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Occurrence and removal of progestagens in two representative swine farms: Effectiveness of lagoon and digester treatment

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ABSTRACT

A total of 21 progestagens were screened in animal wastes and environmental samples from two representative swine farms and surrounding environments of South China using ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) to assess the effectiveness of simple lagoon (and digester) treatment. The results showed that 11, 8 and 8 of 21 target progestagens were detected with the minimum concentration of 2.31 ng/L and maximum of 6150 ng/L in the water samples, with the minimum of 1.36 ng/L and maximum of 98.3 ng/L in the suspended particles, and with the minimum of 1.57 ng/g dry weight (dw) and maximum of 3310 ng/g dw in the solid samples, respectively. Trace levels (a few ng/L or ng/g levels) of dydrogesterone, 5α-dihydroprogesterone, norgestrel and progesterone were found in samples from nearby surface waters and vegetable fields impacted by animal wastes. The residual progestagens at the reported levels may still pose potential risks to aquatic organisms such as fish in the receiving aquatic environments. This finding suggests that swine wastewater and feces could lead to contamination of some detectable progestagens in the surrounding environments. Significant reduction in total progestagen concentrations were observed from the fresh swine wastewaters to the fish ponds, indicating effective removal of these compounds by the lagoon (and digester) treatment. In addition, the biogas digesters provided high removal of the progestagens in the waste streams. This low-cost and eco-friendly treatment system should be promoted in developing countries with concentrated animal operations.

1. Introduction

Steroid hormones in the environment have received great attention from the general public and scientific community since the first report on fish feminization following exposure to estrogens at concentrations as low as 1 ng/L (Purdom et al., 1994). Subsequent studies have documented the masculinization of fish in consequence of their exposure to androgens (Larsson et al., 2000; Parks et al., 2001; Orlando et al., 2004), and

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reproductive and behavior problems of fish associated with exposure of various progestagens at ng/L levels (Sorensen et al., 2005; Zeilinger et al., 2009; Paulos et al., 2010; Runnalls et al., 2013; Svensson et al., 2013). Previous environmental monitoring studies mainly focused on estrogens and androgens (e.g., Ying et al., 2002; Furuichi et al., 2006; Kjaer et al., 2007; Ying et al., 2008, 2009; Arnon et al., 2008). In comparison, relatively few studies have focused on other steroids such as progestogens, and most previous studies deal with only a few progestagen compounds (Kolpin et al., 2002; Chang et al., 2009; Kuster et al., 2009; Chang et al., 2011; Fan et al., 2011; Liu et al., 2012a,b,c).

Natural progesterone and various synthetic progestins are widely used by humans and animals for various purposes, such as human contraception and therapy, animal breeding control and growth promotion (Shelton, 1990; Mortensen et al., 2007; Yang et al., 2009; Liu et al., 2011). As a consequence, municipal wastewater treatment plants (WWTPs) and concentrated animal feeding operations (CAFOs) are deemed to be two important pollution sources for progestagens in the receiving environment, due to the incomplete removal in WWTPs and CAFOs as well as direct discharge of untreated wastewaters (Zheng et al., 2008; Fan et al., 2011; Liu et al., 2012a,b,c). Therefore, more efforts should be made to understand the occurrence and fate of progestagens in the environment.

Swine farms are one of the most important CAFOs. Various synthetic progestagens, such as medroxyprogesterone acetate (MPA), melengestrol acetate (MGA) and chlormadinone acetate (CMA) are known to be used as growth promoters for the fattening of swine due to their gestagenic effect (Mortensen et al., 2007; Lõhmus and Kender, 2007), and to improve the efficacy of feed conversion in animals through increasing bone density, muscular mass and red blood cells (Yang et al., 2009). However, administration of progestagens and other steroid hormones for farmed animal fattening purposes is now prohibited by the Netherlands (Hooijerink et al., 2003), European Union (Hooijerink et al., 2003; Lõhmus et al., 2007; Mortensen et al., 2007) and China (Yang et al., 2009) due to adverse human health effects of hormone residues in animal meats (Hooijerink et al., 2003). Unfortunately, regulations may be ignored for the pursuit of economic benefits in swine farms of some developing countries like China (Yang et al., 2009). To minimize the endogenous and exogenous progestagens in swine wastes, appropriate treatment and disposal of animal wastes are critically important. However, information pertaining to the occurrence and fate of progestagens and their metabolites in swine waste treatment systems is still poorly documented (Liu et al., 2012b,c). Those previous studies only reported a few progestagen compounds (e.g. progesterone (P), norgestrel (N), ethynyl testosterone (ET), medroxyprogesterone (MP), and 19-norethindrone (19-NTD)), thus screening of more progestagens is essential in order to have a full picture of progestagen usage in swine farms. It is also unclear whether those progestagens associated with swine wastes could reach the surrounding environments, and further pose potential risks to the ecosystem.

The objectives of this study were (i) to determine the concentrations of 21 progestagens in swine wastes (flush water and feces) and environmental samples (surface water and sediment, soil, as well as well water) in two representative swine farms using ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), and (ii) to investigate the effectiveness of the wastewater treatment systems for removal of progestagens. The results can assist in assessing contamination of progestagens from swine farms, and treatability of these emerging contaminants by existing waste treatment systems, and then facilitate optimizing waste management practices in swine farms.

2. Material and methods

2.1. Chemicals and reagents

The 21 target progestogens were selected based on the reported detection in the literature and market information from Chinese pharmaceutical companies. The authentic standards, including anordrin (AD), chlormadinone (CMD), chlormadinone acetate (CMDA), cyproterone acetate (CPRA), dydrogesterone (DGT), 5α-dihydroprogesterone(5α-DHP), drospirenone (DPN), ethynyl testosterone (ET), hydroxy progesterone (HP), 17α-hydroxyprogesterone acetate (17α-HPA), hydroxyprogesterone caproate (HPC), mifepristone (MFST), melengestrol acetate (MGA), megestrol (MGT), medroxyprogesterone (MP), medroxyprogesterone acetate (MPA), norgestrel (N), norethynodrel (NTD), 19-norethindrone (19-NTD), norethisterone acetate (NTRA), progesterone (P), and their corresponding internal standards melengestrol acetate-d3 (MGA-d3), mifepristone-d3 (MFST-d3), progesterone-d9 (P-d9), norethindrone-d6 (NTD-d6) were purchased from Meryer Technologies Co. (China), USP, Dr. Ehrenstorfer GmbH (Germany), Steraloids Inc. (USA), Sigma–Aldrich (USA), and TCR (North York, Canada). All reagents of HPLC grade, including methanol (MeOH), ethyl acetate (EtOAc), and hexane (Hex), were obtained from Merck (Darmstadt, Germany) and CNW Technologies (Dusseldorf, Germany). Formic acid was obtained from Tedia company (Fairfield, OH, USA), and ammonium acetate from Sigma–Aldrich (Saint Louis, MO, USA).

2.2. Field sites and sampling

Two representative swine farms were selected in this study. These two farms represent well managed swine farms in South China. Farm A with approximately 10740 pigs including 3700 piglets, 6000 young barrows, 40 boars and 1000 sows is located in Kaiping County, whereas Farm B with 8565 pigs including 2666 piglets, 4000 young barrows, 43 boars and 1857 sows is located in Heshan County of the Pearl River Delta region, and both farms are in Jiangmen city, Guangdong Province, south China. The animal waste treatment and disposal systems are similar in the two farms. In both farms, swine feces are removed directly for sale, then the swine houses are flushed daily using well water and the flush waters are collected and treated with lagoons systems (Fig. 1). But Farm B has extra biogas digesters following the first lagoon. The second lagoon in the two farms is a large fish pond, which is a common practice to use animal waste as fish feed in the region. Basic water quality parameters for wastewaters in the farms at the sampling period are given in Table 1. Farm A

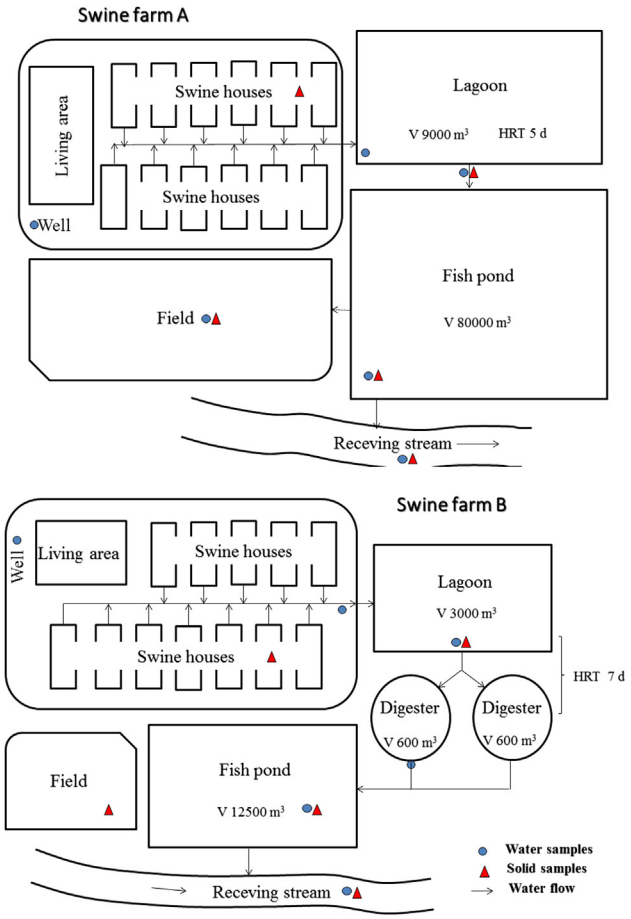

Fig. 1 – The sketch map of selected swine farms with sampling locations. HRT: hydraulic rentention time (days); V: volume of a treatment unit.

generated wastewater of nearly 150 m^3/d, which was first flushed to a waste lagoon with a volume of 9000 m^3 (hydraulic retention time: 5 days), then flowed into a huge fish pond with a volume of 80,000 m^3. Farm B generated wastewater of approximately 120 m^3/d, which was first flushed into a waste lagoon with a volume of 3000 m^3, then flowed to two 600 m^3 digesters (hydraulic retention time for the lagoon and digester system: 7 days), and the digester effluent flowed into a 12,500 m^3 fish pond.

Various samples were collected from the two farms and surrounding environments as shown in Fig. 1. Three samples of each type at each location were collected to determine average concentrations. The feces samples were taken from swine houses by randomly collecting approximately 1000 g each with an aluminum scoop in several different locations and then combining into one composite sample for each swine farm. The flush waters were sampled only in Farm B at the washing time, and they were not accessible at the sampling time in Farm A. Waste waters in the lagoons, biogas digesters and ponds were collected sequentially, while the solid wastes were also collected from the corresponding locations. Meanwhile, environmental samples from the surrounding environments of the farms were also collected, including well water, surface soil from crop fields, water and sediment from the receiving streams, as well as soil samples from nearby forest and water samples from a reservoir (used as reference samples). Solid samples were placed in 1 L glass bottles and liquid samples were stored in 1 L amber bottles. To suppress microbial activity, 1 g of sodium azide was added to each solid sample, and 5% (v/v) of methanol was added to each water sample with its pH adjusted to 3 using 4 M H_2SO_4 in the field. All samples were transported to laboratory in an ice cooler, and then stored in the dark at 4 °C prior to further processing. The liquid samples were processed within 48 h. The solid samples were freeze dried, crushed and homogenized before analysis.

2.3. *Sample preparation and UHPLC-MS/MS analysis*

All samples were processed and extracted according to our previously reported method (Liu et al., 2014). Briefly, aqueous samples were extracted by solid-phase extraction (SPE) using Waters Oasis HLB cartridges (500 mg, 6 mL). Filtered water samples were loaded onto the preconditioned cartridges at a flow rate of 5–10 mL/min, the bottles were rinsed twice by 50 mL of 5% (v/v) MeOH in Milli-Q water after sample loading, then the cartridges were dried under the vacuum for 2–3 h, and the target compounds were eluted by 3 × 4 mL EtOAc. The eluents were dried under a gentle nitrogen stream, then redissolved with 1 mL of MeOH and filtered through a 0.22 μm membrane filter (Anple, Shanghai, China) into a 2 mL amber glass vial (Agilent, USA) prior to UHPLC-MS/MS analysis.

Solid samples were extracted by ultra-sonication extraction (USE), with 10 mL of EtOAc/MeOH (8/2, v/v) as the

Table 1 – Water quality parameters of wastewaters from the swine farms.

	TSS mg/L	BOD_5 mg/L	COD mg/L	TP mg/L	TN mg/L	NH_4–N mg/L	pH
Farm A							
Waste lagoon	1910	755	3050	87.7	330	273	6.3
Lagoon effluent	1248	797	2847	83.1	319	259	6.6
Fish pond	129	7.4	75.4	2.4	4.1	1.7	7.5
Farm B							
Waste lagoon	2260	1030	3730	47.5	505	424	7.7
Digester effluent	96	10.5	34.3	1.5	4.1	1.4	7.2
Fish pond	106	6.1	61.0	1.1	3.8	0.6	6.9

Remarks: TSS, total suspended solids; BOD5, 5d biochemical oxygen demand; COD, chemical oxygen demand; TP, total phosphorus; TN, total nitrogen; NH_4–N, ammonia nitrogen.

extracting solution. The samples were extracted in an ultrasonic bath for 15 min, and centrifuged at 1370 × *g* for 10 min. The clear supernatant was pipetted into a 100 mL flask. The extraction procedure was repeated thrice, and the supernatants were combined and evaporated at 45 °C by a rotary evaporator, then re-dissolved with 1 mL of MeOH and filtered through a 0.22 μm membrane filter (Anple, Shanghai, China) into a 2 mL amber glass vial (Agilent, USA) prior to further cleanup. Suspended particle samples, were extracted just as solid samples and the measured concentrations were calculated on the basis of the volume of corresponding water samples.

All extracts were purified with self-made silica gel columns. Each methanolic extract (200 μL) was loaded to a preconditioned silica cartridge. After the cartridge was rinsed with 6 mL of Hex, the target compounds were eluted thrice with 2 mL of EtOAc/MeOH (9:1, v/v). The eluate was then dried and reconstituted in 200 μL in the buffer MeOH/Milli-Q water-5 mM ammonium acetate-0.05% formic acid (70/30, v/v) before analysis.

The 21 target progestagen compounds were analyzed by an Agilent 1200 series ultra-high performance liquid chromatography (Agilent, USA) coupled to an Agilent 6460 triple quadrupole mass spectrometry (UHPLC-MS/MS). The column oven temperature was maintained at 40 °C and the injection volume was 5.0 μL. The mobile phase consisted of (A) Milli-Q water containing 5 mM ammonium acetate and 0.05% formic acid (v/v) and (B) MeOH. A gradient program of the mobile phase proceeded, and the post run time was set at 5.0 min for column equilibration prior to next injection. The mass spectrometry was operated with ESI in positive ionization mode. The MS operating parameters were optimized by Optimizer (Agilent, USA) to maximize the best signal response and increase detection sensitivity. The quantitative analysis of the target compounds was performed in multiple reaction monitoring (MRM) mode. Detailed instrumental conditions can be referred to our previous method paper (Liu et al., 2014).

2.4. Quantification and quality control

Identification of the target compounds was based on the retention time (within 2%) and the ratio (within 20%) of the two selected precursor-product ion transitions in comparison with the corresponding standards. The quantification was accomplished using the multiple reaction monitoring (MRM) transitions that were most abundant or accompanied with least background interference. Each target compound was quantified by a calibration standard curve containing six points ($R^2 \geq 0.995$). For each type of liquid sample and solid sample, quality assurance and quality control consisted of that at least one water blank or one feces blank, one spiking blank, one matrix spiking blank, and one triplicate sample (Table S1 in the Supporting Information). All samples were analyzed in three replicates. Laboratory blanks, reagent blanks and quality control standard solution (50 μg/L each compound) were also performed with the samples during the instrumental analysis of each batch to assess potential background value and instrument performance. For the various matrices considered, the optimized method showed satisfactory performance with recoveries of 70–110% (except AD, 5α-DHP, DPT, HPC) at the spiked concentration of 50 ng/L or 50 ng/g. Matrix effect for each compound was evaluated by comparing the matrix extracts spiked with the standard solution to the standards in mobile phase at the same concentrations (See Table S1 in the Supporting Information). No target compounds were found in solvent blank analysis. Detailed method performances including the recoveries and limits of quantification (LOQs) of progestagens in the each type of sample can be found in our previous method paper (Liu et al., 2014).

3. Results

3.1. Concentrations of progestagens in aqueous samples

The concentrations of the detected progestagens in dissolved phase and suspended particles of the collected aqueous samples (swine wastewater and surface water) are summarized in Table 2 and Table 3, respectively. The levels of the detected progestagens in suspended particles were much lower than those in dissolved phase, so only those dissolved phase concentrations were discussed in the following section. It should be noted that target progestagen compounds were not detectable in the reference reservoir water and soil. In Farm A, 10 progestagen compounds were detected with the minimum concentration of 1.70 ng/L and maximum of 9330 ng/L, while in Farm B, 11 progestagen compounds were detected with the minimum concentration of 2.31 ng/L and maximum of 5402 ng/L (Table 2). The maximum concentrations of the detected progestagens appeared in the flush water or waste lagoon of both swine farms. Among the detected progestagens, the natural progesterone was detected in all samples. Moreover, HP was detected in the flush water and waste lagoon of Farm B, but not in Farm A. This may be due to different usages of this chemical in the two farms.

The concentrations of the detected progestagens in dissolved phase of wastewater streams decreased from several thousands ng/L in the flush water and waste lagoon to several to tens ng/L or not detected (ND) in the fish pond and receiving stream of both farms (Table 2). Only two compounds (DGT and P) were found in the water samples from the fish pond of Farm B, while eight compounds were still present in the fish pond of Farm A (Table 2). Four compounds (DGT, 5α-DHP, N and P) were detected in the well water and receiving stream of Farm A, whereas one (P) and two compounds (DGT and P) were found in the well water and receiving stream of Farm B, respectively. Contamination of well water is most likely due to animal waste disposal and high rainfall in the region.

3.2. Concentrations of progestagens in solid samples

The concentrations of detected progestagens in solid samples are summarized in Table 4. The detected compounds in the solid samples are the same as those in the water samples from the two farms except for two compounds MP and 17α-HPA that were not found in the solid samples. Seven compounds were detectable in feces from both farms, while seven and five compounds were found in lagoon sludge of Farm A and Farm

Table 2 – Concentrations (ng/L) of detected target compounds in aqueous samples from selected swine farms and surrounding environment.

Compound[a]	Farm A						Farm B					
	Waste lagoon	Lagoon effluent	Fish pond	Field ditch	Well	Receiving stream	Flush water	Waste lagoon	Digester effluent	Fish pond	Well	Receiving stream
AD	237 ± 8.9[b]	165 ± 7.3	ND	ND	ND	ND	1090 ± 7.3	897 ± 8.8	ND	ND	ND	ND
CPRA	444 ± 5.3	232 ± 6.2	10.2 ± 2.8	ND	ND	ND	2330 ± 8.1	565 ± 2.8	ND	ND	ND	ND
DGT	9330 ± 4.1	5630 ± 9.2	26.2 ± 7.4	6.58 ± 4.7	1.82 ± 4.2	3.98 ± 6.9	5400 ± 8.4	4670 ± 8.6	ND	3.73 ± 2.9	ND	3.55 ± 1.8
5α-DHP	1040 ± 8.5	656 ± 8.8	20.5 ± 6.7	4.86 ± 5.1	1.77 ± 3.1	2.59 ± 4.7	1890 ± 6.2	1240 ± 6.3	ND	ND	ND	ND
ET	258 ± 5.3	104 ± 6.5	ND	ND	ND	ND	65.1 ± 4.3	60.5 ± 6.7	ND	ND	ND	ND
HP	ND	ND	ND	ND	ND	ND	100 ± 6.6	48.6 ± 4.7	ND	ND	ND	ND
17α-HPA	250 ± 12.5	94.8 ± 8.4	9.46 ± 5.1	ND	ND	ND	1220 ± 7.2	195 ± 14.2	ND	ND	ND	ND
MP	62.1 ± 3.9	47.8 ± 6.2	11.1 ± 5.5	3.66 ± 5.6	ND	ND	135 ± 3.1	89.9 ± 11.2	ND	ND	ND	ND
MPA	452 ± 7.9	124 ± 4.8	21.5 ± 4.3	ND	ND	ND	506 ± 5.3	297 ± 7.1	ND	ND	ND	ND
N	2380 ± 4.1	1600 ± 1.8	22.8 ± 4.7	9.15 ± 4.3	2.77 ± 2.4	4.77 ± 3.1	4550 ± 7.9	3360 ± 7.5	ND	ND	ND	ND
P	461 ± 9.8	387 ± 7.2	37.6 ± 10.5	5.47 ± 3.8	2.21 ± 1.22	2.89 ± 1.8	2960 ± 8.4	2180 ± 7.9	2.31 ± 1.8	2.64 ± 9.3	2.35 ± 3.1	2.88 ± 4.3
SUM	***14900***	***9040***	***159***	***29.7***	***8.57***	***14.2***	***20200***	***13600***	***2.31***	***6.37***	***2.35***	***6.43***

[a] AD, anordrin; CPRA, cyproterone acetate; DGT, dydrogesterone; 5α-DHP, 5α-dihydroprogesterone; ET, ethynyl testosterone; HP, hydroxy progesterone; 17α-HPA, 17α-hydroxyprogesterone acetate; MP, medroxyprogesterone; MPA, medroxyprogesterone acetate; N, norgestrel; P, progesterone.
[b] Mean ± standard deviation (%) (n = 3); ND: not detected.

Table 3 – Concentrations (ng/L) of detected target compounds in suspended particulate matter samples from selected swine farms and surrounding environment.

Compound[a]	Farm A						Farm B					
	Waste lagoon	Lagoon effluent	Fish pond	Field ditch	Well	Receiving stream	Flush water	Waste lagoon	Digester effluent	Fish pond	Well	Receiving stream
AD	ND	ND	ND	ND	ND	ND	8.1 ± 4.9	4.51 ± 3.7	ND	ND	ND	ND
CPRA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DGT	98.3 ± 5.5[b]	21.5 ± 4.8	ND	ND	ND	ND	27.2 ± 6.3	14.7 ± 1.5	ND	ND	ND	ND
5α-DHP	29.6 ± 7.3	12.5 ± 4.9	ND	ND	ND	ND	53.5 ± 7.2	33.8 ± 8.3	ND	ND	ND	ND
ET	5.56 ± 3.8	0.99 ± 4.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HP	ND	ND	ND	ND	ND	ND	8.6 ± 7.3	4.13 ± 6.4	ND	ND	ND	ND
17α-HPA	4.54 ± 6.6	1.28 ± 4.9	ND	ND	ND	ND	3.31 ± 5.8	ND	ND	ND	ND	ND
MP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MPA	13.9 ± 6.1	ND	ND	ND	ND	ND	2.92 ± 3.6	ND	ND	ND	ND	ND
N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P	19.1 ± 5.3	12.7 ± 4.9	2.86 ± 5.5	ND	ND	ND	52.9 ± 6.8	44.8 ± 7.4	ND	ND	ND	ND
SUM	***171***	***49.0***	***2.86***	-	-	-	***157***	***102***	-	-	-	-

[a] AD, anordrin; CPRA, cyproterone acetate; DGT, dydrogesterone; 5α-DHP, 5α-dihydroprogesterone; ET, ethynyl testosterone; HP, hydroxy progesterone; 17α-HPA, 17α-hydroxyprogesterone acetate; MP, medroxyprogesterone; MPA, medroxyprogesterone acetate; N, norgestrel; P, progesterone.
[b] Mean ± standard deviation (%) (n = 3); ND: not detected.

Table 4 – Concentrations (ng/g dw) of detected target compounds in solid samples from selected swine farms and surrounding environment.

Compound[a]	Farm A					Farm B				
	Feces	Lagoon sludge	Pond sediment	Field soil	Stream sediment	Feces	Lagoon sludge	Pond sediment	Field soil	Stream sediment
AD	ND	6.7 ± 8.0	ND	ND	ND	ND	9.12 ± 5.9	ND	ND	ND
CPRA	2.62 ± 4.4[b]	ND	ND	ND	ND	2.23 ± 4.8	ND	ND	ND	ND
DGT	163 ± 6.8	19.2 ± 1.6	2.93 ± 7.2	3.11 ± 3.8	1.82 ± 3.6	308 ± 7.5	13.7 ± 5.7	1.66 ± 5.4	ND	2.01 ± 2.1
5α-DHP	796 ± 6.8	13.7 ± 8.9	8.55 ± 5.7	3.32 ± 2.1	1.17 ± 5.8	1347 ± 7.7	12.5 ± 9.1	ND	ND	ND
ET	5.56 ± 3.8	0.99 ± 4.7	ND	ND	ND	ND	ND	ND	ND	ND
HP	ND	ND	ND	ND	ND	24.5 ± 4.8	ND	ND	ND	ND
17α-HPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MPA	115 ± 7.4	2.86 ± 5.9	ND	ND	ND	16.9 ± 7.8	ND	ND	ND	ND
N	121 ± 7.2	5.82 ± 4.6	ND	ND	ND	181 ± 9.2	6.16 ± 9.9	ND	ND	ND
P	485 ± 5.8	31.6 ± 5.8	2.56 ± 5.5	2.62 ± 1.7	2.44 ± 1.9	1020 ± 8.4	77.4 ± 2.7	2.41 ± 6.0	1.63 ± 4.3	1.87 ± 5.1
SUM	***1690***	***67.2***	***14.0***	***9.05***	***5.43***	***2900***	***119***	***4.07***	***1.63***	***3.88***

[a] AD, anordrin; CPRA, cyproterone acetate; DGT, dydrogesterone; 5α-DHP, 5α-dihydroprogesterone; ET, ethynyl testosterone; HP, hydroxy progesterone; 17α-HPA, 17α-hydroxyprogesterone acetate; MP, medroxyprogesterone; MPA, medroxyprogesterone acetate; N, norgestrel; P, progesterone.

[b] Mean ± standard deviation (%) (n = 3); ND: not detected.

B, respectively (Table 4). In the two farms, AD was found in lagoon sludge, but not detected in feces.

The total concentrations of detected progestagens in feces were much higher than in other solid samples (Table 4). Two endogenous progestagens P and 5α-DHP were dominant among the detected compounds in feces samples of both swine farms. Following lagoons treatment, only three (DGT, 5α-DHP and P) and two compounds (DGT and P) were present at a few ng/g dw in the pond sediments, field soils and stream sediments of Farm A and Farm B, respectively.

4. Discussion

4.1. *Occurrence of progestagens in the swine farm environments*

Due to natural excretion and intended uses, various progestagens (11 of 21 target compounds) were detected in flush water, waste lagoons and feces of the swine farms at concentrations up to 9330 ng/L for wastewater samples and 1350 ng/g dw for feces samples (Tables 2–4), suggesting animal wastes are an important source for progestagens in the receiving environment. Since solid animal wastes are often directly collected for sale as fertilizer, only flush water is treated by simple lagoon systems. Discharge or leaching of wastewater could lead to contamination of the well water and receiving streams, as demonstrated by detection of several progestagen compounds (DGT, 5α-DHP, N and P) at a few ng/L or ng/g dw levels. Since these progestagen compounds are moderate in polarity with the log K_{ow} values of 3.45–3.87 based on the Estimation Program Interface (EPI) Suite from United States Environmental Protection Agency, they were detected in both water and sediment phases of the receiving streams (Tables 2–4). Irrigation of swine wastewater on the nearby fields in the farms also led to contamination of soils with three progestagens (DGT, 5α-DHP, and P) at concentrations of 1.63–3.11 ng/g dw (Table 4).

Previous studies showed detection of a few progestagens such as P, HP, MPA, N, NTD and 19-NTD in wastewaters from WWTPs with concentrations up to 41 ng/L (Labadie and Budzinski, 2005; Vulliet et al., 2011; Esperanza et al., 2004, 2007; Chang et al., 2008, 2011; Fan et al., 2011; Liu et al., 2012a), but relatively a few studies reported the concentrations of progestagens in animal wastes (Zheng et al., 2008; Hansen et al., 2011; Liu et al., 2012b,c). Natural progestagen P was detected at concentrations up to 1250 ng/g dw in composted animal manure (Zheng et al., 2008; Hansen et al., 2011), and up to 14400 ng/g dw in fresh manure and up to 3470 ng/L in flush water of a swine farm (Liu et al., 2012b,c). Synthetic progestagens MP and N have been reported in swine farms of Guangxi Province, south China with concentrations up to 85.1 ng/L and 10800 ng/L in flush water, and up to 13.3 ng/g dw and 7.6 ng/g dw in feces, respectively (Liu et al., 2012b,c).

In comparison, the present study reported detection of more progestogens such as AD, CPRA, DGT, 5α-DHP and ET in swine farms. Among the detected eleven compounds, P, 5α-DHP and HP are endogenous progestagens, whereas the rest are synthetic progestagens. These synthetic progestagens have been used in animal production for breeding control and growth promotion purposes (Shelton, 1990; Lõhmus and Kender, 2007; Mortensen and Pedersen, 2007). Detection of these synthetic progestagens in flush water and feces indicates their common use in the swine farms for various purposes. Based on the concentrations of progestogens in feces and flush water of the two swine farms in the present study, it is estimated that the daily excretion masses per pig for the three common detected compounds P, DGT and N were 223 μg, 242 μg and 122 μg, respectively.

4.2. *Removal by wastewater treatment systems*

Significant reduction in the progestagens concentrations and compound number was observed in both swine farms following simple lagoon (and digester) treatment (Table 2). The concentrations of those target compounds in the fish

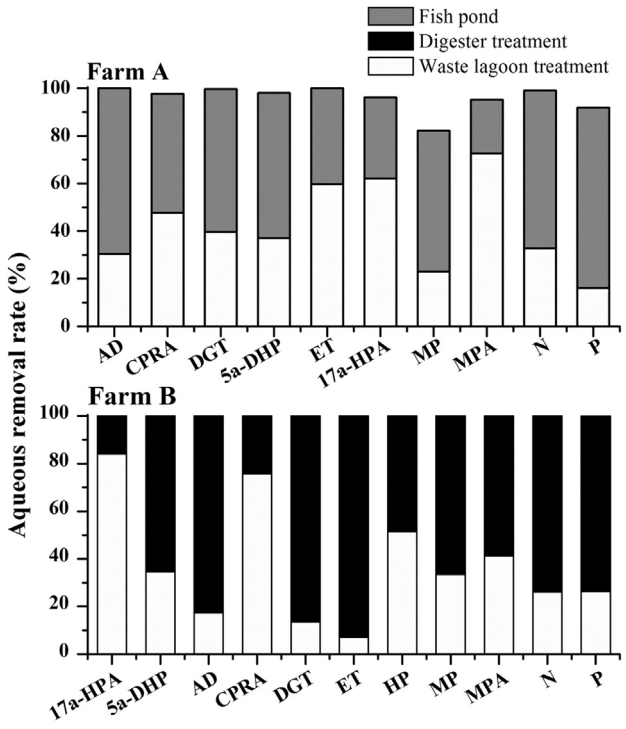

Fig. 2 – Aqueous removal of progestagens by the wastewater treatment systems in the two swine farms. AD, anordrin; CPRA, cyproterone acetate; DGT, dydrogesterone; 5α-DHP, 5α-dihydroprogesterone; ET, ethynyl testosterone; HP, hydroxy progesterone; 17α-HPA, 17α-hydroxyprogesterone acetate; MP, medroxyprogesterone; MPA, medroxyprogesterone acetate; N, norgestrel; P, progesterone.

ponds were almost 2–4 orders of magnitude lower than those in the waste lagoon and flush water (Table 2). The aqueous removal rates for the detected compounds were more than 82% in Farm A, and almost 100% in Farm B (Fig. 2). The large attenuation of progestagens could be attributed to biodegradation, photodegradation, and sorption in lagoon and digester systems, as well as dilution in fish ponds by surface water. In both farms, the lagoon systems contributed aqueous removal of 30–40% of the progestogens in influent (Fig. 2 and Table 2). Approximately 10 times dilution was estimated in the fish ponds according to the farm operators. The biogas digesters in Farm B also showed the effectiveness of anaerobic biodegradation process with almost complete removal of the rest progestagens in influent (Table 2). In addition to anaerobic biodegradation, sorption onto bed materials in the digesters could also contribute to the high removal of progestagens. According to a previous study (Fan et al., 2011), progestagens were decreased by 50% in the anaerobic tank of a WWTP. A previous laboratory study showed that natural progesterone degraded much faster by bacteria in activated sludge than synthetic norgestrel (Liu et al., 2013). Further research is clearly essential to understand contributing factors for progestagen removal in these treatment systems.

4.3. *Environmental implications*

The present study reported 11 progestagens in swine wastewaters and4 progestagens (DGT, 5α-DHP, N and P) in the receiving streams. The presence of these progestagens may affect aquatic organisms in the environment. Limited laboratory experiments showed that some progestagens like N could cause significant decreases in fish fecundity at a few ng/L to 100 ng/L range (Zeilinger et al., 2009; Paulos et al., 2010; Runnalls et al., 2013). Progesterone (P) could elucidate significant changes in expression levels of various genes such as *pgr*, *ar*, *mr*, and *hsd17b3*, as well as global transcriptional profiles in the brain and ovary of female zebrafish occurred at a few ng/L P (Zucchi et al., 2012, 2013). A recent study by Liang et al. (2015) showed that P and N could cause a disruption of sex differentiation at environmentally relevant concentrations based on the skewed sex ratio in the subsequent adult population. Therefore, the dominant compounds P and N in piggery effluent were found at levels above those which have been shown to cause effects in fish at the molecular and genetic level. More field investigations are clearly needed to assess realistic risks to organisms in the environment.

The wastewater treatment systems reported in the present study are commonly used in the region. The systems often consist of anaerobic waste lagoons, followed by biogas digesters, and fish ponds. The present study demonstrated that progestagens could be effectively removed at high rates in the wastewater treatment systems. At the same time, BOD_5, COD and nutrients (TP, TN, and NH_4–N) were also removed at high rates (Table 1). Fish in the ponds make use of the nutrients from swine farms, while biogas generated is used for heating and electricity in the farms. Thus this practice is in fact eco-friendly and low-cost, and it should be further promoted in developing countries.

5. Conclusions

Among the 21 target compounds screened, the present study found eleven progestagens in animal wastes and four in the receiving streams. The results clearly indicate the common use of synthetic progestagens and excretion of natural progestagens in the swine farms. It is also found that the lagoon and digester systems were effective in the removal of progestagens, and should be promoted in developing countries with concentrated animal production. Moreover, biogas digesters and fish ponds should also be encouraged as they are low-cost and eco-friendly and at the same time with economic benefits from energy generated and fish produced.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.03.022.

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