Using Drinking Water Treatment Residuals to Immobilize Tetracyclines in Manures and Manure-Amended Soils: Greenhouse Study

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ABSTRACT

Tetracyclines (TCs) are broad-spectrum antibiotics, which are extensively used for therapeutic purposes in the livestock industry. Studies have shown that tetracyclines can enter the environment in significant concentrations via repeated land application of manure and accumulate in soil, generating potential environmental and human health risks. Our earlier batch sorption and incubation studies revealed high adsorption affinity of Al-based drinking water treatment residuals (WTRs) for tetracycline (TTC) and oxytetracycline (OTC). Based on the successful results from the sorption studies, we hypothesized that Al-WTR could be a promising green sorbent for TTC and OTC rich manure and manure amended soils. A greenhouse column study was set up to evaluate effectiveness of Al-WTR. Cattle manure and two physico-chemically variant soil types (from Immokalee and Belleglade series) were chosen based on their potential differences with regard to TCs reactivity. Bermuda grass (*Cynodon dactylon*) and corn (*Zea mays L.*) were used as control and test plants respectively. Manure and soil samples were spiked with various concentrations of TTC/OTC (0, 1, and 10 mM) and amended at three rates (0, 2.5 and 5%) of Al-WTR. Soil/manure and leachate samples were collected periodically; immediately after spiking (time zero), and after 1, 2, 3, and 4 months. Soil/manure samples were subjected to four different treatments; DI water, 1M KCl (competing ion effect), methanol (hydrophobicity), and 0.25M EDTA (chelating agent) followed by solid phase extraction (SPE) and analysis by HPLC. Results showed that compared to the unamended (no WTR) manure/soils, leaching and mobility of TTC/OTC significantly (p<0.001) decreased by 45-70% within 4 months across all the treatments tested. Leaching of TTC and OTC reduced significantly (p< 0.05) from manure and soils amended with 5% Al-WTR as compared to those with 2.5% Al-WTR. Presence of Bermuda grass and corn reduced leaching of TTC/OTC by 5-9% compared to columns with no plant cover. Highest total leaching (time zero to 4 months) was observed in Immokalee soil, followed by Belleglade soil and manure, showing leaching behavior is related to soil physico-chemical properties. Solid phase extraction results showed less than 10% removal of the initial TTC/OTC concentration in both manure and soils in different phases tested (DI water (< 2%), 1M KCl (< 4%), methanol (< 5%), and 0.25M EDTA (<10%) after 3 months, indicating strong binding of TCs on Al-WTR. The release behavior observed is very encouraging with respect to stability of TTC/OTC sorbed to the retentive surfaces of the WTRs in amended soils and manure. This is the first greenhouse column study documenting WTR as an effective green sorbent for TCs rich manure and manure amended soils.

Keywords: Tetracyclines, Remediation, Drinking Water Treatment Residuals, Greenhouse column study, Manure/Soils

INTRODUCTION

VAs are being used increasingly to protect the health of farm animals and also to accelerate their growth (Boxall et al., 2003). Studies have shown that as much as 50 to 90% of the VAs administered orally may pass through the alimentary canal of cattle unchanged (Chee-Sanford et al., 2001; Kumar et al., 2005). Once excreted in urine and manure, VAs can enter into soils, surface water and/or groundwater via manure applied soils or via sludge storage at concentrated animal feeding operations (CAFOs). The presence of VAs in aquatic and terrestrial environments is of concern because, even at ng/L levels, these molecules are biologically active and can affect critical developmental stages and endocrine systems of aquatic and terrestrial organisms (Aga, 2008; Daughton et al., 1999; Ingham et al., 1994; Levy, 1987). Also, the widespread use and frequent detection of VAs in the environment have raised concerns over proliferation of antibiotic-resistant bacteria, decrease in the effectiveness of medical antibiotics, and other potential adverse human health and ecological effects (Agersø et al., 2006; Campagnolo et al., 2002; Thiele-Bruhn et al., 2003).

OBJECTIVES

- Evaluate the effectiveness of Al-WTR to immobilize TTC and OTC in TCs rich soils and manure in a greenhouse column study.
- Identify the role of soil and manure properties in TTC and OTC retention and release in the presence and absence of Al-WTR
- Investigate the degradants and metabolites of TCs using solid-phase extraction and liquid chromatography–tandem mass spectrometry.

MATERIAL AND METHODS

- Two types of soil – Immokalee and Belleglade series with varying physico-chemical properties were used (Table 1).
- Al-WTR was obtained from the Bradenton, Florida water treatment facility.
- Cattle manure was obtained from Rutgers, Cook Campus, NJ.
- Source of TCs : Tetracycline hydrochloride and Oxytetracycline hydrochloride
- Prior to their use in the greenhouse column study, the soils, manure, and WTR were characterized for physicochemical properties using standard methods.
- The soils were wetted to 70% of their water holding capacity and amended with TTC/OTC rich manure at a rate of 11.2 Mg ha^{-1} to simulate a realistic field loading rate in PVC columns (15" high x 6" diameter) as shown in the figure 1. Al-WTR was added at different rates (0, 25, and 50 g kg^{-1}).
- *Zea mays* (corn) and *Cynodon dactylon* (Bermuda grass) were used as test crop and control grass respectively.
- The TCs rich manure-applied soils were loaded in PVC columns (15" high x 6" diameter). Corn and Bermuda grass seeds were used. Columns were arranged in a randomized block design and were rotated periodically to account for variations in temperature and sunlight within the greenhouse. The plants were maintained and fertilized as per standard guidelines.
- The manure and manure amended soils, sorbent-treated/untreated were analyzed after extraction using HPLC at time zero (immediately after sorbent-amendment), after 1, 2, 3 and 4 months.
- Leachate samples were collected periodically and analyzed for TCs using HPLC.
- Liquid chromatography–tandem mass spectrometry was performed to analyze degradants and metabolites of TTC and OTC in leachate and soils samples after SPE extraction.

RESULTS AND DISCUSSION

Table 1:Properties of Soils, Manure and Al-WTR

	Al-WTR	Immokalee	Belleglade	Manure
pH	5.1 ± 0.34	5.9 ± 0.42	7.85 ± 0.12	6.2 ± 0.1
EC (µs/cm)	363 ± 12.3	59.0 ± 4.5	203 ± 13	240 ± 5.5
OM (g/kg)	240 ± 8.78	8.40 ± 0.2	80.0 ± 1.5	250.5± 2.5
$(Al+Fe)_{Total}$ (g/kg)	110.2 ± 8.5	0.08 ± 0.001	5.42 ± 0.46	<MDL**
$(Al+Fe)_{ox}$ (g/kg)	96.3 ± 4.5	0.02 ± 0.001	1.20 ± 0.005	<MDL**
TCs (mM)	<MDL**	<MDL**	<MDL**	<MDL**

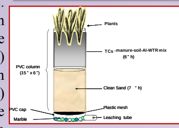

Fig. 1: Design of the PVC column

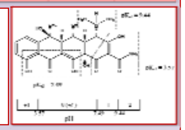

Fig. 2: TCs Structure

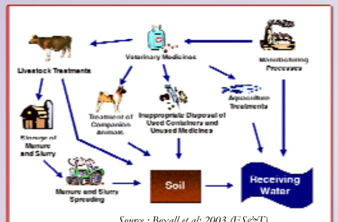

Fig. 3: Pathway of VAs in the Environment

Fig. 4. Photographs from greenhouse column study

Table 2: Mass Balance of TTC and OTC (1mM treatment) in Immokalee and Belleglade series soils. The percent recoveries calculated from TTC and OTC remaining in the soil, lost in the leachates, and potentially accumulated in plants.

Soil Manure TTC conc	Leachate end of 4 months	Extraction DI water	Extraction Methanol	Extraction KCl	Extraction EDTA	% Recovery	% Immobilized/ stabilized in soil/manure
Imm -0% Al-WTR (1mM TTC)	72%	3%	4%	7%	8%	95%	5%
Imm -2.5 % Al-WTR (1mM TTC)	20%	2%	2%	4%	6%	35%	60%
Imm -5% Al-WTR (1mM TTC)	13%	1%	2%	2%	5%	23%	70%
Bellg-0% Al-WTR (1mM TTC)	51%	2%	3%	8%	11%	76%	24%
Bellg-2.5 % Al-WTR (1mM TTC)	15%	2%	2%	3%	8%	29%	71%
Bellg-5% Al-WTR (1mM TTC)	13%	1%	1%	2%	5%	22%	79%
Manure-0% Al-WTR (1mM TTC)	55%	4%	6%	8%	11%	84%	17%
Manure-2.5 % Al-WTR (1mM TTC)	19%	2%	2%	4%	9%	37%	63%
Manure -5% Al-WTR (1mM TTC)	12%	2%	1%	3%	5%	23%	78%

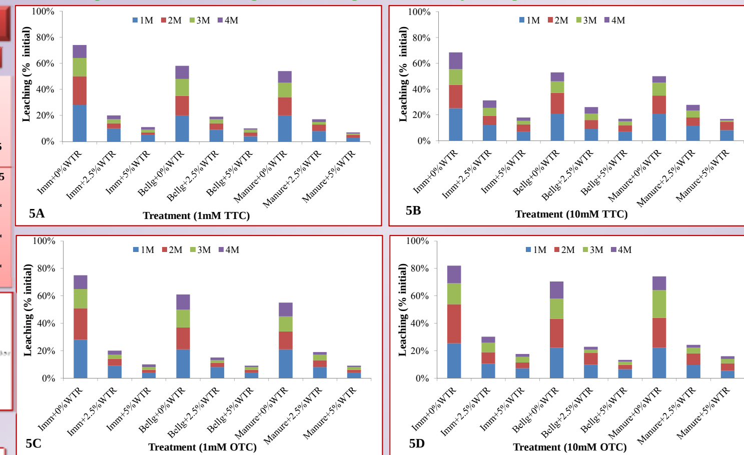

Fig. 5. Leachate analysis from soils (Immokalee and Belleglade) amended with TTC/OTC rich manure, manure rich in TTC/OTC, and treated/untreated with different rates of Al-WTR in presence of plants (Corn). $[TTC/OTC]_{initial}$ = 1 and 10 mM, Al-WTR rates: 0, 2.5 and 5%, and sampling period: 1, 2, 3, and 4 months. After 4 months, highest amount of leaching was observed for Immokalee soil, followed by Belleglade, and manure in all the TTC and OTC concentration tested. Results showed that highest amount of leaching was observed after 1st month for both the soils and manure, at all the TTC and OTC treatment tested. Significant (p < 0.001) decrease in the amount of TTC and OTC leached was observed in Al-WTR treated soils and manure in comparison to control (no Al-WTR) soil and manure. With increase in the amount of Al-WTR, significant (p < 0.05) decrease in the amount of TTC in the leachate was observed .

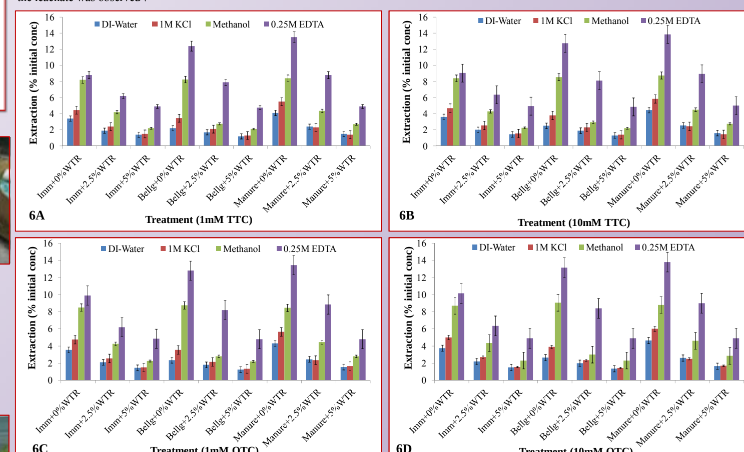

Fig. 6. Extractions were carried out after 4 months to determine potential release of TTC (A and B) and OTC (C and D) from the soils (Immokalee and Belleglade) in presence of corn amended with TTC/OTC rich manure, manure rich in TTC/OTC, and treated/untreated with Al-WTR. Four different treatments (DI water, 1M KCl, methanol, and 0.25M EDTA) were used. Methanol treatment was used to check the role of hydrophobicity on sorption, KCl was used to see the effect of competing ion on sorption, and EDTA was used as a chelating agent to evaluate competitively displaced sorbed TTC/OTC from soil, manure, and Al-WTR. TTC and OTC release from Al -WTR treated soils and manure with all the four treatments was minimal compared to control soils and manure (no Al-WTR), suggesting strong sorption. Less than 7% of the sorbed TTC/OTC was removed in the methanol phase suggesting that hydrophobic interaction between Al-WTR and TTC/OTC was minimal. With the treatment of 1 M KCl, less than 5% of the sorbed TTC/OTC was removed, indicating that the TTC sorption mechanism was stronger than just non-specific electrostatic interaction. Less than 12% of the total sorbed TTC/OTC was released by EDTA in soils and manure treated with Al-WTR suggesting strong TTC/OTC-Al-WTR complexes

CONCLUSIONS

- Amendment of Al-WTR to soils and manure significantly reduce leaching of TTC and OTC via immobilization or stabilization.
- Results from extraction experiments showed minimal release of TTC and OTC with the four treatments tested, indicating strong TTC/OTC-Al-WTR complexes.
- Al-based WTRs could be a promising, cost-effective, "green" medium for immobilization of TCs in manure and manure amended soils.
- The current research is the first step in our endeavor to ultimately develop a "green" remediation technique to immobilize and stabilize TCs in manure and manure-amended soils.
- Future studies being planned include full-scale greenhouse and field studies where dynamic interaction of manure, soil, water, and plants will be evaluated under natural environmental conditions.

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