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Comparative study of heavy metal and pathogenic bacterial contamination in sludge and manure in biogas and non-biogas swine farms

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Abstract

The objective of this study is to determine and compare the heavy metal (Zn, Cu, Cd, Pb) and bacterial (*E. coli*, coliform and *Salmonella* spp.) contamination between swine farms utilizing biogas and non-biogas systems in the central part of Thailand. Results showed that average levels of *E. coli*, coliform, BOD, COD, Zn, Cu and Pb in sludge from the post-biogas pond were higher than the standard limits. Moreover, the levels of *E. coli*, coliform, Cd and Pb were also higher than the standard limits for dry manure. The levels of *E. coli*, coliform and BOD on biogas farms were lower than on non-biogas farms. Following isolation of S*almonella* spp., it was found that *Salmonella* serovars Rissen was the most abundant at 18.46% (12/65), followed by Anatum 12.31% (8/65), and Kedougou 9.23% (6/65). The pathogenic strains of *Salmonella* serovars Paratyphi B var. java and Typhimurium were present in equal amounts at 4.62% (3/65) in samples from all swine farms. This study revealed that significant reduction in *E. coli* and coliform levels in sludge from covered lagoon biogas systems on swine farms. The presence of *Salmonella* as well as Cd and Pb, in significant amount in dry manure, suggests that there is a high probability of environmental contamination if it is used for agricultural purposes. Thus, careful waste and manure disposal from swine farms and the regular monitoring of wastewater is strongly recommended to ensure the safety of humans, other animals and the environment.

Key words: pathogenic bacteria; heavy metal; manure; biogas; sludge; swine

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Introduction

In recent years, livestock production systems, especially those common in intensive swine farming has been utilizing zinc oxide (ZnO) and copper sulfate ($CuSO_4$) as growth promoters (Salyer et al., 2004). When added to feeds, these two compounds improve feed utilization and the growth of farm animals (Hojberg et al., 2005; Li et al., 2006). At the same time, there has been a rise in environmental problems and public conflicts associated with intensive livestock production. Similarly, over the past few decades, there has been an increase in environmental pollution from the disposal of excess animal manure. As a result, many new regulations and directions are being implemented at the national and European Union (EU) levels to mitigate these pollution problems (Gassmann and Bouzaher, 1995).

Zinc oxide administered at a pharmacological level improves performance and reduces the incidence of diarrhea/colitis in the post-weaned piglets (Mavromichalis et al., 2000). However, the high rate of its excretion into the environment has let to interest in the potential of organic minerals. Only 2% to 10% is recovered in the urine, while the remainder is lost in the feces (Hambidge et al., 1986). Supplements to the diet containing copper significantly increased the concentration of sulfides in the supernatant, creating a condition that is toxic to phototrophic bacteria. In contrast, a decrease in sulfide concentration resulting from the addition of dietary zinc favored phototrophic bacteria. Since pig slurry is therefore high in copper and zinc, these can accumulate in the topsoil and in crops. Care must be taken when grazing sheep due to their

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particular susceptibility to copper toxicity (Conway and Pretty, 1991).

Intensive livestock production generates large quantities of manure, most of which is applied to limited areas of land in close proximity to the manure source. Continuous heavy manuring may pose environmental problems due to heavy metal accumulation in the soil (Wadman et al., 1987). Excess application of manure to the soil is one of the greatest concerns regarding livestock production (Abdel-Shafy, 1996), since it creates an accumulation of nutrients, such as phosphorus, zinc and copper, which can potentially enrich water sources during run-off or through leaching into ground-water supplies, and may contribute to euthrophication of groundwater and freshwater sources (CAST, 1996; Jongbloed & Lenis, 1998). Excessive accumulation of heavy metals in soil is toxic to humans and other animals. Exposure to heavy metals is normally chronic, due to food chain transfer. Although acute poisoning from heavy metals through ingestion or dermal contact is rare, it is possible. Soil and crop management can help preventing the uptake of pollutants by plants. Once heavy metals are precipitated in the soil (the soil-plant-animal or human cycle), their toxic effects will become apparent (Brady and Weil, 1999). Plants transfer larger quantities of metals to their leaves than to their fruits or seeds. The greatest risk of food chain contamination is from leafy vegetables like lettuce or spinach, and from forage eaten by livestock.

Most of the large-scale swine farmers in Thailand that utilize biogas technology on their farms for recycling and reusing organic waste gain significant benefits. Biogas technology provides cheap fuel and high-quality fertilizers. In contrast, manure disposal, such as by spreading it on land, by burning, or simply by collecting it into piles, is not environmentally acceptable (Abdel-Shafy, 1996). Different anaerobic bacteria act upon complex organic materials to produce biogas rich in methane. Anaerobic bacteria are able to withstand quite high concentrations of total heavy metals, which contain a percentage of metal ions, and which can be precipitated out of solution as sulfides or carbonates. The material drawn from the digester, sludge or effluent is rich in nutrients (ammonia, phosphorus, potassium, and more than a dozen trace elements), and is an excellent soil conditioner. The toxic compounds (pesticide, etc.) that are in the digester feed stock material may become concentrated in the effluent. In high concentrations, heavy metal ions react to form toxic compounds in cells (Nies, 1999; Codina et al., 2000).

Meargeay et al. (1985) tested the minimal inhibitory concentrations (MICs) of several different metal ions for *Escherichia coli* on agar medium, and found that the most toxic metal (with the lowest MIC) was mercury at 0.01 mmol/L, followed by Cr, Cd, Cu and Zn at 0.2, 0.5, 1 and 1 mmol/L, respectively. Thus, in an environment with multiple stresses, for example antibiotics and heavy metals, it would be more ecologically favorable in terms of survival for a bacterium to acquire resistance to both stresses. If the resistance is plasmid mediated, those bacteria with clustered resistance genes are more likely to simultaneously pass on those genes to other bacteria, and those bacteria would then survive. The high percent of bacteria that were tolerant to metals were also antibiotic resistant (Calomiris et al., 1984). Moreover, *E. coli* and *Salmonella* spp. express diverse resistance mechanisms and toxic to excess Zn (Lee et al., 2005). Disinfectants, sterility agents and heavy metals used in industry and in household products, along with antibiotics, can create selective pressures in the environment that lead to mutations in microorganisms that may allow them to better survive and multiply (Baquero et al., 1998).

The purpose of this study was to determine the fate of heavy metals (Zn, Cu, Cd, Pb) and bacterial contamination in wastewater and dry manure on swine farms. In addition, the heavy metal and bacterial contaminations on swine farms utilizing biogas and non-biogas systems, was investigated.

1 Materials and methods

1.1 Swine farms selection

Six medium-sized swine farms (sow numbers ranged from 1000 to 1500) in the central part of Thailand were randomly selected. They were divided into 2 groups: 3 implemented a covered lagoon biogas system while the remainder were non-biogas farms. All 6 farms were using zinc oxide (ZnO) and copper sulfate ($CuSO_4$) as a growth promoter in their piglet feeding formula. The samples were collected from four different locations: sludge in nursery sewage drains, sludge before it entered the biogas system, sludge that had passed through the biogas system, and dry manure (Fig. 1).

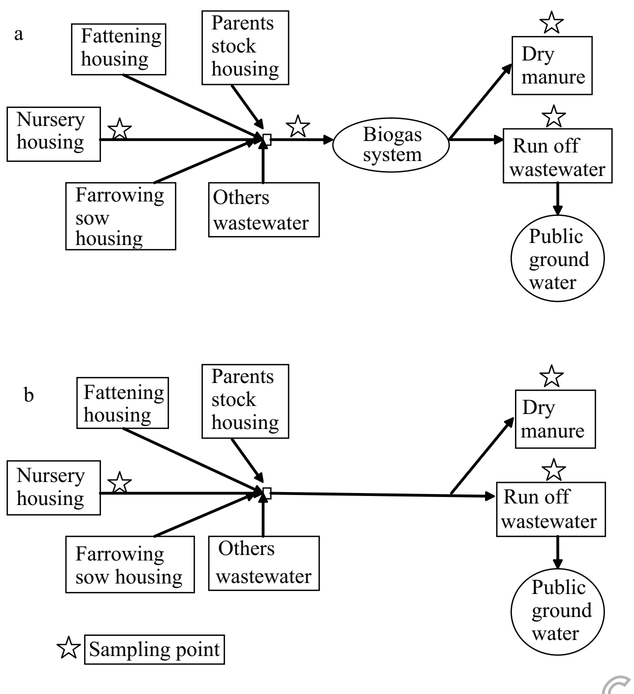

Fig. 1 Sampling points of wastewater and dry manure taken in swine farms. (a) biogas farm; (b) non-biogas farm.

1.2 Samples collection

The sludge and dry manure were pooled and sampled every 3 months over a period of 12 months (March 2007 – February 2008). Sludge samples of 500 mL were taken from different locations and kept at 4°C before analysis. *E. coli* and *Salmonella* spp. from each sample were cultured and counted following the Conventional Method for Determining Coliform and *E. coli* (BAM, 2001). The physical properties of the sludge from each location were determined. The isolation of *Salmonella* spp. was performed using official methods of analysis (BAM, 2001), while the serological tests were performed by Serotest® (S&A Reagents LAB Ltd., Part., Thailand). The homogeneous representative samples of sludge and dry manure from the selected pig farms were dried in an oven at 80°C until the stable weights were reached. Then, 1 g (dry weight, dw) of each sample was digested using acid digestion following the US EPA method (SW-846; method 3050B available at http://www.epa.gov/sw-846/3_series.htm), and then it was analyzed using flame atomic absorption spectrometry (FLAAS, GBC Avanta, Australia).

1.3 Calculations

Each element concentration was fitted to a standard curve. The same concentration (C_s, mg/kg) can be calculated using the following equation:

$$C_s = \frac{C \times V \times D}{W}$$

where, C (mg/L) is the concentration in the extract; V (L) is the volume of extract; D is the dilution factor (undiluted = 1); and W (g) is the weight of sample aliquot extracted.

1.4 Data analysis

Mann-Whitney U test or *t*-tests were performed to compare the means or medians of selected parameters for biogas and non-biogas systems using the NCSS 2007 software program.

2 Results

It was found that the 65 samples contaminated with *Salmonella* were from wastewater and dry manure from all six swine farms (Table 1). Isolated *Salmonella* spp. were classified into six groups; groups B, C, D, E, G, O:30 and O:38. *Salmonella* group C was the most abundant, followed by groups E and B at a level of 30.77%, 27.69% and 23.08%, respectively. Among the *Salmonella* isolates, there were 22 different serovars of which Rissen was the most frequently found at 18.46% (18/65 samples), followed by Anatum at 12.31% (8/65 samples), Kedougou at 9.23% (6/65 samples) and Stanley at 7.69% (5/65 samples). The serovars Typhimurium and Paratyphi B var. java, Lexinton and Albany were all present with equal frequency, 7.69% (5/65 samples). However, the most abundant of *Salmonella* serovars Rissen occurred in group C (12/20 in group C).

Table 2 shows *Salmonella* spp. classified by sample location. *Salmonella* contamination was found most frequently in post-biogas samples (20/65), followed by pre-biogas (19/65) and dry manure samples (18/65).

Table 3 shows the determined physiological and chemical properties of the sampled wastewater. It was found that the heavy metal levels, as well as the pH, conductivity, temperature and COD, were not significantly different between biogas and non-biogas swine farms. However, BOD and coliform levels on biogas farms were significantly lower ($p < 0.01$ and $p < 0.05$, respectively) than in the wastewater from non-biogas farms.

Interestingly, the levels of *E. coli*, coliform, BOD, COD, Zn, Cu and Pb in sludge taken from the last pond before

Table 1 *Salmonella* spp. isolated from wastewater and dry manure in swine farm

Salmonella (serovar)	Group							Total samples	Percentage (%)
	B	C	D	E	G	O:30	O:38		
Albany		3						3	4.62
Anatum				8				8	12.31
Bangkok							1	1	1.54
Corvallis		1						1	1.54
Give				2				2	3.08
I4,5,12;i	4							4	6.15
Kedougou					6			6	9.23
Lexington				3				3	4.62
Mbandaka		1						1	1.54
Montevideo		1						1	1.54
Newport		1						1	1.54
Orion				2				2	3.08
Panama			2					2	3.08
Paratyphi B var. Java	3							3	4.62
Rissen		12						12	18.46
Senftenberg					1			1	1.54
Soerenga		1				1		1	1.54
Stanley	5							5	7.69
Thompson		1						1	1.54
Typhimurium	3							3	4.62
Weltevreden				2				2	3.08
Worthington					2			2	3.08
Total samples	15	20	2	18	8	1	1	65	100

Table 2 *Salmonella* spp. isolated from samples taken in swine farm

Salmonella (serovar)	Dry manure	Nursery wastewater	Pre biogas	Post biogas	Raw water	Treated water	Total Samples	Percentage (%)
Albany	1			2			3	4.62
Anatum	2	1	3	2			8	12.13
Bangkok	1						1	1.54
Corvallis	1						1	1.54
Give			1	1			2	3.08
I4,5,12;i		2	2				4	6.15
Kedougou	3			3			6	9.23
Lexington			2	1			3	4.62
Mbandaka						1	1	1.54
Montevideo			1				1	1.54
Newport	1						1	1.54
Orion			2				2	3.08
Panama	2						2	3.08
Paratyphi B var. Java	3						3	4.62
Rissen		1	3	8			12	18.46
Senftenberg			1				1	1.54
Soerenga				1			1	1.54
Stanley	1		3			1	5	7.69
Thompson	1						1	1.54
Typhimurium	1			2			3	4.62
Weltevreden					1	1	2	3.08
Worthington	1		1				2	3.08
Total samples	18	4	19	20	1	3	65	100

Table 3 Comparison of the means of various parameters in wastewater from the last pond on biogas and non-biogas farms

Parameter	Biogas farm		Non-biogas farm	
	n	Mean ± SD	*n*	Mean ± SD
Temperature	15	32.4±3.16	15	31.16±2.65
pH	15	7.14±0.32	15	7.30±0.19
Conductivity (mV)	15	3.66±19.28	15	12.57±13.69
E. coli (cfu/mL)	15	28,913±28,791	15	49,562±59,691
Coliform(cfu/mL)	15	38,400±33,109*	15	161,468±27,826*
BOD (mg/L)	13	1371±2554**	13	4967±5049**
COD (mg/L)	13	51,454±76,000	13	80,004±88,720
Zn (mg/kg)	13	4600±4682	15	2468±4040
Cu (mg/kg)	13	1143±1068	15	1155±1353
Cd (mg/kg)	11	21.25±31.63	15	4.31±7.26
Pb (mg/kg)	10	44.24±33.14	13	31.09±26.38

*, ** Significant difference at $p < 0.05$ and $p < 0.01$, respectively (Mann-Whitney U test).
cfu: colony forming unit; *n*: wastewater sample number.

being released into the public ground water, were found to be significantly higher than the standard levels on both types of swine farm, but the level of Cd was lower than the standard level as shown in Table 4. Moreover, a comparison of the levels of *E. coli*, coliform and heavy metals in dry manure taken from both farm types to the standard levels, showed differences in the levels of *E. coli*, coliform, Cd and Pb. In contrast, there was no significant difference in the levels of Zn and Cu between biogas and non-biogas farms (Table 5).

A comparison of the pH levels in wastewater samples taken from each sample location on the farms showed that the pH of wastewater from nursery housing was lower ($p < 0.05$) than at other sample locations (Fig. 2a). Furthermore, the mean *E. coli* level in nursery sludge was really significantly different from that in pre-biogas sludge, post-biogas sludge, and also in dried manure (Fig. 2b). However, the other parameters did not show any significant differences between the sample locations.

Table 4 Comparison of sludge from the last pond and standard limits for sample (sludge from last pond) collected from biogas and non-biogas swine farms

Biogas system (Yes/No)	Dependent variables	*p*-Value	Standard limitation
No	*E. coli*	< 0.05	Not found[a]
Yes	*E. coli*	< 0.05	
No	Coliform	< 0.05	< 2.2 MPN/100 mL[a]
Yes	Coliform	< 0.05	
No	BOD	< 0.05	60 mg/L[b]
Yes	BOD	< 0.05	
No	COD	< 0.05	300 mg/L[b]
Yes	COD	< 0.05	
No	Zn	< 0.05	150, 300 mg/L[c]
Yes	Zn	< 0.05	
No	Cu	< 0.05	50, 140 mg/L[c]
Yes	Cu	< 0.05	
No	Cd	0.494	1.0, 3.0 mg/L[c]
Yes	Cd	0.085	
No	Pb	< 0.05	150, 300 mg/L[c]
Yes	Pb	< 0.05	

[a] Pollution Control Department, 2010; [b] Pollution Control Department, 2010; [c] Webber and Kloke, 1984.

3 Discussion

Environmental contamination by *E. coli* and *Salmonella* spp. is of concern because it causes health problems for humans and other animals. *Salmonella* contamination in the six sampled farms (three biogas and three non-biogas system farms) was restricted to the wastewater and dry manure samples. Significantly, *Salmonella* contamination was found in wastewater and dry manure from these farms, along with far higher levels of *E. coli* the housing for nursery piglets, although the frequency of detection was lower than that in other sampled locations. The contamination by *Salmonella* on farms may come from public untreated water pumped onto farms, contamination in nursery feeds, or cycling between the

Table 5 Comparison of various parameters between dry manure and the standard limitation from sample (dry manure) taken in biogas and non-biogas swine farm

Biogas system (Yes/No)	Dependent variables	*p*-Value	Standard limitation
No	*E. coli*	< 0.05	Not found[a]
Yes	*E. coli*	< 0.05	
No	Coliform	< 0.05	< 2.2 MPN/100 mL[a]
Yes	Coliform	< 0.05	
No	Zn	0.061	< 300 mg/L[b]
Yes	Zn	0.042	
No	Cu	0.082	< 100 mg/L[b]
Yes	Cu	0.085	
No	Cd	< 0.05	< 2.0 mg/L[b]
Yes	Cd	< 0.05	
No	Pb	< 0.05	< 100 mg/L[b]
Yes	Pb	< 0.05	

[a] Pollution Control Department, 2010; [b] Webber and Kloke, 1984.

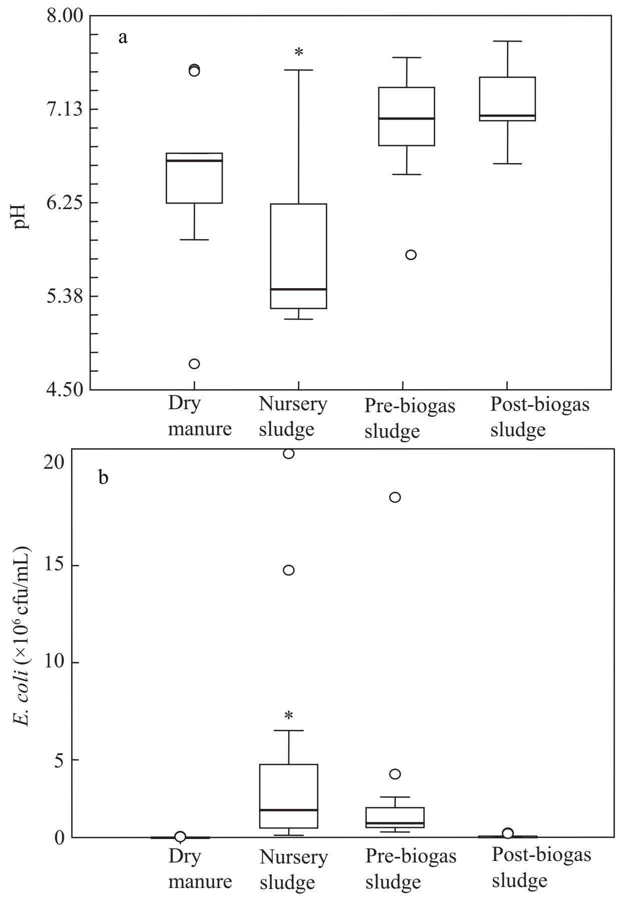

Fig. 2 pH (a) and *E. coli* (b) levels of each sampling location in biogass farms. Samples were repeatedly measured every 3 months for 1 year. * Significant difference at $p < 0.05$, $n = 5$.

swine and their environment as reported by Callaway et al. (2005). *Salmonella* contamination is usually found in the raw animal feedstuffs for example soybean oil, palm, rice brand, and cotton seeds, and from moving new animal onto farms (Davies and Hinton, 2000; Bahnson et al., 2006). Moreover, Percival et al. (2004) reported that most *Salmonella* found in the environment were mainly non-motile strains. However, contamination by *S. typhi* (typhoid fever) and *S. paratyphi* was found in 4.62% of dry manure and post-biogas wastewater samples although these were not normally found in the natural environment and only forming colonies in human bodies as reported by Percival et al. (2004) and Berends et al. (1996). While these kinds of *Salmonella* are basically found when the environment is contaminated by human stools via faecal-oral transmission, the non-typhoidal *Salmonella* are mainly found in the natural environment where they usually causes problems in animals rather than in humans. Davies et al. (1999) reported that *Salmonella* can be isolated from wastewater which is rich in nutrients, but the number of bacteria can be reduced by wastewater treatment. It can survive in open-air soils for at least 6 months and for more than 5 months in wallows (Callaway et al., 2005).

When comparing the chemical properties of wastewater from the last tank so-called post-biogas on biogas farms and at the last pond on non-biogas farms, before it is released to the public groundwater, it was found that the coliform and BOD levels in biogas farm samples were significantly lower than in non-biogas swine farm samples. *E. coli* and COD levels in biogas farm samples were lower than in non-biogas farms, but the differences were not significant. This finding strongly suggests that biogas farms have lower environmental impacts. However, the levels of Zn, Cu and Pb in wastewater from biogas farms were higher than those for non-biogas farms. The levels of *E. coli* in wastewater from biogas farms were lower than those for non-biogas farms but, unfortunately, these differences were not significant at $p < 0.05$ (Table 3), but they should be considered for future evaluations.

Interestingly, when the parameters for sludge samples taken from the last pond on both types of farms were compared to the standard limits, the *E. coli*, coliform, BOD, COD, Zn, Cu and Pb levels, but not the Cd levels, were found to be significantly different at $p< 0.05$. However, when using dry manure samples alone, it was found that *E. coli*, coliform, Cd and Pb levels were significantly higher than the standard limits, but Zn and Cu levels on all farms failed to follow this trend. The high levels of Cd and Pb in dry manure from swine farms might come from animal feed. However, this proposition is unproven since heavy metal concentrations in the raw materials used in animal feeds, were not determined in this study. It can be inferred, nevertheless, that the dry manure sold for agricultural used as fertilizer is at risk of being contaminated by *E. coli, Salmonella* spp. and coliform and thus could contaminated agricultural products. Thus, increasing the temperatures used in the preparation of fertilizers is necessary to reduce these bacteria in the contaminated soil from swine farms. The reported disappearance of *E. coli* and *Salmonella* contamination after 21 days of ploughing had taken place and 7 days of harrowing the soil by Boe et al. (2005) supports this conclusion.

The dry manure from swine farms in this area was found to contain high levels of Zn, Cu, Pb and Cd, which is similar to the findings of Moral et al. (2008). They found that Zn and Cu were important heavy metals which can contaminate the soil when dry manure is used as organic fertilizer in agriculture. Significantly, a study of Zn and Cu contaminants in various types of animal manure from farms showed that they were highest on swine farms, followed by chicken farms and then ruminant farms (Nicholson et al., 1999). Therefore, vegetables from soil that is heavy contaminated by Zn and Cu, can potentially affect consumers. Katanda et al. (2007) found that toxic levels of Zn and Cu can accumulate in Mustard rape and lettuce without having any effect on their growth rates. In addition, in Zimbabwe, higher levels of Zn, Cu, Cd and Pb contaminants than the standard limits for maize, bean, pepper and sugarcane, for which the EU standard limits for agricultural products are 50, 20, 0.2, and 0.3 mg/kg, respectively, have been reported (Muchuweti et al., 2006). This suggests that special concern for the health effects of contaminated agricultural products throughout the food chain is warranted. In addition, the pH level has a high correlation with C and N levels in the soil. It was found that agricultural soil fertilized with dry manure from swine had a low pH than that containing ruminant manure, and that the application of swine manure in agriculture for a long time caused an increase in bacteria levels, Actinomycete fungi, and nitrogen in the soil (Wood and Hattey, 1995).

4 Conclusions

This study revealed that biogas systems, especially covered lagoon implementations on swine farms, showed significant reductions in *E. coli* and coliform levels in sludge. *Salmonella* could be detected in dry manure which was to be sold for the agricultural uses. The occurrence of *Salmonella* was similar to that of *Listeria* spp. and *Salmonella* spp. in swine lagoon reported in the study by McLaughlin et al. (2009). Even though the levels of Zn and Cu in dry manure were below EU standard levels, the levels of *E. coli*, coliform, BOD, COD, Zn, Cu and Pb in wastewater, and the levels of *E. coli*, coliform, Cd and Pb in dry manure, were above the standard limits. Thus, swine disease control and food safety in swine production are issues of concerns. Disposal of excess animal manure may result in environmental problems.

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