out in 4 and 20°C according to the following schema:

a. soil + bacteria suspension,
b. soil + bacteria suspension + slurry.

Soil samples for microbiological studies (3 replications) were taken in 3-week intervals during 36 weeks. In all laboratory studies 144 samples were investigated.

Microbiological and physicochemical soil analysis

The number of bacteria in soil was calculated with MPN method in 10 g of soil sample. For *E. coli* equation Mac Conkey bouillon was used (43°C for 24 hours). Then the material was sieved on agar with tergitol and TTC (43°C for 24 hours). The test for decarboxylase of glutamine acid was made for confirmation. The ability of *E.coli* of lactose utilization with gas deliberation was also tested (44°C for 48 hours).

Broth bouillon with glucose and azide was used as an enriching medium for selective growth of fecal streptococci. After 48 hours of incubation in 37°C the material was streaked to agar with esculine and azide (37°C for 48 hours). The serological test for confirmation of presumption colony was made (Phadabac-test).
For isolation of *Salmonella* two media were used. In the first stage soil samples were blended in 1% peptonic water (24 hours in 37°C) and then 0.1ml of material from each test-tube was streaked to selective fluid medium acc. to Rappaport (43°C for 24 and 48 hours). Afterwards the material was sieved to selective agar BPLA acc. to Kaufmann (37°C for 24 hours). The identification was confirmed by using serological tests with polivalent serum Hm.

At the same time physicochemical soil studies were carried out with methods commonly used in agricultural chemistry.

Statistical analyses

Microsoft Excel software was used to perform statistical analyses. The significance of differences between the counts of investigated microorganisms in particular stages of the experiment was determined with Student's t-test. The critical P-value for the t-test was set at 0.05.

RESULTS

The results of investigation are presented in Table 1 and in Figs. 1-3. Soil taken from the layer of 0-20 cm was investigated. It was fine-grained boggy soil, silted up with carbonates, gray and brown in color, with medium-shaped aggregate structure. Organic matter content was 32.35%, and pH measured in KCl ranged from 7.1 to 7.5.

Table 1. Regression lines for survival of investigated bacteria according to temperature and slurry addition

	Temperature °C	*Salmonella*	*Escherichia coli*	*Streptococcus* D-group streptococci D-group
Peat without slurry	4	36.9 weeks y = 8.657 – 0.234x r = - 0.984	32 weeks y =8.074 – 0.252x r = - 0.974	33.8 weeks y = 7.677 – 0.227x r = - 0.944
	20	28 weeks y = 8.343 – 0.296x r = - 0.967	24.6 weeks y = 8.287 – 0.336x r = - 0.956	16.5 weeks y = 9.525 – 0.575x r = - 0.982
Peat with slurry	4	28 weeks y = 8.801 – 0314x r = - 0.983	30 weeks y = 8.280 – 0.276x r = - 0.975	43 weeks y = 7.853 – 0.181x r = - 0.956
	20	34.5 weeks y = 7.945 – 0.230x r = - 0.956	26.8 weeks y = 8.619 – 0.321x r = - 0.978	24.8 weeks y = 8.433 – 0.340x r = - 0.982

During the process of storage the elimination of fecal bacteria in soil was noticed. It was observed in all investigated microorganisms and had different effectiveness depending on storage temperature, slurry addition, and the species of fecal bacteria. In peat soil with an addition of suspension of fecal bacteria longer survival time of bacteria incubated in 4°C than 20ºC was noticed. Statistical calculations (Table 1) indicate that the survival of investigated bacteria in 4°C varied from 28 to 43 weeks, and in 20°C – from 16.5 to 34.5 weeks. This tendency was clearly visible in fecal streptococci which survived in 4°C 17.3 weeks longer than in 20°C. Similar reaction for temperature was determined in *Escherichia coli*. They were isolated 7.4 weeks longer in 4°C in comparison to samples stored in 20°C. *Salmonella* was more persistent in lower temperature as well. The shortest survival in 20°C was noticed in fecal streptococci. Their number decreased by 0.575 log·week $^{-1}$. *E. coli* and *Salmonella* were eliminated more slowly – 0.336 log·week $^{-1}$ and 0.296 log·week $^{-1}$, respectively.

Fig. 1. Survival of *Escherichia coli* in peat soil with/without slurry according to temperature

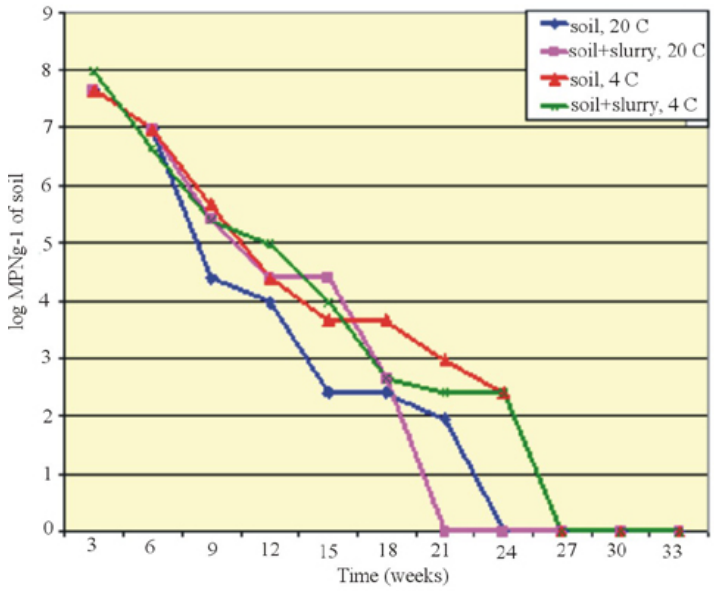


Fig. 2. Survival of group D streptococci in peat soil with/without slurry according to temperature

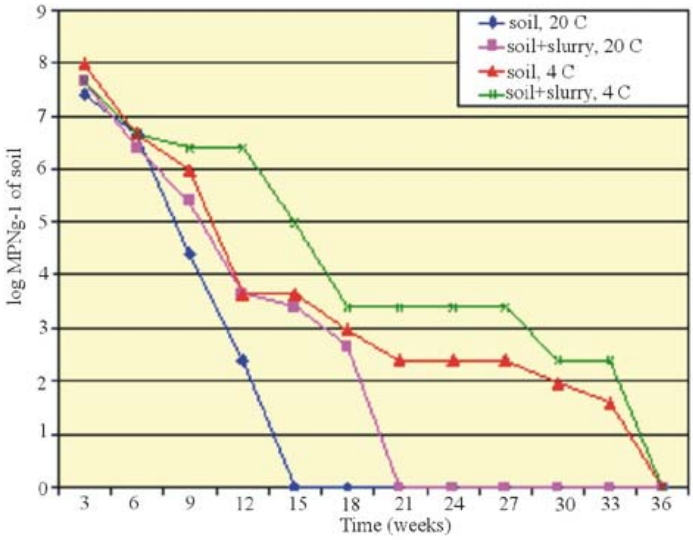
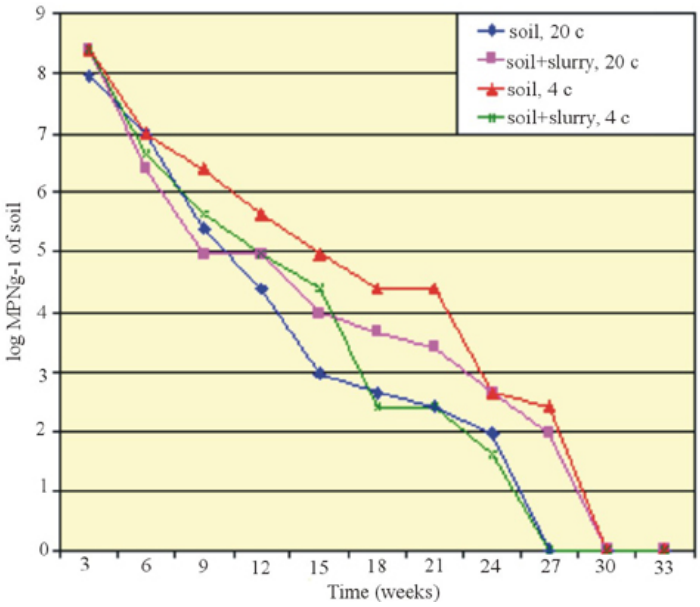


Fig. 3. Survival of *Salmonella* in peat soil with/without slurry according to temperature



The kinetics of quantitative changes of investigated bacteria in soil amended with slurry was quite different. The survival rates varied from 24.8 to 43 weeks in comparison with 16.5- 36 weeks observed in samples without slurry. Streptococci D-group were determined 9,2 weeks later in soil with slurry in lower temperature and 8.3 weeks later in 20°C. The survival time of *E. coli* in 4°C was 2 weeks longer in soil without slurry, while in 20°C the bacteria were eliminated more slowly (by 2.2 weeks) in soil with slurry. In 4°C *Salmonella* was eliminated 8.9 weeks later in a sample without slurry, and in 20°C – persisted 6.5 weeks longer in soil with slurry.

The results obtained indicate that all investigated bacteria were eliminated faster from soil with slurry in 4°C. In higher temperature *E. coli* and *Salmonella* lived longer in soil with slurry, while streptococci were eliminated faster in samples without slurry.

DISCUSSION

As soil does not provide favorable conditions for fecal bacteria due to small amount of nutrients, low temperature, and the presence of autochtonic microflora, they are gradually declined to different extent [12, 13].

Our investigations showed that the number of microorganisms introduced to soil stored in higher temperature declined much more rapidly than in 4°C. Thus, *Salmonella* persisted the longest (34.5 weeks) and streptococci – the shortest (16.5 weeks) in soil in 20°C. However, in lower temperature, on the contrary, elimination rate of *Salmonella* was the fastest (after 28 weeks) and of fecal streptococci – the slowest (43 weeks). It is generally accepted that high temperature do not favor vitality of fecal bacteria in soil. *E. coli* survival rate, determined at 10, 26, and 37°C, decreased with the increase of temperature [4, 6, 7]. Similarly, *Salmonella typhimurium* in animal feces stored in 20°C and 4°C persisted 63 and 111 days, respectively [21]. Within the range from 5 to 30°C elimination rate of fecal microorganisms in soil increased two times with temperature growth of 10°C [16]. Our results suggest that the decline speed of indicator bacteria was less in soil samples with slurry. It was connected with the presence of nutrients easily available for bacteria.

Apart from temperature and organic fertilizers, a kind of soil has also a great effect on survival rate of allochtonic bacteria in soil. It seems that soil rich with organic matter favors longer survival of microorganisms. It has been proved by previous studies on pathogenic bacteria in mud, where they lived much shorter than in peat [14].

The most dangerous of investigated fecal microorganisms is *Salmonella*. Slurry contaminated with *Salmonella* and spread out on grassland may infect grazed animals [8]. Consumption of raw not washed vegetables from crops fertilized with slurry may also pose hazard for people health [17]. Our investigations indicate that *Salmonella* was especially resistant to environmental factors in the laboratory experiment. It survived in 20°C: in soil without slurry – 28 weeks, and in samples with slurry – 34.5 weeks, respectively. There are extremely different data in literature regarding *Salmonella* survival rates in soil. Pioch and Bräuing [15] observed elimination of bacteria in soil after 6 days, while Thunegard [20] – within the range from 6 to 64 weeks, and Hess et al. [5] isolated them in soil even 500 days after application.

The behavior of *E. coli* and streptococci D-group in our investigations was similar, though a tendency to longer survival time of fecal streptococci was observed (especially in 4°C in peat with slurry). The results are confirmed with the report of Crane and Moore [2], who observed longer survival time of enterococci in comparison with *E. coli*.

The number of fecal bacteria added to soil in the study was relatively big, but it did not exceed upper limit observed in animal feces. The number of *E. coli* may vary from 10^5 to 10^7, while enterococci from 10^4 to 10^8 bacteria in 1g of cattle feces [3]. Soil contamination with such high amount of fecal bacteria enabled both to isolate them easier and to investigate precisely the rate of their inactivation.

In favorable conditions, especially after abundant rainfall, fecal bacteria may migrate into soil profile and contaminate ground waters. Long-term presence of the microorganisms in soil proves the necessity of constant monitoring of slurry sanitization effectiveness before spreading out on arable lands.

CONCLUSIONS

1. Elimination rate of allochtonic bacteria in soil was much faster in 20^{o}C than in 4^{o}C, and addition of slurry made the process slower.
2. *Salmonella* survived the longest, and streptococci - the shortest in soil without slurry; while in soil with slurry added streptococci survived the longest of all investigated bacteria in lower temperature and the shortest in higher temperature.
3. Due to possibility of presence of pathogenic bacteria in slurry and their long survival rate in soil it should be monitored in order to minimize the sanitary and epidemiological hazard.

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