

Concentration and Environmental Loading of Veterinary Antibiotics in Agricultural Irrigation Ditches

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The concentration of veterinary antibiotics in aqueous and sediment matrices was measured in agricultural irrigation ditches bordering several animal-feeding operations (AFOs) and then compared to its concentration in the watershed. Analytical determination in aqueous samples was based on solid phase extraction (SPE) and appropriate buffer solutions were used to extract residuals in sediment samples. Separation and detection of extracted veterinary antibiotics were performed with high performance liquid chromatograph tandem mass spectrometry (HPLC/MS/MS). In general, higher concentrations of antibiotic were observed in the aqueous phase of irrigation ditches, with the highest concentration of erythromycin hydrochloride (ETM-H₂O) of 0.53 $\mu\text{g L}^{-1}$, than in aqueous watershed samples. In contrast, higher concentrations were measured in river sediment than in irrigation ditch sediment with the highest concentration of oxytetracycline of 110.9 $\mu\text{g kg}^{-1}$. There was a high calculated correlation (> 0.95) between precipitation and measured concentration in aqueous samples from the irrigation ditches for five of the ten targeted veterinary antibiotics, indicating that surface runoff could be an important transport mechanism of veterinary antibiotics from field to environment. Further, environmental loading calculation based on measured concentrations in aqueous samples and flow information clearly showed that irrigation ditches were 18 times greater than river. This result suggests the likelihood that veterinary antibiotics can be transported via irrigation ditches to the watershed. The transport via surface runoff and likely environmental loading via irrigation ditches examined in this study helps identify the pathway of veterinary antibiotics residuals in the environment.

Key words: Veterinary antibiotics, Irrigation ditch, Sediment, Transport, Environmental loading.

Introduction

Pharmaceutical antibiotics are used to prevent and treat disease in humans and to prevent illness and promote growth in livestock (Cromwell, 2002). It has been estimated that three million pounds of antibiotics, or 9% of total consumption in the U.S., are involved in human treatment annually, and more than 29 million pounds, or 84% of total consumption, are used in therapeutic and non-therapeutic animal application (Mellon et al., 2001).

Concern grows over the occurrence of antibiotics in the environment because most antibiotics are excreted

either as the parent compound or as a slightly modified form generally conjugated to polar molecules that can be cleaved easily under certain environmental conditions (Diaz-Cruz et al., 2003; Heberer, 2002). Depending on physicochemical properties such as water solubility, octanol/water partitioning coefficient, dissociation coefficient (pKa), and Henry's Law constant, pharmaceutical antibiotics can persist in soil or sediment and mobilize to ultimately reach the ground water (Bruhn, 2003; Tolls, 2001).

Occurrence of antibiotics in surface water was first reported in the late 1980's (Richardson and Bowron, 1985); subsequently, several researchers have found residuals of different classes of antibiotics in surface water, wastewater, and even ground water at sub-microgram concentrations (Hirsch et al., 1999; Lidberg et al., 2007;

Lindsey et al., 2001; Zhu et al., 2001). In 1999 and 2000, the first nationwide reconnaissance reported the occurrence of pharmaceuticals, hormones, and other organic wastewater contaminants (OWCs) in U.S. streams (Kolpin et al., 2002); the identified OWCs included pharmaceutical antibiotics originating from industrial, residential, and agricultural application. Based on such findings, an increasing level of research has focused on the pathways through which antibiotics enter the environment. Municipal sewage treatment plants (STPs) are a primary point source of human-use pharmaceutical antibiotics that reach the environment, prompting research on concentrations of antibiotics in STP influent and effluent (Castiglioni et al., 2006; Gobel et al., 2004; Hirsch et al., 1999; McArdell et al., 2003; Rooklidge, 2004).

Meanwhile, animal-associated antibiotics can enter the environment through the application of manure and slurries on agricultural fields as fertilizers and even the leaking of animal-waste handling equipment (Hamscher et al., 2002; Kay et al., 2005a; Kolpin et al., 2002). After manure and slurries are applied in the field, veterinary antibiotics can be found in both aqueous and sediment phases. High levels of veterinary antibiotics have been reported in lagoons, liquid manures, and fertilized agricultural fields. As much as 4.0 mg/kg of tetracycline has been measured in manure, a much higher concentration than found in an aqueous matrix (Blackwell et al., 2004; Campagnolo et al., 2002; Haller et al., 2002; Hamscher et al., 2002; Jacobsen et al., 2004; Liguoro et al., 2003; Schlusener et al., 2003).

While the measured concentration of antibiotics in the environment (e.g. surface water, ground water, soil, and sediment) was several orders of magnitude lower than levels that can be toxic or directly detrimental to ecological systems, accumulated trace amounts of antibiotics can result in bacterial resistance. Tetracycline-resistant bacteria were identified in lagoons and ground water underlying large swine production operations (Chee-Sanford et al., 2001). Also, different classes of antibiotic resistance genes (ARGs) were found in sediment and animal manure (Pei et al., 2006; Storteboom et al., 2007). In addition, resistance to three different antibiotic groups (tetracycline, macrolides, and streptomycin) was observed in soil treated with pig manure (Sengelov et al., 2003).

This study targeted the concentration of veterinary antibiotics in irrigation ditches bordering several animal-feeding operations. In addition, environmental loading calculations based on flow information and measured concentrations were made at irrigation ditches and in-river. Through these concentration measurements and calculated environmental loading indicators, more comprehensive understanding of the potential intermediate pathways of veterinary antibiotics from the animal farm to surface water should be achieved.

Materials and Methods

Materials Ten veterinary antibiotics were targeted in this study; tetracycline (TC), chlortetracycline (CTC), oxytetracycline (OTC), sulfathiazole (STZ), sulfamethazine (SMT), erythromycin (ETM), tylosin (TYL), monensin

Table 1. Properties of veterinary antibiotics used in this study.

Compound	Acronym	CAS Number	pKa ^a	Log K _{ow} ^a	Water Solubility (mg/L) ^a
Tetracycline	TC	60-54-6			
Chlortetracycline	CTC	64-72-2	3.3/ 7.7/ 9.3	-1.3 to 0.05	230 to 52000
Oxytetracycline	OTC	79-57-2			
Sulfathiazol	STZ	72-14-0			
Sulfamethazine	SMT	57-68-1	2 - 3/ 4.5 - 10.6	-0.1 to 1.7	7.5 to 1500
Erythromycin-H ₂ O	ETM-H ₂ O	114-07-8			
Tylosin	TYL	1401-69-0	7.7 to 8.9	1.6 to 3.1	0.45 to 15
Monensin	MNS	22373-78-0			
Salinomycin	SLM	53003-10-4	6.4	NA	NA
Narasin	NRS	55134-13-9			

^a reference from Thiele-Bruhn, 2003; NA: No information is available.

(MNS, 90-95%), and narasin (NRS, 97%) as a sodium salt were purchased from Sigma Aldrich Co. (St. Louis, MO, USA), except salinomycin sodium salt (SLM, 96%) obtained from ICN (Aurora, OH, USA). A stock solution of 100 mg L⁻¹ was prepared in HPLC grade methanol (99.9%) for each compound and stored at 4°C until use. HLB (Hydrophilic-Lipophilic-Balanced) cartridges used for solid phase extraction (SPE) had 3mL 60mg⁻¹ capacity and were obtained from Water Oasis Co. (Milford, MA, USA). High purity DI water (18.3 MΩ cm⁻¹) was used throughout the study. Properties of the selected compounds are summarized in Table 1.

Hydrology of Cache La Poudre River The study area, the Cache la Poudre River basin in northern Colorado, USA, represents one of the most highly managed and complex hydrologic systems in the western United States. The Cache la Poudre River, or Poudre River, flows north and east through the Roosevelt National Forest as it tumbles down the slopes of the Colorado Front Range and meanders through the City of Fort Collins, Colorado. From its headwaters to the confluence with the South Platte River east of Greeley, Colorado, the Poudre River drops 7,000 feet. In the mountainous areas upstream of Fort Collins, native flow occurs throughout the year on the river's main stem. Significant diversions on the main stem begin approximately 62 river miles upstream of the river's mouth, and return flows from agricultural irrigation and municipal/industrial wastewater treatment plants are an integral part of the water supply in the lower basin during the storage season, which generally lasts from October through April, and the irrigation season, which generally lasts from May to September. In the reach of the Poudre River from Fort Collins to the City of Greeley, returns from agricultural irrigation contribute approximately 4.5 m³ s⁻¹ to the river's main stem, some of which is in the form of seepage, discharge from various drains, and influent from natural tributaries.

Sample Collection and Pretreatment Samples from agricultural irrigation ditches and the Poudre River were collected at three different time periods (August 2004 and July/August 2005). Four sampling points were located along with the Larimer and Weld Canal, which connects with the Eaton Canal, and three sampling points were selected along the Cache la Poudre Canal, locally known as the Greeley #2 Ditch. An additional two collection points were chosen in the Graham Seep Ditch,

another river tributary. The Larimer and Weld Canal, located 47 miles upstream from the mouth of the Poudre River, and the Greeley #2 Canal, located 33 miles upstream from the river's mouth, are major river diversions during irrigation season, normally from June to September. Several feedlots and dairy farms, including a small breeding operation, are located between these two ditches. Sampling sites along with agricultural irrigation ditches and watershed are shown in Fig. 1. Average flow of the three ditches and the main Poudre River was summarized in Table 2.

Aqueous samples were collected in sampling bottles previously rinsed with DI water. The sample was collected vertically at three different depths at each sampling point. The collected sample was carefully mixed in one bottle and kept in a cooler before transporting to the lab, where it was filtered through a 0.2-μm glass fiber filter and stored at 4°C until analysis. Sediment samples were collected at only three ditch locations due to limited depth and at two river locations downstream of the ditches. Collected sediment samples were completely air dried at 20°C in a dark room to prevent any possible photolysis loss.

Air-dried sediment samples were sieved at 2 mm and 75 μm. The 2-mm-sieve fraction was used for physico-chemical property determinations, and the 75-μm-sieve

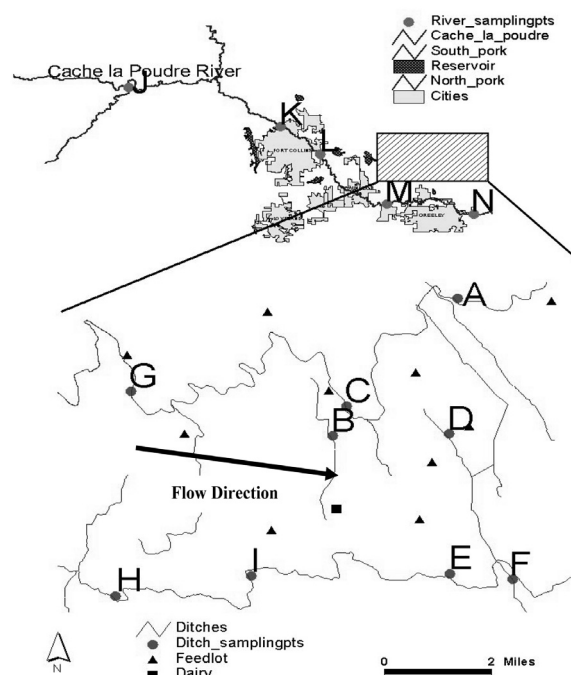


Fig. 1. Map of studied area: upper figure shows the watershed area from which aqueous and sediment river samples were collected; lower figure shows ditch sampling locations.

Table 2. Mean stream flow at sampling sites.

Sampling Sites	Site Name or Description	Average Monthly Mean Stream Flow ($\text{m}^3 \text{s}^{-1}$) ^a
A, C, G	Eaton Canal	6.8
B	Local Ditch	0.2
D, F	Graham Seep Ditch	0.1
E, H, I	Greeley #2 Canal	2.8
J	Pristine Area (no urban or agricultural activity)	NA
K	Urban Area	0.8
L	Urban Area (near wastewater facility)	0.6
M	Agricultural Area	1.4
N	Urban and agricultural combined area	1.4

^aAverage monthly mean stream flow at canal or ditch site was calculated for August 2004 and July/August 2005. Monthly mean stream flow of river sites was adapted from U.S. Geological Survey (USGS). NA: No information is available.

Table 3. Physical and chemical properties of (a) water and (b) sediment at sampling sites in ditches and river

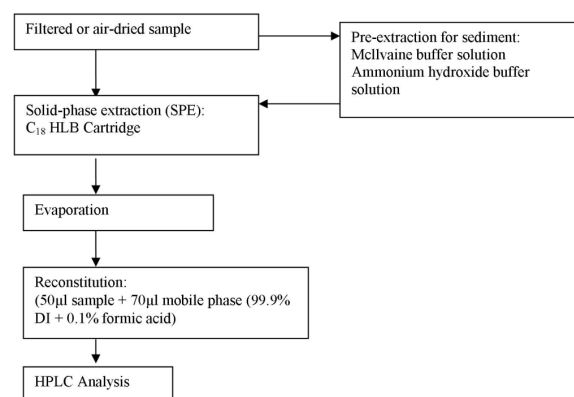
(a) Water						
Sampling Sites	pH	Ca	HCO ₃	SO ₄	NO ₃ -N	TDS ^a
		----- mg L ⁻¹ -----				
A	7.6	32.2	76.9	89.1	0.2	228.0
F	8.2	120.0	246.0	468.0	6.5	773.0
I	8.5	65.1	161.0	204.0	0.6	521.0
L	7.8	47.9	150.0	80.7	11.2	433.0
M	8.3	69.4	117.0	232.0	6.3	600.0
(b) Sediment						
	pH	EC	OM	NO ₃ -N	PO ₄ -P	Fe
		(dS m ⁻¹)	(%)	----- mg kg ⁻¹ -----		
D	7.7	1.5	1.8	8.8	20.5	14.4
M	7.4	2.9	0.8	11.2	18.6	82.9
N	7.5	1.8	0.5	15.3	32.6	68.7

^a TDS: Total Dissolved Solids.

fraction was used to extract antibiotic residuals since the clay portion is known to sorb the antibiotics (Kim et al., 2004). Physicochemical properties of collected samples were determined at the Soil, Water, and Plant Testing Laboratory at Colorado State University (Fort Collins, Colorado) and are summarized in Table 3.

Analytical Method The methodology for extraction and cleanup of the collected samples is shown in Fig. 2. The general procedure for aqueous sample preparation was adapted from a previous study (Kim and Carlson, 2007; Lindsey et al., 2001); the extraction procedure for MNS, SLM, and NRS was slightly modified to eliminate pH adjustment of samples prior to the SPE process (Kim and Carlson, 2006). The procedure for extracting antibiotics

in sediment was modified from a USDA (United States Department of Agriculture) guideline (USDA, 2003);

**Fig. 2. Extraction Procedure for Samples from River and Ditches.**

two different buffer solutions were used to extract antibiotics from solid to liquid phase - McIlvaine buffer solution (pH 4.0) for TC, CTC, OTC, STZ, and SMT; and ammonium hydroxide buffer solution (pH 10.0) titrated with formic acid for ETM-H₂O, TYL, MNS, SLM, and NRS - all followed by SPE.

The HPLC system (HP 1100 Series Liquid Chromatograph, Agilent, Palo Alto, CA, USA) consisted of an Agilent 1100 Series Thermostatted Auto Sampler and a variable-wavelength UV detector. The analytical column was an XTerra MS C₁₈ (Waters, Milliford, MA, USA), 2.1 × 50 mm (diameter × length, 2.5-μm pore size, end-capped). A reversed-phase C18 guard column (Phenomenex, Torrance, CA, USA) was installed to filter any particulates from the sample.

Mobile phase A was composed of 99.9% water and 0.1% formic acid, mobile phase B was 99.9% acetonitrile mixed with 0.1% formic acid, and mobile phase C was 100% of MeOH. Once the mobile phase condition was set, the composition of the mobile phase was gradually increased or decreased with time, and no isocratic condition was applied except for MNS, SNM, and NRS determinations. Injection volume was 20 μL for all compounds; a ten-minute column equilibrium period was included after each sample run.

A ThermoFinnigan LCQ Duo ion trap mass spectrometer (ThermoQuest, Woburn, MA, USA) equipped with a heated capillary interface and electrospray ionization (ESI) was used for mass spectrometric determinations. A 10 μM standard solution of each compound was made in

DI water and injected using the LCQ Duo syringe pump at a flow rate of 5 μL min⁻¹ to optimize the mass spectrometry parameters as needed. HPLC conditions for specific compounds and optimized mass spectrometry parameters are summarized in Table 4.

Positive ion mode was utilized for all compounds. Once the precursor ion was identified - [M+H]⁺ for seven compounds and [M+Na]⁺ for MNS, SNM, and NRS - fragment ions were produced with different collision energy. It should be noted that ETM-H₂O was used as a precursor ion based on a previous study (Hirsch et al., 1999). The dehydrated form of ETM can be achieved using an acidic mobile condition. The precursor and product ion for each compound, and tandem mass spectrometry parameters, are identified in Table 5.

Recovery and Limit of Quantification (LOQ) To validate recovery and limit of quantification (LOQ), the sample from river sampling site J was chosen as control sample for both water and sediment samples; this particular sample was chosen because of the site's pristine condition and the confirmation of no residual antibiotics. Average recovery in three aliquots spiked at different concentrations ranged from 91 to 115% for water samples and 46 to 119% for sediment samples. The LOQ was calculated based on documented statistical methodology (Zhu et al., 2001) and results are presented in Table 6. An external calibration curve constructed to calculate concentration demonstrated linearity of $r^2 > 0.97$ for all compounds from 0.01 to 5 μg L⁻¹.

Table 4. (a) Compound-specific HPLC conditions and (b) optimized mass spectrometry parameters.

	TC, CTC, OTC, STZ, and SMT	ETM-H ₂ O and TYL	MNS, SLM, NRS
Flow Rate (mL min ⁻¹)	0.32	0.32	0.25
(a) Mobile Phase Composition (%) Time	A: 4 + B: 96; 1 min	A: 20 + B: 80; 1 min	A: 50 + C: 50; 1 min
	A: 30 + B: 70; 29 min	A: 35 + B: 65; 13 min	A: 10 + C: 90; 14 min
	A: 4 + B: 96; 1 min	A: 20 + B: 80; 1 min	A: 50 + C: 50; 1 min
Column Temp. (°C)	15	45	15
Wavelength (nm)	360 for TC and CTC; 260 for STZ and SMT	No measurable wavelength	
(b) N ₂ gas:dryingandnebulizing	N ₂ gas:dryingandnebulizing		
	Spray voltage: 4.5kV		
	Capillary voltage: 21V		
	Capillary temperature: 165°C		
	Sheath gas flow rate: 40 units (arb)		
Auxiliary gas: off			

Table 5. Precursor and fragment ions associated with collision energy and isolation width.

Compound	Precursor Ion [M+H] ⁺ (m z ⁻¹)	Isolation Width	Collision Energy (%)	Fragment Ion (m z ⁻¹)
Simatone ^a	198			
TC	445	2.0	26	427 [M+H-H ₂ O] ⁺
CTC	479	2.0	32	462 [M+H-NH ₃] ⁺
OTC	461	2.0	28	443 [M+H-H ₂ O] ⁺
STZ	256	2.0	32	156
SMT	279	2.0	38	204
ETM-H ₂ O	716	3.0	26	558 [M-desosamine-H ₂ O+H] ⁺
TYL	916	3.0	30	772 [M-cladinose+H] ⁺
Compound	Precursor Ion [M+Na] ⁺ (m z ⁻¹)	Isolation Width	Collision Energy (%)	Fragment Ion (mz ⁻¹)
MNS	693	2.0	28	675 [M+Na-H ₂ O] ⁺
SLM	773	2.0	30	755 [M+Na-H ₂ O] ⁺
NRS	787	2.0	30	769 [M+Na-H ₂ O] ⁺

^a Internal standard. Simatone was quantified with selective ion monitoring (SIM).

Table 6. Summary of analysis recovery and limit of quantification (LOQ).

	(a) Water		(b) Sediment	
	Recovery (%) ^a	LOQ (μg L ⁻¹)	Recovery (%) ^a	LOQ (μg kg ⁻¹)
TC	107	0.006	88	1.9
CTC	112	0.009	75	0.9
OTC	102	0.007	46	0.5
STZ	91	0.002	98	1.2
SMT	105	0.005	93	1.8
ETM-H ₂ O	102	0.008	119	1.7
TYL	102	0.009	77	1.1
MNS	115	0.001	83	0.4
SLM	100	0.002	81	3.6
NRS	105	0.003	53	0.7

^a Average value of three spiked concentrations, 0.1, 1, and 5 μg L⁻¹, for water and 1, 30, and 90 μg kg⁻¹ for sediment. Relative standard deviation (RSD) for water was 3 to 13 % and 3 to 24 % for sediment.

Results and Discussion

Concentration of Veterinary Antibiotics in Irrigation Ditches and River The concentrations of ten veterinary antibiotics at the irrigation ditches and the Cache la Poudre River in aqueous and sediment samples are summarized in Tables 7 and 8, respectively. Higher concentrations of all compounds were observed in ditch aqueous samples compared to river aqueous samples. Compounds TC, CTC, ETM-H₂O, MNS, SLM, and NRS showed the highest concentrations at sampling site A located downstream in the Eaton Canal. Specifically, the highest concentration of TC and CTC was measured at

sampling sites A, E, and F, the last sampling points of each irrigation ditch. Measured CTC concentration at sampling points G, B, C, and A in the Eaton Canal were 0.08 μg L⁻¹, 0.11 μg L⁻¹, 0.03 μg L⁻¹, and 0.22 μg L⁻¹, respectively, while CTC concentrations at sampling sites H, I, and E in the Greeley #2 Canal were 0.05 μg L⁻¹, 0.02 μg L⁻¹, and 0.06 μg L⁻¹ respectively. These measurements show the concentration at sampling site A equaled the combined concentrations at sampling sites G, B, and C, while the concentration at sampling site E was 86% of the combined levels measured at sampling sites H and I, indicating CTC was being added to the irrigation ditches as water flowed through animal

Table 7. Summary of measured antibiotic concentration in water (values are the calculated mean of three individual measurements at three different sampling events, N = 9).

Sampling Sites	Measured concentration ($\mu\text{g L}^{-1}$) ^a									
	TC	CTC	OTC	STZ	SMT	ETM-H ₂ O	TYL	MNS	SLM	NRS
A	0.12	0.22	0.10	0.01	0.08	0.53	0.06	0.006	0.005	0.010
B	0.05	0.11	0.11	0.05	0.05	0.09	0.07	0.005	0.003	0.005
C	0.01	0.03	0.04	0.01	0.15	0.03	0.03	0.003	0.002	0.004
D	0.02	0.02	0.22	0.01	0.02	0.02	0.03	0.004	0.003	0.007
E	0.03	0.06	0.05	0.01	0.02	0.02	0.02	0.005	0.003	0.004
F	0.03	0.06	0.06	0.01	0.07	0.05	0.04	0.006	0.002	0.003
G	0.02	0.08	0.03	<BDL	0.08	0.02	0.01	0.002	0.003	0.003
H	0.01	0.05	0.03	<BDL	0.12	0.02	0.01	0.003	0.003	0.003
I	<BDL	0.02	0.07	<BDL	0.10	0.06	0.01	0.005	0.003	0.004
J	<BDL	<BDL	<BDL	<BDL	<BDL	<BDL	<BDL	<BDL	<BDL	<BDL
K	0.01	0.01	0.03	<BDL	<BDL	0.12	0.05	<BDL	<BDL	<BDL
L	0.02	0.04	0.03	0.03	0.02	0.15	0.05	<BDL	<BDL	<BDL
M	0.01	0.02	0.16	0.00	0.02	0.03	0.04	0.002	0.003	0.003
N	0.01	0.01	0.06	<BDL	0.01	0.08	0.03	0.003	0.002	0.003

^a More significant figures are shown for MNS, SLM, and NRS due to low concentration. <BDL: Below Detection Limit

Table 8. Summary of measured concentration in sediment (values are the calculated mean of three individual measurement at three different sampling events, N = 9).

Sampling Sites	Measured concentration ($\mu\text{g kg}^{-1}$)									
	TC	CTC	OTC	STZ	SMT	ETM-H ₂ O	TYL	MNS	SLM	NRS
B	35.3	31.2	155.2	1.4	<LOQ	18.1	20.3	4.7	7.3	5.8
D	6.8	7.3	20.4	<LOQ	1.1	19.8	11.7	6.1	6.1	6.2
F	11.8	4.0	63.5	<LOQ	<LOQ	7.9	7.3	2.2	5.7	6.6
M	28.4	7.8	91.4	4.7	9.1	30.7	19.6	9.4	10.6	8.5
N	27.9	7.9	110.9	<LOQ	2.7	59.0	37.1	10.8	8.9	6.9

^a LOQ: Limit of Quantification

feeding operations, despite slightly lower concentration at sampling sites C and I. Although STZ was rarely found along the irrigation ditches, the highest STZ concentration was detected at sampling site B ($0.05\mu\text{g L}^{-1}$). In contrast, SMT was detected at all irrigation ditch sampling sites, and concentrations were higher than those in river sample levels at all locations.

Another recent study also verified that runoff is one of the transport mechanisms for veterinary antibiotics and that amended manure can increase the transport efficiency of veterinary antibiotics (Burkhardt et al., 2005). In the current study, fairly consistent levels of MNS, SLM, and NRS were observed in the irrigation

ditches and river samples within the agricultural area (sampling sites M and N). However, no detectable levels were found in upstream of heavily agricultural influenced region, indicating irrigation ditches affected transport of these three compounds.

For sediment samples, sampling sites M and N located below the irrigation ditches were sampled. In contrast to aqueous samples, generally higher concentration was observed in river sediment samples than in ditch sediment samples. The exception was the three tetracycline compounds TC, CTC, and OTC, which showed highest concentrations in ditch sampling site B (Table 8). This contradiction in observed concentrations in sediment and

aqueous samples can be explained by higher flow energy in the ditches that might minimize the sorption of antibiotics to sediment, compared to a more static flow in the river that would favor sorption of antibiotics in the sediment.

Correlation Analysis Several mechanisms might be involved in transporting veterinary antibiotics from the field to the watershed. Preferential flow has been identified as a transport mechanism (Kay et al., 2004), and surface runoff might serve as an important transport mechanism (Davis et al., 2006). Previous studies showed transport of applied veterinary antibiotics from field to watershed via surface runoff (Burkhardt et al., 2005; Kay et al., 2005b; Pedersen et al., 2005). Although mass losses of veterinary antibiotics via surface runoff was usually less than 1%, surface runoff after severe rainfall still could affect significant transport.

Consequently, this study included estimation of the correlation coefficient between average monthly precipitation and measured concentration of target antibiotics in aqueous samples at selected irrigation ditches to assess the impact of surface runoff on residual concentration. Average monthly precipitation in Greeley, located in Northern Colorado, was obtained from the Western Region Climate Center (WRCC, Reno, Nevada, USA)

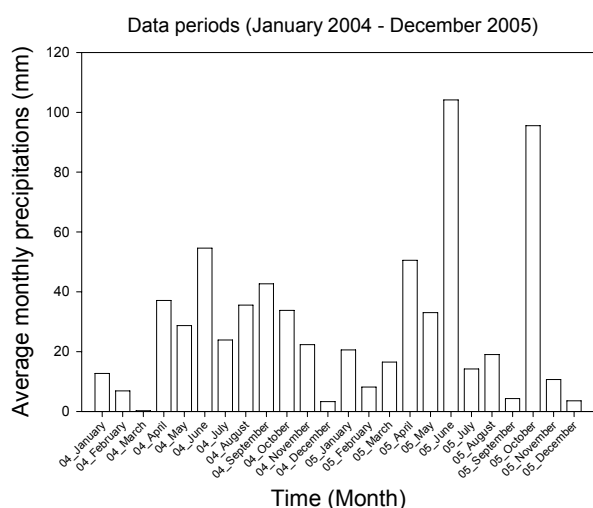


Fig. 3. Average Monthly Precipitation in Greeley (Northern Colorado).

and is shown in Fig. 3. During the study periods, the greatest rainfall - 104 mm - was recorded in June 2005; generally, more precipitation events are reported during the irrigation season from April to September. The average monthly precipitation at three sampling events was 36 mm for August 2004, 14 mm for July 2005, and 19 mm for August 2005.

Calculated correlation coefficients are shown in Table 9. Five of the ten antibiotics - TC, CTC, OTC, TYL, and MNS - showed high correlation (> 0.95) between recorded precipitation and measured concentration in the irrigation ditches. Compounds SMT and NRS showed moderate correlation, and a negative correlation was calculated for STZ and ETM-H₂O. While the exact cause of the negative correlation could not be identified, it is evident that the highest average concentration of STZ and ETM-H₂O was measured during the lowest and second-lowest precipitation periods. Overall results, however, clearly show that runoff might be significant in the transport of veterinary antibiotics from field to environment.

Environmental Loading at Irrigation Ditches and Watershed Environmental loading of veterinary antibiotics was calculated based on measured concentration in aqueous samples at each site (Table 7) and flow information (Table 2) and is summarized in Table 10. Because sampling sites M and N are located downstream of the study irrigation ditches, only those sites were included for comparison of environmental loading between irrigation ditches and river. When the concentration was below detection limit (< BDL), half of the BDL was used for environmental loading calculation. The results of environmental loading calculation clearly show that much higher mass was estimated along the irrigation ditches, compared to the river. Calculated masses at all irrigation ditches for eight of the ten compounds were greater than river-site concentrations, except for sampling sites D and F due to low-flow conditions. The highest environmental loading in irrigation ditches was calculated for ETM-H₂O with 1,432 g yr⁻¹ followed by CTC with 995 and SMT with 927 g yr⁻¹ respectively. In addition, the ratio of average calculated

Table 9. Calculated correlation coefficient between average monthly precipitation and measured concentration in irrigation ditches.

Compounds	TC	OTC	CTC	STZ	SMT	ETM-H ₂ O	TYL	MNS	SLM	NRS
Correlation Coefficient	0.99	0.97	0.98	-0.92	0.85	-0.01	0.95	0.96	0.13	0.63

Table 10. Summary of calculated environmental loading (g yr⁻¹).

Sampling Sites ^a	TC	CTC	OTC	STZ	SMT	ETM-H ₂ O	TYL	MNS	SLM	NRS
G	542	1446	542	0	1446	362	181	36	54	54
B	904	1989	1989	904	904	1627	1266	90	54	90
C	181	542	723	181	2712	542	542	54	36	72
A	2170	3977	1808	181	1446	9584	1085	108	90	181
H	151	376	226	0	903	151	75	23	23	23
I	75	151	527	0	753	452	75	38	23	30
E	151	452	376	75	151	151	151	38	23	30
D	3	6	71	3	6	6	10	1	1	2
F	10	19	19	3	22	16	13	2	1	1
Avg.	465	995	698	150	927	1432	378	43	34	54
M	36	72	579	0	72	108	145	7	11	11
N	36	36	217	0	36	289	108	11	7	11
Avg.	36	54	398	0	54	199	127	9	9	11

^a Location is ordered according to flow direction, from upstream to downstream of irrigation ditches

ditch site mass (N = 9) and average calculated river-site mass (N = 2) ranged from 2 to 18, depending on the compound. These results suggest that irrigation ditches might act as intermediate transport pathways of veterinary antibiotics from soil, where they might be exposed by surface runoff or seepage flow, to the watershed.

Conclusions

To verify potential transport pathway of veterinary antibiotics from field to watershed, concentration of veterinary antibiotics was measured at several agricultural irrigation ditches inter-connected with watershed. In general, higher concentration was observed at irrigation ditches compared to river samples in water samples while no noticeable trend was observed for antibiotic concentration in sediment. Also, calculated environmental loading at irrigation ditches was 18 times greater than river depending on compounds. The result of our study clearly showed that irrigation ditches might act as potential transport pathway of veterinary antibiotics from source to watershed.

Acknowledgement

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008961)" Rural Development Administration, Republic of Korea.

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