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EFFECT OF PIG FARM ON MICROBIAL CONTAMINATION OF SOIL*

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Abstract

The objective of the study was to assess microbial contamination of soil collected in a swine farm and manure from animals housed there depending on the season of the year and the sampling site. The study was conducted from October to September. The soil samples were taken immediately at the pig house wall (GI), and at the distance of 15 m (GII) and 45 m (GIII) from the house wall. Besides, manure samples were collected inside the pig housing facility: at the entrance to the pig house (KI) and at 1/4 (KII) and 1/2 length of the animal facility (KIII). The soil and manure samples underwent qualitative and quantitative bacteriological evaluation. The study was conducted according to the procedure laid out in the Polish Standards. There was also assessed air temperature and relative moisture, air motion and cooling as well as sample moisture in the sampling site. The greatest number of all studied bacteria was determined in soil collected 15 m from the piggery (GII) in December/January. The highest coli titre (0.0001) was also established in the samples (GII) at that time. The qualitative analysis of soil showed solely the presence of *E. coli* bacteria which were recovered in the GII soils taken from November to May. The largest bacterial load in swine manure was determined in the samples collected at 1/2 length of the pig house (KII) at the end of December and January. The growth of all the analysed microbes was favoured by sample moisture, while air relative moisture prompted development of psychrophilic and proteolytic bacteria. *E. coli* were isolated in manure samples throughout the entire research period, whereas *Enterobacter* spp. were detected in the KI and KII samples from June to August and in KII samples from June to September. The winter period was shown to affect significantly microbial contamination of swine farm environment as at that time the highest bacterial load was determined in soil and manure. This is most likely to be associated with the climatic and microclimatic conditions observed in those days.

Key words: pigs, soil, faeces, bacteria

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Pig farming produces emissions of biological (microbes), mechanical (dusts) and chemical (gases) contaminants. Microbial contamination of animal environment constitutes one of the most profound health and life hazards to animals during the raising period. It is associated with confinement of high numbers of animals per unit area that contributes to considerable pollution of air and bedding material in pig facilities (Buczyńska and Szadkowska-Stańczyk, 2010). Studies determined the presence of numerous microorganisms in air of the swine facilities, the most frequently isolated bacteria included *Escherichia coli*, *Staphylococcus xylosus*, *Micrococcus lentus*, *Streptococcus uberis*, *Leuconostoc lactis* and *Shigella* spp., *Enterococcus faecium* and *Enterococcus faecalis* (Jurek et al., 2006; Kluczek, 2002). Besides, *Salmonella* rods were commonly identified among bacterial pathogens (Nowak et al., 2007). Sandvang et al. (2000) showed in their study the presence of Salmonella in more than half of the samples collected in the immediate vicinity of a pig facility. Whereas Letellier et al. (1999) evaluating the samples taken from various sites in the swine unit (doors, floor, ventilation system, litter), recovered *Salmonella* rods in 70.7% of the samples. Bacterial pollution of air in different units of pig facility was studied by Tombarkiewicz et al. (2000) who determined the highest total bacterial count (55600 per m^3 air) in the swine farrowing unit.

Bacteria habitually identified in a pig house also include *E. coli*, which is a component of the gastrointestinal tract flora and often trigger conditions associated with diarrhoea (Kiers et al., 2007; Weiner et al., 2004). It is also thought that better knowledge of the factors affecting the survival of pathogenic strains of *E. coli* in the soil facilitates their more efficient control and prevents the transfer of these microbes to food products (Habteselassie et al., 2008). Bacterial development and survival in soil is favoured by high temperature and moisture (Boes et al., 2005). Takahashi et al. (2000) investigated seasonal changes in bacterial flora of manure from pigs before and after implementation of membrane filtration treatment. Microbes frequently isolated from animal faeces, such as *Streptococcus* spp. and *Lactobacillus* spp. dominated in July and October, *Clostridium* spp. in February and July, whereas *Corynebacterium* spp. in August and October.

Substantial bacterial contamination also pertains to the area surrounding large-scale livestock farms. Tymczyna et al. (1999) studying groundwater samples taken from the surroundings of a swine farm showed the presence of *E. coli*, fecal streptococci, *Clostridium perfringens* and *Pseudomonas* spp., whereas *Corynebacterium pseudotuberculosis*, *E. coli*, *Clostridium perfringens*, faecal streptococci, *Bacillus subtilis* and *Proteus* spp. were determined in soil samples. It is noteworthy to highlight a vital role of the environmental reservoir in the incidence of Salmonella-induced infections in pigs (Hoelzer et al., 2011).

Microbial contamination of animal faeces and natural environment, especially the presence of pathogenic bacteria, may pose human and animal health hazard.

The above findings have prompted the evaluation of bacterial contamination of soil collected from a pig farm and manure from these animals depending on the season of the year and sampling sites.

Material and methods

The studies were conducted at a swine farm with pigs of PLW (Polish Large White) and PL (Polish Landrace) breeds crossed with Duroc maintained under litter housing system. The animals were kept in two buildings, 100 pigs each. They were aged 25 months at the beginning of the study. The research period lasted for a year, from October to September (T1 – October, T2 – November, T3 – December/January, T4 – February/March, T5 – April/May, T6 – June, T7 – July/August, T8 – September). During the research period, the animals were under constant veterinary supervision. There were not recognized any symptoms of animal disease or death.

Soil samples were collected from the following three locations: GI – immediately at the pig house wall, GII – 15 m off the house wall, GIII – 45 m from it. Whereas manure samples were taken inside the pig housing facility, i.e. at the entrance to the pig facility (KI), at 1/4 length (KII) and at 1/2 length of the building (KIII). A total of 48 samples were collected, 7 from each research location, then averaged into one pooled sample. The samples were taken twice each month. The soil samples were collected according to the Polish Norm (PN-ISO 10381 – 6: 1998), employing sterile soil augers in the plots of 25 m^2 area at ca. 20 cm depth. The soil around the research objects was not cultivated or manure-contaminated but it was slightly grass overgrown.

The soil and manure samples underwent quantitative and qualitative bacteriological evaluation, estimation of total count of mesophilic, psychrophilic, proteolytic bacteria, actinomycetes, coliforms and *E. coli*. The values are given in log (cfu/g soil) and log (cfu/g manure). Besides, the value of coli titre of soil was estimated by the multiple-tube fermentation technique. The pH of the soil was determined to be in the range of 6.28–7.09. The organic matter content in the soil was between 76.39% and 88.94%.

Immediately after the samples were delivered to the laboratory, they were evaluated bacteriologically. A 10 g pooled sample was added to sterile distilled water containing Tween 80 surfactant and the soil solution shaken thoroughly. Afterwards, 1 ml of the solution was transferred to test tubes with 90 ml of Ringer lactate to obtain a dilution of 10^{-3}. Finally, serial dilutions were made.

With the aim of establishing the numbers of mesophilic bacteria, incubation was performed at 37°C for 24 h, while for psychrophilic bacteria at 22°C for 72 h. After incubation, the number of arising colonies was counted.

When assessing proteolytic bacteria, the inoculation procedures were made on Frazier's medium according to PN-A-82055-14: 1997, using the dilutions prepared before in Ringer lactate. After incubation at 26°C, the bacterial colonies were counted. The number of actinomycetes was determined on the nutrient medium for Actinomycetae performing the surface inoculation (PN-C-04615-27: 1981). Incubation process was carried out at 26°C for 5 days and subsequently the count of the characteristic colonies estimated.

Coliforms were inoculated into selective and differential medium Endo Les and incubated for 24 h (Oliver et al., 2010, PN-ISO 9308-1). Following this incubation period, the colonies were counted, transferred to the test tubes containing peptone water with lactose and incubated at 37°C for 48 h. Gas generation in the test tubes

was an evidence of the presence of coliform bacteria. *E. coli* bacteria were inoculated on the mFC medium and incubated at 44°C for 24 h. Then, the arising colonies were transferred to the test tubes with tryptone water (PN-ISO 9308-1). Coli titre value was determined according to PN-75052-11: 1990.

In order to isolate bacteria from the *Enterobacteriaceae* family, the technique of preincubation in liquid medium BPW (buffered peptone water) was used followed by multiplication on the RV medium (Rappaport-Vassiliadis) and reducing inoculations on solid selective-differential media XLD, BGA and SS (Nayak et al., 2003). Besides, biochemical analysis was performed using API 20E tests.

Air temperature, relative moisture, air motion and cooling as well as the moisture of the samples were evaluated in the area where the samples were taken. The soil and manure samples were placed in weighing bottles, then dried at 105°C for 24 h for dry matter. After drying, the samples were reweighed and the difference of weight served to calculate a percentage of water content.

Statistical calculations were conducted using single factor analysis of variance and multiple comparison Duncan's test. SAS Enterprise Guide 4.2 was applied with two levels of significance of differences $P \leq 0.05$ and $P \leq 0.01$.

Results

The results of quantitative bacteriological evaluation of soil subject to sampling sites are presented in Table 1. The highest total count of mesophilic, psychrophilic, proteolytic bacteria, actinomycetes, coliform and *E. coli* was determined in the soil samples collected 15 m off the building wall (GII). Regarding psychrophilic bacteria, significance of differences ($P \leq 0.05$) was found between the soil samples taken 15 m off the building wall (GII) and at the building wall (GI) as well as between GII and those collected 45 m off the piggery (GIII).

Table 1. Bacterial contamination of soil and swine manure (log cfu/g) related to sampling site

Bacteria	Distance from building			Distance within the building		
	soil			manure		
	GI	GII	GIII	KI	KII	KIII
Mesophilic	5.54	6.30	5.26	8.35	8.89	9.08
Psychrophilic	6.25 b	7.31 a	6.26 b	8.51	8.63	9.38
Proteolytic	4.83 a	4.98 a	3.59 b	4.49 b	5.01 b	5.57 a
Actinomycetes	4.43 a	4.60 a	3.77 b	-	-	-
Coliform	2.54 b	3.80 a	0	5.59 b	5.70 b	6.65 a
E. coli	2.09 b	2.72 a	0	5.16 b	5.35 b	6.37 a

Denotation: a, b – values in rows with different letters differ significantly ($P \leq 0.05$).
GI – soil samples collected immediately at the pig house's wall.
GII – soil samples collected at the distance of 15 m from the pig house's wall.
GIII – soil samples collected at the distance of 45 m from the pig house's wall.
KI – manure samples taken at the entrance to the pig house.
KII – manure samples taken at ¼ length of the pig house.
KIII – manure samples taken at ½ length of the pig manure.

Table 2. Microbial contamination of soil and manure (log cfu/g) depending on sampling date

Bacteria	Bacteria in soil							
	T1	T2	T3	T4	T5	T6	T7	T8
Mesophilic	5.68 b	5.83 b	6.51 a	6.28 a	5.46 b	5.30 b	4.23 c	3.86 c
Psychrophilic	6.79 b	6.24 b	7.32 a	7.24 a	6.15 b	5.85 b c	5.20 c	4.53 c
Proteolytic	4.75 a	4.58 a	5.04 a	4.82 a	4.97 a	4.69 a	4.42 a	3.87 b
Actinomycetes	4.47 a	3.33 b	4.86 a	4.66 a	4.23 a	3.85 b	3.05 c	2.85 c
Coliform	0	2.81 b	4.19 a	3.10 b	2.57 b	0	0	0
E. coli	0	1.92 c	3.19 a	2.66 b	2.50 b	0	0	0
	Bacteria in manure							
Mesophilic	8.10 b	8.92 a	9.61 a	8.83 a	8.01 b	7.68 b	7.46 b	6.32 c
Psychrophilic	8.46 b	9.17 a	9.54 a	9.44 a	8.29 b	8.19 b	7.70 b	6.47 c
Proteolytic	5.30 a	4.63 b	5.77 a	5.53 a	5.19 a	4.69 b	4.56 b	3.69 c
Coliform	5.96 b	6.01 b	6.90 a	6.55 a b	5.79 b	5.42 b c	5.01 b c	4.40 d
E. coli	5.50 b	5.84 b	6.53 a	6.40 a	5.14 b	4.90 b c	4.66 c	3.99 d

Denotation: a, b, c, d as in Table 1.
T1 – October, T2 – November, T3 – December/January, T4 – February/March, T5 – April/May, T6 – June, T7 – July/August, T8 – September.

As for proteolytic microbes and actinomycetes – statistical significance was stated between GI and GII and GIII, while for coliform and *E. coli*, significance of differences occurred only between the samples GII and GI. Thereby, *Escherichia* genus bacteria were only present in soil samples taken at a distance of 15 m from the pig house from November to May.

Effect of sampling dates on microbial contamination of soil is shown in Table 2. The highest total number of bacteria under investigation was reported at the turn of December and January (T3). For mesophilic, psychrophilic, proteolytic bacteria and actinomycetes a statistical significance ($P \leq 0.05$) was confirmed between sampling dates. Whereas for coliform bacteria and *E. coli* between T3 and the samples collected, the statistical difference was seen in November (T2), February/ March (T4) and April/May (T5).

The influence of a sampling site and date on coli titre of soil is presented in Table 3. The highest microbial contamination (coli titre – 0.0001) was also exhibited by soil samples taken 15 m off the pig facility (GII) at the end of December/January. The lowest contamination level was observed in soil samples taken 45 m from the building wall (GIII) as throughout the entire research period the coli titre was ≤0.01.

Table 3. Coli titre in soil as related to sampling site and date

Date	GI	GII	GIII
T1	≤0.01	≤0.01	≤0.01
T2	≤0.01	0.001	≤0.01
T3	0.001	0.0001	≤0.01
T4	≤0.01	0.001	≤0.01
T5	≤0.01	0.001	≤0.01
T6	≤0.01	≤0.01	≤0.01
T7	≤0.01	≤0.01	≤0.01
T8	≤0.01	≤0.01	≤0.01

Denotation: T1 – T8 – as in Table 2.

The impact of sampling locations on bacterial pollution of manure from the pigs is shown in Table 1.

The greatest bacterial load was found in the manure samples collected at 1/2 length of the pig facility (KIII). Statistically significant differences ($P \leq 0.05$) were noted in the case of proteolytic bacteria, coliform and *E. coli* between the samples KIII and those collected at the entrance to the building (KI) and KIII and the samples taken at ¼ length of the pig house (KII).

Assessment of the basic climatic parameters during the soil sampling showed that air temperature ranged between 0.18°C in December/January and 30.7°C in July/ August. Relative moisture reached 39% in winter and 63% in April/May. The air motion measurements oscillated from 0.23 m/s in September up to 1.45 m/s at the end of December/January. The lowest soil water content (1.02%) was established in July/August, whereas the highest (4.98%) in December/January.

For the pig housing facility, the highest air temperature (24°C) was determined in September, whereas the lowest (15°C) at the turn of February and March. The highest air relative moisture was recorded in October (80%) and the lowest in April/May (61%). While the range of air motion values was from 0.10 m/s at the end of December/January, February/March and July/August up to 0.16 m/s in October. The cooling was within the interval of 0.15 W/dm^2 in April/May to 3.73 W/dm^2 in June and in July/August. A moisture level of the manure samples ranged between 3.10% in February/March and 9.01% in April/May.

The effect of a sampling date on microbial contamination of manure is presented in Table 2.

Similarly to the case of soil, the highest bacterial count under investigation was determined at the turn of December and January. Significance of differences ($P \leq 0.05$) referring to all bacteria was established between each manure sampling date.

Furthermore, correlations between the chosen microclimate parameters and moisture of samples and bacterial count in the pig manure were calculated. It was found that increasing air relative moisture significantly ($P \leq 0.05$) raised the number of mesophilic and proteolytic bacteria. An elevated moisture level of samples had significant influence ($P \leq 0.01$) contributing to increased numbers of all the bacterial groups under study (Table 4).

Table 4. Correlations between parameters of microclimate and sample humidity and bacterial count in swine manure

Pearson correlation coefficients, True > |r| at H0: Rho=0					
Parameter	Bacteria				
	mesophilic	psychrophilic	coliform	*Escherichia coli*	proteolytic
Air relative humidity (%)	0.490*	0.372	0.322	0.318	0.403*
Air temperature (ºC)	–0.053	–0.050	–0.038	–0.023	–0.100
Sample humidity (%)	0.527**	0.732**	0.703**	0.729**	0.735**

Denotation: * $P \leq 0.05$. ** $P \leq 0.01$.

In qualitative assessment of bacteria occurring in manure samples subject to a sampling site and date, the presence of *E. coli* was determined in three sampling locations throughout the research period. Whereas bacteria from the *Enterobacter* genus were recovered from the samples collected at the entrance to the pig facility (KI) and at 1/2 length of the building (KIII) from June till August and those taken at 1/4 length of the building from June to September.

Discussion

Soil proves to be the natural habitat for bacteria. It harbours numerous saprophytes, yet pathogenic bacteria can also enter it with animal faeces. As manure is

commonly applied for field fertilization, the appropriate withdrawal period must be observed. Otherwise, manure can introduce pathogenic bacteria and viruses to the soil (Amin et al., 2013).

The soil biota, including their microbial activity, is affected by organic fertilizers obtained from high production farms (Plaza et al., 2004).

The highest numbers of investigated bacteria were established in the soil samples collected at the distance of 15 m from the piggery. A probable cause may be the close proximity to the manure pad – 12 m from the farm buildings that may contribute to increased microbial counts in soil. The number of *E. coli* in the soil samples GII was log 2.72. Similar results were reported by Oliver et al. (2010) who sampled the soil at the distance of 15 m off the piggery and recovered *E. coli* in concentration of 5.20×10^2 cfu/g (colony forming units/g). Besides, actinomycetes count was estimated and their numbers ranged from log 3.77 in the soil obtained 45 m from the farm buildings (GIII) up to log 4.60 in the soil samples collected 15 m off the piggery (GII). These microorganisms make up a pivotal component of bacterial population in soil, while in some types of soil, they can occur even in a larger number than other microbes (Jayasnighe and Parkinson, 2008).

The highest load of the studied bacteria was noted at the turn of December and January (T3) when the moisture conditions were likely to favour the growth of microbes.

There was also assessed coli titre value as a sanitary-hygienic indicator of soil. The lowest value was determined in the soil samples taken 15 m off the pig house at the turn of December and January. That gives evidence of the most severe bacterial contamination of soil at that time as just then a higher moisture level was observed which could be conducive to elevated microbial pollution. Soil moisture and temperature have influence on survival of coliform (Ngole et al., 2006). While Topp et al. (2003) studying the effect of soil moisture on *E. coli* bacterial numbers found that growing moisture was responsible for increased bacterial count, especially in early spring. Likewise, Lenehan et al. (2005) demonstrated elevated numbers of these bacteria in soil and faeces collected in March and April.

Qualitative assessment of bacteria present in soil identified only *E. coli* in the samples obtained in the autumn-winter and spring period from soil collected at the distance of 15 m from the building (GII); *Salmonella* rods were not recovered. Whereas Rajic et al. (2005) evaluating soil and faeces from the swine farm reported the presence of Salmonella in 20.1% of environmental samples and 14.3% faecal samples. Gessel et al. (2004) evaluating the effect of swine manure application on soil pointed to the presence of *Salmonella anatum* and *E. coli* bacteria. The authors suggest that bacteria from coli group persist in the soil, with survival over winter up to 143 days.

Evaluation of bacterial contamination of manure collected inside the pig housing facility showed the highest total bacterial load in the samples collected at 1/2 length of the pig house (KIII). The numbers of psychrophilic bacteria oscillated between log 8.51 in manure samples taken at the entrance to the pig unit (KI) and log 9.38 in those collected at half length of the building. Petkov et al. (2006), however, demonstrated that total count of psychrophilic bacteria in the bedding material sam-

pled in the pig unit was lower and reached 8.2×10^6 cfu/g. Heinonen-Tanski et al. (2006) reported that fresh faeces can contain a high count of bacteria, i.e. $10^9 – 10^{10}$ cfu/g.

The highest number of studied bacteria in manure was established in winter, similarly to the case of soil (T3). But Sargeant et al. (2004) determined the greatest load of *E. coli* 0157 in bovine manure sampled in summer.

The studies on the influence of chosen microclimate parameters and sample moisture manifested that the manure moisture favoured increased numbers of all the microbes under investigation, while the rise of air relative moisture affected only mesophilic and proteolytic bacteria. According to Tombarkiewicz et al. (2000), excessively high moisture level makes the animal housing a sort of incubator, a place promoting microbial development.

E. coli were isolated in swine manure throughout the entire research period, while *Enterobacter* spp. were recorded in some samples (KI, KII, KIII) from spring, summer and early autumn. Kluczek and Kluczek (1999) found a number of Gram-positive and Gram- negative bacterial species in faeces collected from gilts. The dominant species of bacteria were *Kluyvera* spp., *Serratia marcescens*, *Pseudomonas cepacia*, *Staphylococcus faecalis*, *Gemella haemolysans*, *Leuconostoc* spp., *Staphylococcus aureus* and *Micrococus varians*. However, Kluczek and Kluczek (2000) also reported that identification of bacterial species inhabiting the floor of grower house revealed the presence of the following genera: *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Streptococcus* and *Staphylococcus*.

Summing up, the winter period had significant effect on bacterial contamination of environment in a swine farm as the highest bacterial load in soil and manure was determined at that time. This is probably associated with climatic and microclimatic conditions, especially the increase in air relative moisture and sample moisture. Therefore, it is advisable to maintain appropriate zoohygienic conditions in pig houses.

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