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Removal of veterinary antibiotics, alkylphenolic compounds, and estrogens from the Wuluo constructed wetland in southern Taiwan

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This study investigated the treatment in the constructed Wuluo wetland, Taiwan, of 13 veterinary antibiotics, including five classes (tetracyclines, sulfonimides, chloramphenicol, fluoroquinolone, and dyes), five alkylphenolic compounds (nonylphenol di-ethoxylates [NP_2EO], nonylphenol mono-ethoxylates [NP_1EO], nonylphenol [NP], octylphenol [OP], and bisphenol A [BPA]), and three estrogens (17β-estradiol [E_2], estriol [E_3], and 17α-ethynylestradiol [EE_2]). The veterinary antibiotics oxytetracycline (OTC), ciprofloxacin (CIP), chloramphenicol (CAP), oxolinic acid (OXO), sulfamonomethoxine (SMM), and sulfadimethoxine (SDM) were detected in 7.1–96.4% of samples with concentrations varying widely from not detected to 552 ng/L. Removal efficiencies within different units of the wetland system exceeded 87% for OTC, CAP, SMM, and SDM, excluding those for IP and OXO, which were 72.1% and 43%, respectively. The other seven antibiotics (tetracycline [TC], enrofloxacin [ENR], chlortetracycline [CTC], sulfamerazine [SMR], sulfamethazine [SMZ], malachite green [MG], and leucomalachite green [LMG]) were all below detection limits in all samples. Additionally, detection rates were as follows: NP_1EO, 70.0%; NP_2EO, 70.0%; 4-n-NP, 72.9%; 4-OP, 50.0%; BPA, 81.3%; E_2, 52.1%; E_3, 57.1%; and EE_2, 31.3%. Concentrations of the alkylphenolic compounds were as follows: NP_1EO, ND-1092.7; NP_2EO, ND-643.7; 4-n-NP, ND-6812.3; 4-OP, ND-10400.1; and BPA, ND-1733 ng/L. Natural and synthetic estrogens E_2, E_3, and EE_2 in samples were found in the ranges of ND–907.4, ND–749.5, and ND–226.0 ng/L, respectively. Analytical results show that with the exception of EE_2 throughout the wetland system, target compounds were largely removed.

Keywords: Alkylphenolic compounds, estrogens, constructed wetland, veterinary antibiotics.

Introduction

The toxicological and ecological effects of well-known xenobiotics (*i.e.*, metals, polycyclic aromatic hydrocarbons [PAHs], pesticides, and polychlorinated biphenyls [PCBs]) have been widely studied over recent decades. Lately, other contaminants, such as pharmaceuticals and personal care products (PPCPs), including antibiotics, phenolic endocrine disruptors, and estrogens, have become a concern due to their continuous release into aquatic environments and persistence.[1] Many studies show that common PPCPs used in daily life, such as antibiotics, anti-inflammatories, and antiseptic agents, have been detected in aquatic environments and effluents from conventional wastewater treatment plants (WWTPs) in concentrations of ngL^{-1} to μgL^{-1}.[2–5] Some technologies and processes have been used to treat these organic contaminants in an attempt to minimize their potential adverse effects on ecosystems and human health.[6,7]

Constructed wetlands (CWs) are used worldwide because of their low cost, ease of operation, and ecological importance.[8] Different types of CWs have been used successfully to treat municipal, industrial, and agricultural wastewaters. Research has shown that CWs have very high removal efficiencies for suspended solids, heavy metals, microorganisms, and selected endocrine-disrupting chemicals.[9,10] Hijosa-Valsero et al.[11] assessed the removal of eight classes of antibiotics in seven mesocosm-scale CWs and found that sulfamathoxazole was the most efficiently removed in all types of CWs. Dan et al.[12] assessed sulfonamide and trimethoprim antibiotics from domestic wastewater in pilot scale CWs, and research has shown that flow types were the most significant factor in antibiotic removal. Carvalho et al.[13] reported the potential for the common reed, *Phragmites australis,* in CWs to eliminate veterinary drugs from livestock wastewater. Matamoros demonstrated that a horizontal sub-surface flow system can treat wastewater containing PPCPs.[14,15]

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Antibiotics like tetracycline, sulfonamides, quinolones, nitrofuran, and chloramphenicol are commonly used in livestock farming. Most antibiotics are excreted and enter aquatic environments, possibly increasing antibiotic resistance in microorganisms. Environmental contamination by antibiotics in many countries has been investigated, revealing that higher concentrations of antibiotics exist in intensive-farming wastewater.[16–19]

Oxytetracycline, chlortetracycline, and tetracycline are the three most common antibiotics for treating disease, promoting growth, and targeting respiratory infections. These antibiotics are highly sensitive to pH, light, and heat.[20] Therefore, the threat of high doses of antibiotics in the environment is important. Additionally, residual oxytetracycline inhibits immunological functioning and drug resistance in fish (*Salvelinus alpinus L.*).[21,22] Uno et al.[23] showed that the residues of oxytetracycline in water accumulate in fish, shrimp, and shellfish, and therefore in humans through bioaccumulation. Further, sulfonamide antibiotics were effective drugs for controlling and eliminating bacterial infections until they were replaced by penicillin and other antibiotics. However, they are still used as antimicrobial agents to prevent or treat *Streptococcus* (and other bacteria) infections in poultry. Huang et al.[24] identified sulphamethoxazole in concentrations as high as 3.8 $\mu g\ L^{-1}$ in a sewage treatment plant (STP) in Georgia, USA.

The fluoroquinolone antibiotics that have been investigated include enrofloxacin (ENR), oxolinic acid (OXO), and ciprofloxacin (CIP). Enrofloxacin is used in the poultry industry as an antibacterial agent; it persists in poultry and enters the environment as ciprofloxacin, a breakdown product. Ma et al.[25] detected enrofloxacin in feces after 15 days of feeding 199 mg/kg/day to pigs. Moreover, oxolinic acid, a regulated aquaculture drug in Taiwan, is also used at fish farms in Japan and other countries. However, it is administered during feeding because it has strong antibacterial activity and poor resistance.[26]

Wang and Wu[27] showed that different concentrations (0–80 ppm) of OXO can inhibit the growth of bacteria more than in other microorganisms in sediment. Lin et al.[28] identified a strong relationship between OXO concentrations in sediment and the degree of photo-transformation. Lai et al.[29] examined eels exposed to 5, 10, and 20 mg L^{-1} OXO. The residue concentration reached 3.32 mg/L, with 5 mg L^{-1} spiking after 691 days of exposure, indicating that low concentrations of OXO degrade slowly. Chloramphenicol, a broad-spectrum antibiotic, was used in the early 1980s to prevent and treat poultry pathogens. However, chloramphenicol can cause serious side effects for humans such as bone marrow damage and inhibition of regeneration of the hematopoietic system via adverse anemia and leukopenia granules.[30] The residual chloramphenicol in animal tissue can induce pathogen resistance, and therefore its use is prohibited worldwide.[31]

In addition to antibiotics, endocrine-disrupting chemicals (EDCs) like alkylphenolic compounds and estrogens, nonylphenol-mono-ethoxylate (NP_1EO), nonylphenol-diethoxylate (NP_2EO), 4-n-nonylphenol (4-n-NP), 4-octylphenol (4-OP), and bisphenol A (BPA), and estrogenic compounds 17β-estradiol (E_2), estriol (E_3), and 17α-ethinylestradiol (EE_2) are common contaminants from industrial and municipal pollution. Numerous studies have reported EDCs in river waters downstream of wastewater or industrial discharges.[32–34] These EDCs cause adverse endocrine effects in male animals and their toxicity to humans has been widely evaluated.[35–37] The removal of these contaminants by wastewater treatment plants is well documented. The literature reported that the removal rates of compounds E_2 and EE_2 ranged from 36–41% at CWs.[38] The long-term impacts of these exogenous agents on aquatic biota warrant investigation.

Wastewater treatment CWs have become valuable alternatives to wastewater treatment plants in Taiwan. However, studies examining the distribution within and removal efficiency of contaminants from wetlands are limited. This study focused on the distribution of the select veterinary antibiotics, alkylphenolic compounds, and estrogens in the Wuluo CW system that receives wastewater from concentrated animal feeding operations (CAFOs) and evaluated the removal efficiencies within the various sections of CWs.

Materials and methods

Site description

The study was conducted at the 4-year-old Wuluo CW, one of the largest wetland systems in southern Taiwan. This wetland combines filter beds, lotus ponds, and a free water surface (FWS) system to treat highly polluted wastewater discharged into the Wuluo River from livestock operations and aquaculture farms. The filter bed contains six sections arranged in a series, with each section containing six ladder filter beds covered with gravel and sized 340 cm (L) × 140 cm (W) × 150 cm (H) (Fig. 1). Several macrophytic species in the wetland system were planted in the lotus pond and substrate. Predominant plant species include native cattail, *Cyperus, Phragmites*, and *Vetiveria*. Each of these taxa grows rapidly, has a deep root system, and is economical. The FWS system consists of three basins with an impermeable bottom soil and shallow water depth. Table 1 lists the operational parameters of the different units. The wetland treats approximately 32,870 $m^3\ d^{-1}$ (CMD) (average) with a hydraulic retention time (HRT) of 53 hours. This wastewater flows through each cell and purification (pollutant removal) is achieved via three main sub-units. Treated water from the system is discharged to outlet ditches and back into the lower reaches of the Wuluo River, which merges into the Kaoping River,

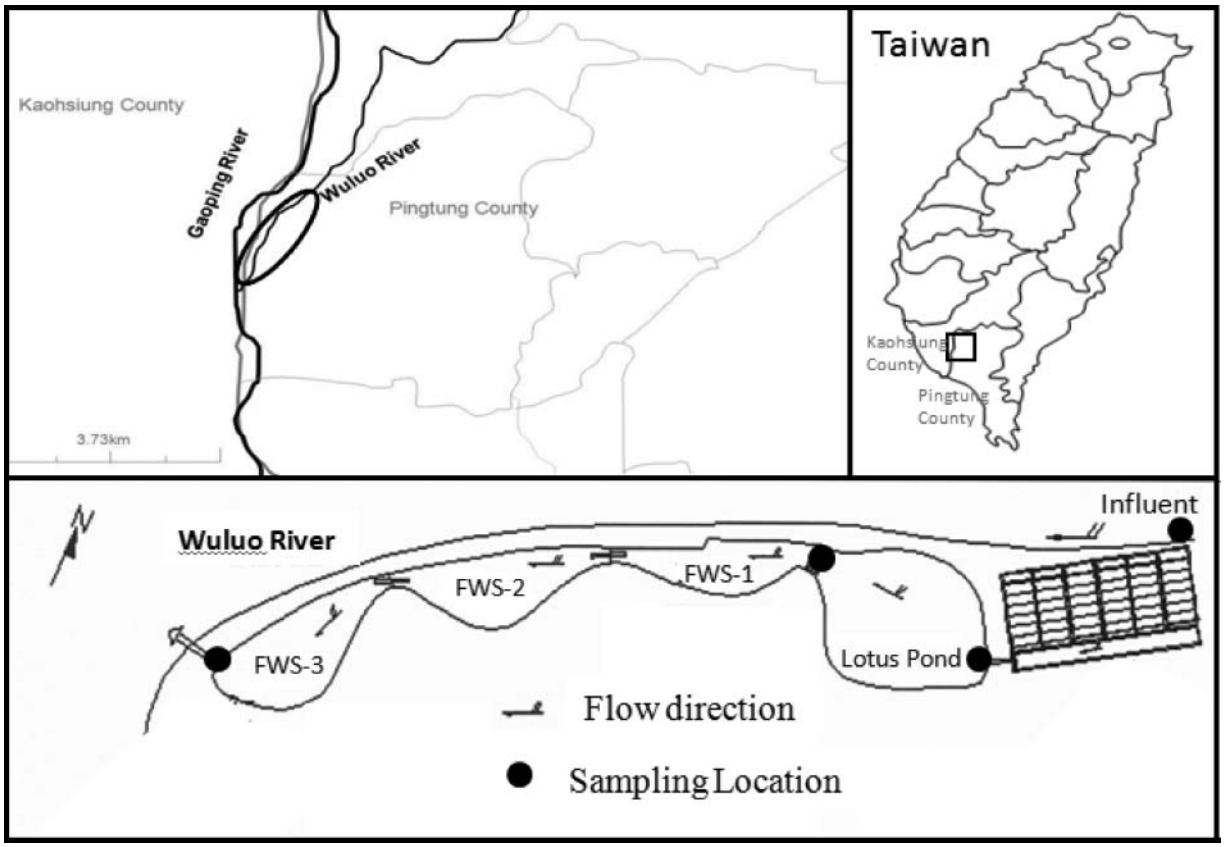

Fig. 1. Schematic diagram of Wuluo constructed wetland.

a drinking water resource for Kaohsiung County, southern Taiwan.

Chemicals

The following 21 chemical standards were purchased from Sigma-Aldrich (Steinheim, Germany): chloramphenicol (CAP), oxolinic acid (OXO), chlortetracycline (CTC), oxytetracycline (OTC), tetracycline (TC), enrofloxacin (ENR), ciprofloxacin (CIP), sulfamerazine (SMR), sulfamonomethoxine (SMM), sulfadimethoxine (SDM), sulfamethazine (SMZ), malachite green (MG), leucomalachite green (LMG), nonylphenol diethoxylates (NP_2EO), nonylphenol monoethoxylates (NP_1EO), 4-nonylphenol (NP), 4-octylphenol (OP), bisphenol A (BPA), 17β-estradiol (E_2), estriol (E_3), and 17α-ethynylestradiol (EE_2). All chemicals were of analytical grade unless stated otherwise. All solvents, such as methanol and acetonitrile, were purchased form Merck (Darmstadt, Germany). Analytical grade dichloromethane was obtained from Supelco (TEDIA, Fairfield, OH, USA).

Sample collection and preparation

Water samples were collected from four sampling sites within the wetland between January 2008 and July 2009. Grab samples from different units, including raw water (influent), at the inlets of the lotus ponds and free water surface system 1 (FWS-1), and at the outlet of the free water surface system 3 (FWS-3) (effluent) of the wetland

Table 1. Main parameters of the Wuluo constructed wetland (32870CMD).

	Filter Beds	*Collection Tank*	*LP*	*FWS 1*	*FWS 2*	*FWS 3*
Amount	36	6	1	1	1	1
Depth (m)	1	0.8	2	0.8	0.8	0.8
Area (m^2)	29	8	4375	30,000	30,000	30,000
HRT	7.6 min	1.7 min	3.15 hr	0.73day	0.73 day	0.73 day

*LP: A combined small lotus pond and large lotus pond; FWS: free water system.

system were collected in 1-L bottles and refrigerated during transportation to the laboratory where they were stored at 4°C before pretreatment.

Samples were then acidified and filtered through a 1.6-μm glass microfiber filters (GF/A (Whatman, UK) and 0.45-μm glass fiber filters (Advantec MFS, Dublin, CA, USA) to remove particles that could interfere with the extraction procedure. Selected compounds from filtrates were recovered after solid-phase extraction through Oasis Hydrophilic Lipophilic Balance (HLB) cartridges (200 mg, 6 mL; Waters, Taunton, MA, USA). Cartridges were placed on a Varian vacuum manifold connected to a tank and pump and conditioned sequentially with 10 mL of acetonitrile/methanol, 5 mL of methanol, and 10 mL of milli-Q water at a flow rate of 4 mL min^{-1}. Filtrates (500–1000 mL) were passed through the cartridges and dried under a vacuum and then eluted with 2 × 3 mL dichloromethane. Eluates were reduced to 1 mL by rotary evaporation and dried under a gentle nitrogen stream. The final extracts were transferred into amber vials until further analysis to prevent photo-degradation of selected analytes. All extracts were stored at −20°C in a freezer until further analysis.

Chemical analyses and quality control

Liquid chromatography mass spectrometry atmospheric-pressure ionization (API 3000; Applied Biosystems, Foster City, CA, USA) (LC-MS/MS) equipped with an electrospray source was employed to identify and quantify the target compounds. Analyses were performed in positive mode for 12 compounds and negative mode for chloramphenicol. A ZORBAX SB-C8 column (4.6 mm × 15 cm, 5 μm; Agilent, Germany) was used for chromatographic separation of target compounds. A binary gradient with a flow rate of 0.8 mL min^{-1} was used. Mobile phase A contained 0.1% formic acid (v/v) in water with formic ammonium. Mobile phase B contained 0.1% formic acid (v/v) in methanol for chloramphenicol and acetonitrile for the other 12 antibiotics. The method detection limits (MDLs) were determined as the ratio of signal to noise (S/N = 10) and taken from the chromatograms of calibration. The MDLs for 13 antibiotics were between 0.1–5.0 ng L^{-1}. Samples with concentrations less than MDLs may have detectable levels of the analyte of interest but those levels are non-quantifiable.

The alkylphenolic compounds and estrogens were determined with a Waters 2695 High Performance Liquid Chromatograph equipped with a fluorescence detector (HPLC/FLD). The separation of alkylphenolic and estrogen compounds was achieved with a Waters XTerra RP18 column (25 cm) having a film thickness of 5 μm and internal diameter of 4.6 mm; injection volume was 10 μL. Acetonitrile and ultrapure water were the mobile phases. The MDLs for alkylphenolic compounds and estrogens were between 1.5–10 ng/L^{-1}.

For quality control in the analytical process, a five-point calibration curve was generated for each target compound. The correlation coefficients (R^2) of the calibration curves were in the range of 0.9984–0.9999. Additionally, sample

Table 2. Occurrence and operational conditions of thirteen selected antibiotics.

	Linearity	*Detection Freq*	*Precursor Ion*	*Products Ions*		*Concentration (ng/L)*	
Compound	*(R^2)*	*(%)*	*(m/z)*	*(m/z)*		*Min. Conc.*	*Max. Conc.*
$[M+H]^+$							
Tetracycline antibiotics							
Tetracycline (TC)	0.9992	—[a]	445	410	427	<MDL	<MDL
Oxytetracycline (OTC)	0.9996	46.4	461	426	444	<MDL	552
Chlortetracycline (CTC)	0.9986	—	479	444	462	<MDL	<MDL
Sulfonimide antibiotics							
Sulfamerazine (SMR)	0.9991	—	265	156	172	<MDL	<MDL
Sulfamethazine (SMZ)	0.9992	—	279	124	186	<MDL	<MDL
Sulfamonomethoxine (SMM)	0.9993	89.3	281	126	156	<MDL	133
Sulfadimethoxine (SDM)	0.9992	82.1	311	156	245	<MDL	176
Quinolones antibiotics							
Ciproflovxacin (CIP)	0.9993	10.7	332	288	315	<MDL	27.9
Enrofloxacin (ENR)	0.9999	—	360	316	342	<MDL	<MDL
Oxolinic acid (OXO)	0.9984	3.6	262	216	244	<MDL	12.2
Nitrofurun antibiotics							
Malachite green (MG)	0.9997	28.6	329	313	208	<MDL	det
Leucomalachite green (LMG)	0.9997	21.4	331	239	316	<MDL	det
$[M-H]^-$							
Chloramphenicol antibiotics							
Chloramphenicol (CAP)	0.9998	96.4	321	152.194	257	<MDL	158

Det.: detected, quantification not possible.

duplicates, trip blanks, and lab procedure blanks were also used for quality control.

Results and discussion

Using free-flow wetland systems to treat different conventional pollutants in wastewater is very common in Taiwan.[39–41] Most studies have focused on the removal of conventional water parameters, operational processes, or specific antibiotic-resistant microorganisms in treatment systems. The distribution and removal of veterinary antibiotics, estrogens, and alkylphenolic compounds by wetland systems in Taiwan is seldom addressed.

Occurrence of the antibiotics in the constructed wetland

Thirteen veterinary antibiotics (CAP, OXO, CTC, OTC, TC, ENR, CIP, SMR, SMM, SDM, SMZ, MG, and LMG) were analyzed in samples collected from different units of the Wuluo CW. Table 2 lists the analytical conditions and concentrations of antibiotics. Seven of the 13 antibiotics (SMR, SMT, ENR, TC, CTC, MG and LMG) were not detected in the samples. Detection rates were in the order of CAP > SMM > SDM > OTC > CIP > OXO. CAP was the most common antibiotic, followed by SMM and SDM, with detection frequencies of 96.4, 89.3, and 82.1%, respectively. Among these six antibiotics, the concentrations of OTC were highest with an average of 217.9 ng L^{-1}, followed by SMM with an average of 93.2 ng L^{-1}. The concentrations of MG and LMG were relatively low compared to those of other compounds.

The concentration of CAP at the wetland's inflow was 21.4–158 ng L^{-1}, which decreased to 10.3–18 ng/L in the lotus pond; its mean concentration in the effluent samples was <1 ng L^{-1}. The concentrations of CAP in influent samples were several times higher than that of the samples from the nearby Nichou River (data not shown). Hou et al.[42] examined the effects of *Chlorella* sp. exposed to different concentrations (0–40 ppm) of CAP, showing that a concentration of 5 ppm inhibited the growth of algae and that the median inhibitory concentration was 12.9 ppm, which was much higher than the levels found in this study.

SMR, SMM, SDM, and SMZ are common sulfonamide antibiotics used to prevent bacterial infection. Although sulfonamide antibiotics were replaced by other antibiotics at present, its residues pose a health risk. The concentrations of SMM in influent were 75.2–133 ng L^{-1} and decreased to a mean concentration of 12.1 ng L^{-1} at FWS3 (effluent). Moreover, influent SDM concentrations were 26.8–176 ng L^{-1} with a mean concentration of 84.3 ng L^{-1}. The concentration increased from a mean of 64.8 ng L^{-1} in the lotus pond to 83.3 ng L^{-1} at FWS1 with a negative removal efficiency. However, the mean concentration decreased sharply to 7.95 ng/L at FWS3. The SDM concentrations in wetland units were similar to concentrations of 60–210 and 47–68 ng L^{-1} in two effluent streams from WWTP in the United States.[43,44] Average concentrations of SDM in China's STP influents are 83 ± 4.7, similar to those of this study.[45] The other two sulfonamide antibiotics (SMR and SMT) were not detected in our survey.

The three tetracycline-based antibiotics in this study were OTC, CTC, and TC. Among these, OTC was the only compound detected, and its detection rate was 42.9%. Its concentration in influent was ND–552 ng L^{-1}, with a mean of 217.9 ng L^{-1}, decreasing to 58.6 ± 18.2 ng L^{-1} in the small lotus pond. The mean concentration was lowest (<5.8 ng/L) in FWS3 effluent with the exception of one sample at 42.7 ng L^{-1}. The concentrations of OTC were in the same range as effluent samples measured in Canada (220 ng L^{-1}) and similar to the concentration of 340 ng L^{-1} found in other watersheds.[46,47] This OTC level is also similar to that reported by Kolpin et al.[4] for U.S. surface waters (0.34 μg L^{-1}). Jacobsen and Berglind[48] investigated the occurrence of OTC residues in sediment samples, detecting concentrations at ≤ 4.9 mg kg^{-1} that were detectable after 12 weeks, showing the persistence of antibiotics in the aquatic environment. Huang[24] detected OTC in sludge from a WWTP in Georgia, USA, with a concentration as high as 1064 μg g^{-1} dw, indicating that OTC was easily adsorbed by activated sludge.

Babin et al.[49] studied two fish cells, RTG-2 and RTL-W1, to assess the inhibition of growth caused by OTC. They found that concentrations exceeding 20 mg L^{-1} will inhibit the growth of exposed fish cells, and derived an EC_{50} of 22.1 mg L^{-1}. A study by Eguchi et al.[50] also demonstrated that veterinary drugs, including sulphamethoxazole, trimethoprim, and oxytetracycline, adversely affect the growth of *Chlorella vulgaris* and *Cyclotella striata* (especially by oxytetracycline).

Ciprofloxacin, the metabolite of antibacterial agent ENR, is commonly detected in surface water and soil. This compound was only found in three influent samples, in the range of ND–27.9 ng L^{-1}, which was much lower than in samples examined by Gibs et al.[51] Further, oxolinic acid has been widely used as an antimicrobial compound and was detected in the influent in only 2 of the 28 samples, at 12.2 and 6 ng L^{-1}.

Malachite green has been used to treat fungal infections on fish eggs at aquaculture farms and is known to cause hepatic tumors in rodents.[52] Its use is restricted at aquaculture farms in many countries because of its health risks to fish consumers.[53] However, MG is used anyway because it is inexpensive and highly effective at preventing parasites and fungi.[54] Notably, MG was detected in three influent water samples in our study; however, concentrations were

Table 3. Occurrence and operational condition of eight selected alkylphenolic and estrogenic compounds.

				Concentration (ng/L)	
Compound	*Linearity (R^2)*	*Retention Time (min)*	*Detection Freq (%)*	*Min. Conc.*	*Max. Conc.*
Alkyphenolic compounds					
Nonylphenol (NP)	0.998	10.16	72.9	<MDL	6812.34
Nonylphenol monoethoxylates (NP_1EO)	0.999	8.32	70	<MDL	1092.72
Nonylphenol diethoxylates (NP_2EO)	0.999	7.69	70	<MDL	643.7
Octylphenol (OP)	0.997	9.64	50	<MDL	5657.64
Bisphenol A (BPA)	0.999	3.61	81.3	<MDL	1733
Estrogenic compounds					
17β-estradiol (E_2)	0.999	7.30	52.1	<MDL	907.4
Estriol (E_3)	0.999	2.86	57.1	<MDL	749
17α -ethynylestradiol (EE_2)	0.999	4.50	33.3	<MDL	226

relatively low and unquantibile. Further, MG was not detected in samples collected from any lotus pond or FWS unit. The distribution of LMG shows a similar trend in different units in the Wuluo wetland system. Analytical results show LMG was detected in most influent samples (only one sample in the FWS1 unit), however, non-quantifiable due to low concentrations. The presence of low concentrations of MG and LMG in samples was unsurprising despite their use being illegal. Although their use has been outlawed in Taiwan for many years, the illegal use of this drug to treat fish remains a possibility.

Presence of estrogens and alkylphenolic compounds in wetland

Five alkylphenolic compounds and three estrogens were all found in quantifiable levels in different wetland units, and their levels were higher than those of some antibiotics (Table 3). The concentrations of alkylphenolic compounds were higher than those of estrogenic compounds, likely due to the widespread use of alkylphenolic compounds. Mean influent concentrations were in the order of NP > OP > BPA > E_3 > NP_1EO > E_2 > NP_2EO > EE_2, and mean effluent concentrations were in the order of NP > OP > BPA > E_3 > NP_1EO > NP_2EO > EE_2 > E_2. The highest mean concentration was in the lotus pond and was for NP, while the lowest was found in FWS1 and was for E_2. The average concentration of EE_2 was also the lowest among seven contaminants.

The detection rates for the five alkylphenolic compounds and three estrogens were as follows: NP_1EO, 70.0%; NP_2EO, 70.0%; NP, 72.9%; OP, 50%; BPA, 81.3%; E_2, 52.1%; E_3, 57.1%; and EE_2, 31.3%. The alkylphenolic compounds (NP_1EO, NP_2EO, NP, OP and BPA) exhibited concentrations of ND–1092.7, ND–643.7, ND–6812.3, ND–10400.1, and ND–1733 ng L^{-1}. Natural and synthetic estrogens (E_2, E_3, and EE_2) were at concentrations of ND–907.4, ND–749.5, and ND–226.0 ng L^{-1}, higher than those reported by Clara et al.[55] and Baronti et al.[56] of 23–660 ng L^{-1}, 0.4–13 ng/L, and 4.7–25 ng L^{-1} for E_3, EE_2, and E_2, respectively. Moreover, concentrations for E_2 were also higher than those in influent at WWTPs (35–125 ng L^{-1} [57] and ND–161.6 ng L^{-1} [58]) in other studies.

Comparison of removal efficiencies of different units by this CW system and in other studies

This study analyzed the concentrations of veterinary antibiotics, alkylphenolic compounds, and estrogens to

Table 4. Mean concentrations and removal efficiencies in influent and effluent at the Wuluo CW during the 18-month monitoring study.

	Concentrations (ng/L)		*Removal*
	Influent	*Effluent*	*Efficiency (%)*
CAP	59.0 ± 46.4	0.25 ± 0.015	98.2
MG	det	ND	n.e.
LMG	det	ND	n.e.
OXO	12.2	ND	100
SMR	ND	ND	n.e.
SMT	ND	ND	n.e.
SMM	93.2 ± 19.7	12.08 ± 0.94	87
SDM	84.3 ± 50.7	7.95 ± 0.54	90.6
ENR	ND	ND	n.e.
CIP	17.6 ± 9.2	ND	100
TC	ND	ND	n.e.
OTC	217.6 ± 166.9	8.24 ± 15.2	97
CTC	ND	ND	n.e.
NP	1652.6 ± 1809.4	169.9 ± 147.9	89.7
OP	1119.1 ± 3017.2	166.6 ± 289.9	85.1
BPA	931.7 ± 684.2	104.8 ± 185.3	88.8
E_2	189.0 ± 274.1	9.01 ± 15.9	95.2
E_3	156.2 ± 139.6	282.8 ± 121.9	76.6
EE_2	24.8 ± 38.5	16.9 ± 38.6	31.8
NP_1EO	290.97 ± 456.8	68.5 ± 68.7	76.5
NP_2EO	172.5 ± 274.5	25.6 ± 27.9	85.2

Det: detected, quantification not possible; ND: not detected; n.e: not evaluated.

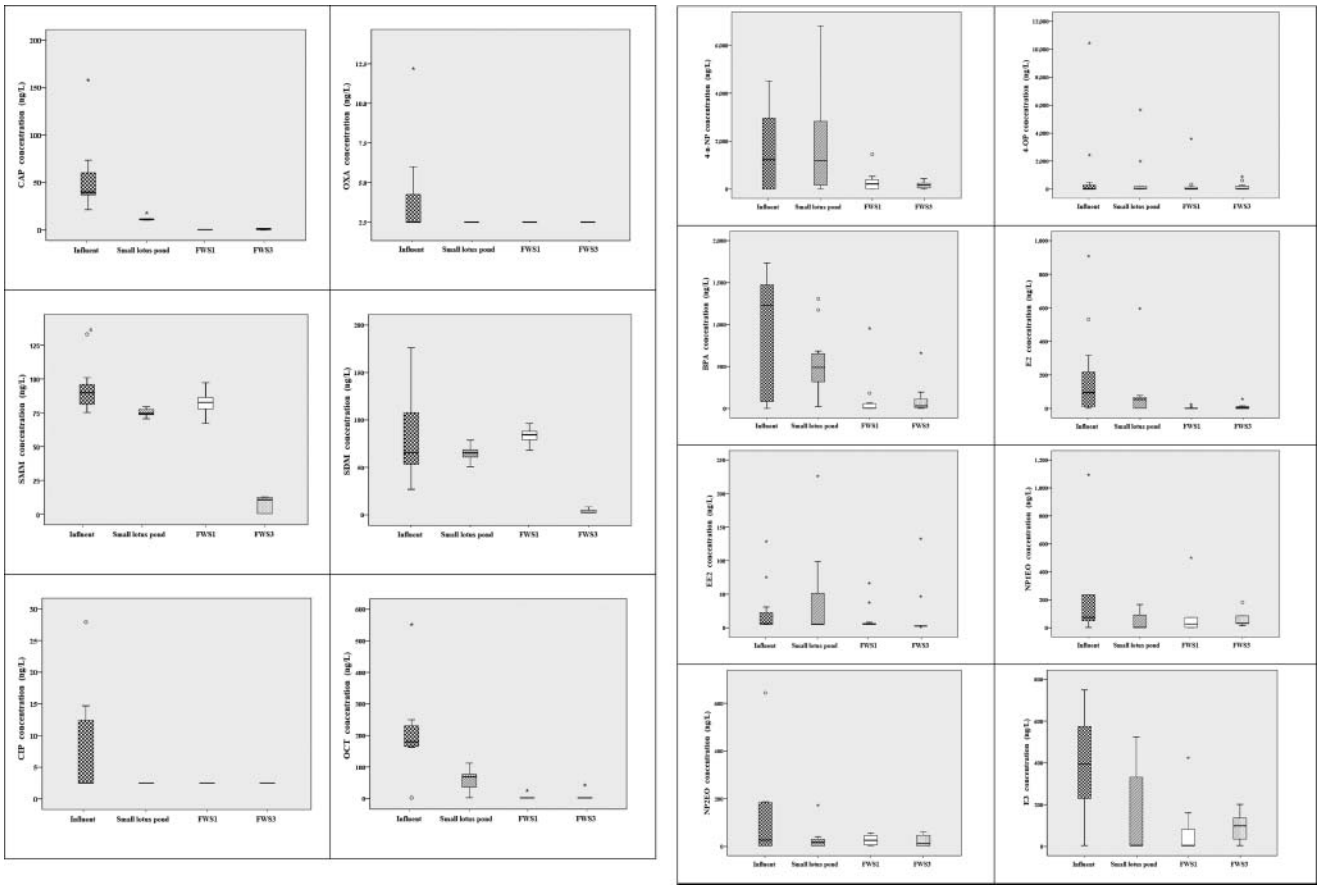

Fig. 2. Box plot indicating concentration ranges of selected analytes in different units of the CW.

identify their distribution in the Wuluo CW. Table 4 summarizes the concentrations and removal efficiencies at the inlets and outlets within the wetland system. The most efficient treatment compartment for NP_1EO, NP_2EO, E_3, OXO, and CIP was the filter bed section. The water collected from the sedimentation tank at the bottom of the filter beds removed large amounts of suspended solids and the water flowed into a rock-bed filter via gravity. The microbial degradation process occurred within biofilms growing on the filter in the aerobic environment. That is, the major removal mechanism was dominated by solids removal, specifically those solids with high hydrophobicity, which may be adsorbed by suspended particles.

The alkylphenolic compounds, including the plasticizer BPA, nonionic surfactant degradates (NP), and estrogens (E_2 and EE_2), were removed at relatively higher rates by the lotus pond relative to other units. That is, contaminant removal was higher in the lotus pond than in filter beds and FWS units (47.5–95.5%), similar to the rates for CAP and OTC, reflecting uptake by aquatic macrophytes. For the other compounds, removal rates were high in the FWS units.

Figure 2 shows the variation in compounds in different units. The greatest overall removal rates from influent to effluent for estrogenic, alkylphenolic, and veterinary compounds were 95.2% for E_2, 89.7% for 4-NP, and 98.2% for CAP, respectively. The removal efficiencies from influent to FWS3were in the range of 31.8–98.2% for all compounds except EE_2. In fact, the removal rate for the Wuluo CW was higher than for WWTPs in Guangzhou Province, China, which were 2430 and 1730 ng L^{-1} in influent and >2430 and 1460 ng L^{-1} in effluent.[59]

The overall removal rates (influent to effluent) for estrogenic and alkylphenolic compounds in decreasing order were E_2 > NP > BPA > NP_2EO > OP > NP_1EO > EE_2. Elimination rates were 76.5% for NP_1EO to 89.7% for NP. All alkylphenolic compounds had high removal rates (>80%) except for NP_1EO, which was slightly lower. Removal rates were high (>75%) for E_3, SMM, SDM, CIP, and OTC, with that of SMM exceeding 87%. The average concentration of SDM decreased from 84.3 ng/L to 7.95 ng/L, with an overall removal efficiency of 91%. However, removal efficiencies for EE_2 at the Wuluo CW were not obvious due to wide variations in its concentration.

Antibiotic removal by conventional wastewater treatment has been investigated in various studies.[60,61] Matamoros et al.[62] showed that the veterinary medicine flunixin was either not removed or only moderately removed (64 ± 3%) after 720 h. Although their wetland had a short retention time, selected antibiotics were removed efficiently (>80%). CIP in the Wuluo constructed wetland was at lower concentrations than those found in

three STPs in New York, USA (Lackawana, East Aurora, and Holland; 43–76 ng L^{-1} [63]) and other wastewater treatment plants (7–71 ng L^{-1}).[64,65]

To identify variations in selected compounds that were treated or not treated by the wetland, grab samples from the Wuluo River were collected and analyzed for the highest proportion of analytes, which were alkylphenolic compounds and estrogens. The concentrations of NP_2EO, NP_1EO, NP, OP, and BPA in the upper portion of the river were in the range of ND–166.8, ND–20.5, ND–2038.2, ND–1505.8, and ND–1390.9 ng L^{-1}, respectively, while concentrations of E_2 and EE_2 were ND–117.3 and ND–64.4 ng L^{-1}, respectively. The downstream concentrations of NP_2EO, NP_1EO, NP, OP, BPA, E_2, and EE_2 were 17.5–64.8, ND–69.9, ND–105.2, ND–3173.3, 41.2–443.2, ND–12.6, and ND–499.8 ng L^{-1}, respectively. Decreasing concentrations from upstream to downstream are not obvious, indicating that the efficiency of contaminant removal by the wetland was greater than that of the river.

Conclusion

The occurrence and fate of these veterinary antibiotics, alkylphenolic compounds, and estrogens in aquatic environments is recognized as an emerging issue because of their potential adverse effects on ecosystems and human health. Discharging these contaminants into watersheds and their removal by different processes (e.g., WWTPs) has been widely discussed in other studies. However, it is not possible to compare in-country findings because similar contaminant removal efficiency has not been investigated in any other constructed wetland in Taiwan.

Our experimental results demonstrate that veterinary antibiotics, estrogens, and alkylphenolic compounds existed in the Wuluo CW and that their concentrations were markedly reduced within a short period of time. The potential threat to drinking water quality from aquaculture and livestock operations that discharge wastewater into the Wuluo and Kuoping Rivers is reduced after wetland treatment. Although the wetland was seriously damaged by flooding during Typhoon Morag in 2009, the experimental results obtained herein provide valuable information for the restoration or design of similar wetlands in Taiwan. Additionally, a detailed study of the removal mechanisms and level of risk posed by these contaminants to the aquatic biota within a treatment wetland is warranted.

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