Remediation of Tetracyclines in Soils and Manure Amended Soils: A Long-Term Greenhouse Column Study

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ABSTRACT

The presence of Tetracyclines (TCs) in manure, soil, sediment, sewage, surface water, and groundwater has been reported in several recent studies. The presence of TCs in the environment is of great concern because even at ng L-1 levels, these molecules are biologically active and can affect critical developmental stages and endocrine systems of aquatic and terrestrial organisms. Also, there are concerns over proliferation of antibiotic-resistant bacteria, decrease in the effectiveness of medical antibiotics, and other potential adverse human health and ecological effects. In the current one year greenhouse column study, we evaluated the effectiveness of a waste by-product from drinking water treatment process; namely Al-based drinking water treatment residuals (WTRs) to immobilize and stabilize tetracycline (TTC) and oxytetracycline (OTC) in soils and manure amended soils. Two physico-chemically variant soil types (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. Cattle manure was collected from Rutgers Cook campus. 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. Soils, manure-applied soils, plants, and leachate samples were collected periodically for one year. Soil and manure-applied soil samples were subjected to solid phase extraction to understand retention and release mechanisms of antibiotics. Results show that compared to the unamended (no WTR) soils, leaching and mobility of TTC/OTC significantly ($p<0.001$) decreased by 44-68% within 12 months across all the WTR treatments tested. Leaching of TTC and OTC reduced significantly ($p< 0.05$) from soils and manure-applied 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 6-9% compared to columns with no plant cover. Highest total leaching (time zero to 12 months) was observed in Immokalee, followed by Belleglade soil and manure-applied soil, showing physico-chemically dependent leaching behavior. Data from SPE showed less than 12% release of the initial TTC/OTC concentration in both manure-applied soils and soils in different phases tested, indicating strong binding of TCs on Al-WTR. Results obtained from the current greenhouse column study are encouraging and will potentially help in developing an optimal low-cost remediation technique for TCs and other veterinary antibiotics using a waste by-product.

Keywords: Tetracyclines, Soil, Remediation, Drinking Water Treatment Residuals, Greenhouse column study.

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 amended soils in a greenhouse column study.
- Identify the role of soil properties in TTC and OTC retention and release in the presence and absence of manure and Al-WTR.

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 Al-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 soils and manure amended soils treated/untreated with Al-WTR were analyzed after extraction using HPLC at time zero (immediately after sorbent-amendment), after 3, 6, 9 and 12 months.
- Leachate samples were collected periodically and analyzed for TCs using HPLC.
- Plant samples were harvested after time of maturity (6 months). Plant samples were extracted using citric acid and methanol (Boxall et al., 2006).
- Four different treatments, DI water, 1 mol L^{-1} KCl, methanol, and 0.25 mol L^{-1} EDTA were used as extractant.
- The TCLP was determined using USEPA SW-846 Method 1311.
- Statistical analysis was performed using JMP IN version 10 pro (Sall et al. 2005).

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**

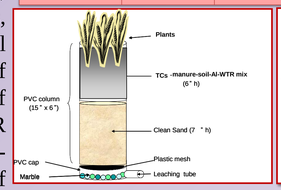


Fig. 1: Design of the PVC column

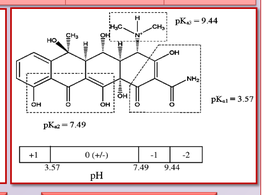


Fig. 2: TCs Structure

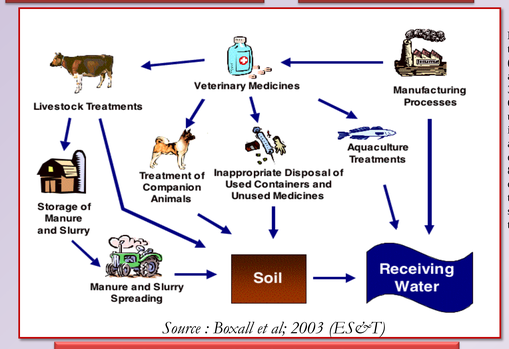


Fig. 3: Pathway of VAs in the Environment

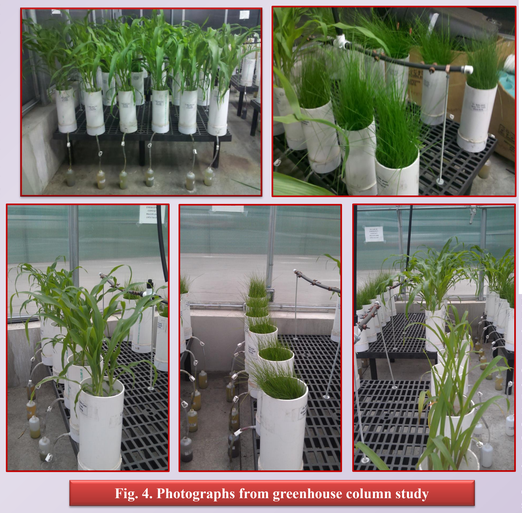


Fig. 4. Photographs from greenhouse column study

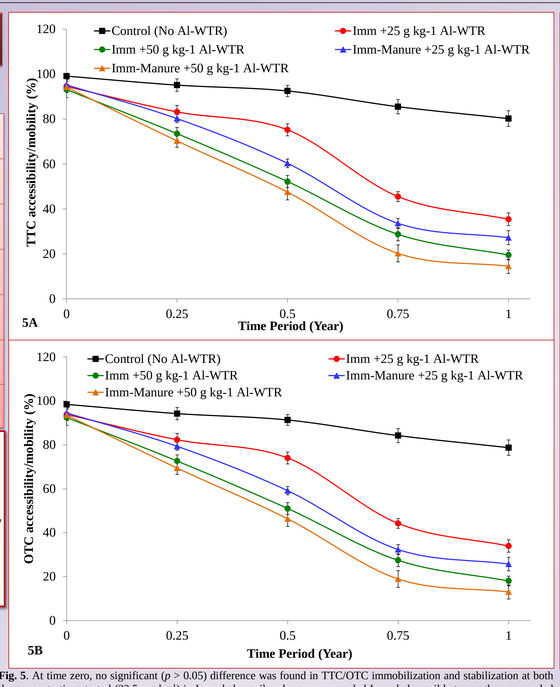
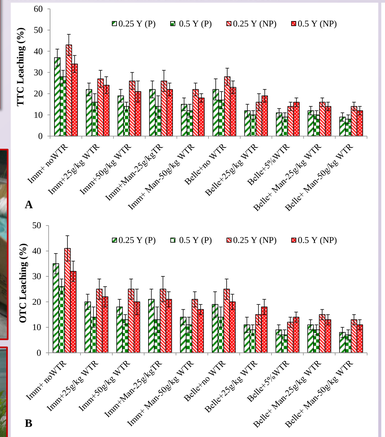
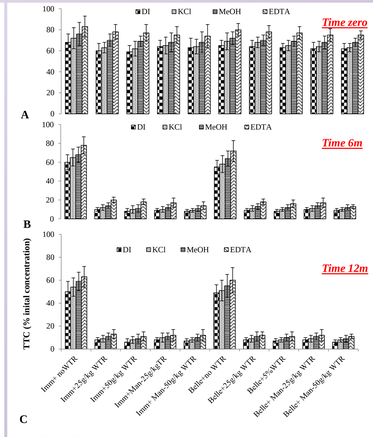


Fig. 5. At time zero, no significant ($p > 0.05$) difference was found in TTC/OTC immobilization and stabilization at both the concentrations tested (22.5 mg kg^{-1}) in Immokalee soil and manure amended Immokalee soil between the unamended (control) and the Al-WTR-amended soil (treatment) at two different rates (25 and 50 g kg^{-1}). However, the trend changed after 0.25 year of the equilibration time for both the TTC/OTC concentrations and Al-WTR rates tested (Figure 5-2 and 5-3). After 0.25 year of equilibration time, the effect of Al-WTR amendment on soil and manure amended soils, TTC and OTC immobilization became significant ($p < 0.001$), with increase in extent of immobilization compared to the unamended control at both the Al-WTR application rates and TTC/OTC concentrations tested (Figure 5-1). Further with increase in the equilibration time from 0.25 to 0.5 year, the effect of Al-WTR amended soil became pronounced on TTC and OTC immobilization. The trend remained unchanged until 1 year of equilibration period. At the end of 1 year equilibration period the TTC immobilization rate increased by 58-64 % and 72-79% in Immokalee soil, 63-69% and 79-84% in manure amended Immokalee soils applied with 25 and 50 g kg^{-1} Al-WTR, respectively compared to unamended control at both the TTC concentrations tested (Figure 5-2). Similar immobilization behavior was seen for OTC. However, the rate of OTC immobilization by Al-WTR was slightly lower compared to TTC. Al-WTR application rate had a significant positive effect ($p < 0.001$) on the rate of TTC and OTC immobilization in both the TTC/OTC concentrations tested and Immokalee and manure amended Immokalee soil.

Fig. 6. At time zero, no significant difference ($p > 0.11$) in Al-WTR amended Belleglade soil and unamended control was seen (6A and 6B). At the end of 1 year equilibration period, the TTC immobilization rate increased by 67-71 % and 75-81% in Belleglade soil, 72-75% and 82-87% in manure amended Belleglade soils applied with 25 and 50 g kg^{-1} Al-WTR, respectively compared to unamended control at TTC concentrations tested (6A). In case of OTC the immobilization behavior was similar to TTC. However, as seen in Immokalee soil, the rate of OTC immobilization by Al-WTR was slightly lower compared to TTC. After 1 year of equilibration period, the OTC immobilization rate increased by 64-69 % and 72-76% in Belleglade soil, 70-74% and 80-85% in manure amended Belleglade soils applied with 25 and 50 g kg^{-1} Al-WTR, respectively compared to unamended control at OTC concentrations tested (6B). There was no significant effect ($p > 0.05$) of initial TTC and OTC concentrations observed on the rate of immobilization by Al-WTR in Belleglade and manure amended Belleglade soil. However, as observed in case of Immokalee soils, Al-WTR application rate had a significant positive ($p < 0.001$) effect on the rate of TTC and OTC immobilization in both the TTC/OTC concentrations tested in manure amended and unamended Belleglade soil. The observed results are in agreement with the previous short time incubation study conducted with Belleglade and TTC/OTC rich manure amended Belleglade soil in the absence of plants with application of Al-WTR with different initial concentrations of TTC and OTC.





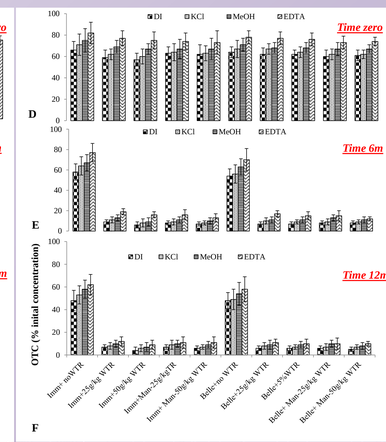


Fig. 7. The effect of different Al-WTR application rates (0, 25, and 50 g kg^{-1}) on leaching of TTC (7A) and OTC (7B) was investigated as a function of initial TTC and OTC concentrations (22.5 mg kg^{-1}) after 0.25 and 0.5 year of equilibration time in Immokalee and Belleglade soils unamended and amended with TTC/OTC rich manure in presence and absence of Bermuda grass (control plant) and corn (test crop). Soils amended with manure and spiked with TTC/OTC without Al-WTR amendment were used as control. . In the unamended Immokalee soil (no Al-WTR), the highest amount of leaching was observed; due to sandy nature, low organic matter, and very low content of Fe, Al, Ca, and Mg. In presence and absence of plant cover 25-32% and 38-42% , TTC and OTC leached from Immokalee soil after equilibration period of 0.25 and 0.5 year. However, in unamended Belleglade soil relatively low amount of leaching was observed; 15-20% and 20-25% in presence and absence of plants after equilibration period of 0.25 and 0.5 year. Application of Al-WTR in Immokalee and Belleglade soils significantly ($p < 0.01$) decreased the downward movement of TTC and OTC via leachate, by immobilization and stabilization. Similar trend was observed for manure amended Immokalee and Belleglade soil; However, the amount of leaching was lower than soils unamended with manure.

Fig. 8. Experiments were performed to determine the potential release of TTC and OTC from Immokalee and Belleglade soil amended and unamended with manure and treated with different Al-WTR rates at high initial TTC (8A, B, and C) and OTC concentration (8D, E, and F) as a function equilibration time. Four different treatments namely; DI water, 1 mol L^{-1} KCl, methanol, and 0.25 mol L^{-1} EDTA were used. DI water extraction was used to determine soluble-water extractable phase, methanol treatment was used to check the role of hydrophobicity, KCl was used to see the effect of competing ion, and EDTA was used as a chelating agent to evaluate competitively displaced bound TTC and OTC from Soil-Manure-Al-WTR mixture at time zero, 0.5, and 1 year equilibration time. At time zero, no significant difference ($p > 0.05$) was observed in the amount of TTC and OTC released/extracted between the Al-WTR applied Immokalee and Belleglade soil amended and unamended with manure compared to control (no Al-WTR application) in all the treatments tested. The application of manure and Al-WTR in Immokalee and Belleglade soil at time zero did not exert any significant effect on lowering the TTC (8A) and OTC (8D) soluble fraction. The addition of Al-WTR at both the rates tested in soils and soils amended with manure significantly decreased ($p < 0.01$) extraction by all treatments at 0.5 and 1 year of equilibration time for TTC (8B and C) and OTC (8E and F) indicating immobilization of TCs. Al-WTR was highly effective in decreasing the release of TTC and OTC from Immokalee and Belleglade soils manure amended and unamended and thereby increasing the immobilization and stabilization under dynamic system with soil, water, manure, and plants.

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.
- Further, long-term (≥ 3 years) simulated field based studies under a dynamic system with repetitive application of TCs rich manure (due to persistent nature of TCs), cyclic crop rotation, and uncontrolled natural condition are required to validate the current findings of Al-WTR in immobilizing and stabilizing TCs in soil system.

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Table 2: Toxicity characteristics values of several metals and metalloids measured for soils and soils applied with manure amended with Al-WTR at different rates using the EPA 1311 TCLP extraction method. The results are expressed as mg L^{-1}.

	mg L^{-1}								
Sample ID	Al*	Cr	Fe*	Ni	Cu	Zn	As	Ag	Cd	Pb
Immokalee control	0.65	0.01	0.18	0.01	0.06	0.53	0.01	0.01	0.01	>MDL
Imm + 2.5% Al-WTR	14.63	0.05	0.75	0.01	0.11	1.16	0.01	0.01	0.01	>MDL
Imm + 5% Al-WTR	24.30	0.10	0.80	0.01	0.11	0.78	0.01	0.01	0.01	>MDL
Belleglade control	1.11	0.01	1.63	0.02	0.01	0.25	0.02	0.01	0.02	>MDL
Belle + 2.5% Al-WTR	1.59	0.01	1.87	0.02	0.01	0.28	0.01	0.01	0.02	>MDL
Belle + 5% Al-WTR	1.85	0.01	1.95	0.02	0.01	0.05	0.01	0.01	0.02	>MDL
Imm + Manure control	0.15	0.01	0.19	0.01	0.01	0.02	>MDL†	>MDL	>MDL	>MDL
Imm + Manure+ 2.5% Al-WTR	26.15	0.03	0.22	0.01	0.01	0.02	>MDL	>MDL	>MDL	>MDL
Imm + Manure+ 5% Al-WTR	32.35	0.04	0.16	0.01	0.01	0.01	>MDL	>MDL	>MDL	>MDL
Belle + Manure control	1.45	0.04	1.25	0.01	0.01	0.07	>MDL	>MDL	>MDL	>MDL
Belle + Manure+ 2.5% Al-WTR	1.70	0.01	1.89	0.01	0.01	0.01	0.01	0.01	>MDL	>MDL
Belle + Manure+ 5% Al-WTR	2.07	0.01	1.95	0.01	0.01	0.01	0.01	0.01	>MDL	>MDL

< MDL †:(Below Method Detection Limit for As, Ag, Cd, and Pb 10 mg L^{-1} using ICP-MS)
**NR: Not regulated under the Toxicity Characteristics Leaching Protocol by USEPA*

Table 3: Dry plant biomass of corn plants (without corn cob and kernel/seeds) harvested after full maturation period grown in Immokalee and Belleglade soils applied with manure and amended with different rates of Al-WTR spiked with high TTC/OTC (22.5 mg kg^{-1}) concentration. Data are expressed as mean of three replicates (n=3) with one SD

Sample ID	**TTC**	**OTC**
Treatment with presence corn plants	-------g----	
Immokalee Control*	189.4 ± 13.6	179.1 ± 15.2
Immokalee + 2.5% Al-WTR	192.8 ± 17.1	182 ± 17.8
Immokalee + 5% Al-WTR	179 ± 15.4	194 ± 13.1
Belleglade Control	242.5 ± 18.9	258 ± 22.4
Belleglade + 2.5% Al-WTR	235.6 ± 21.5	243.2 ± 21.5
Belleglade + 5% Al-WTR	228.1 ± 20.3	245.9 ± 11.2
Immokalee + Manure Control	212.5 ± 20.5	208 ± 21.5
Immokalee + Manure + 2.5% Al-WTR	202.5 ± 19.2	219 ± 15.9
Immokalee + Manure + 5% Al-WTR	207.8 ± 24.2	218.5 ± 16.2
Belleglade + Manure Control	268.5 ± 15.4	259 ± 25.9
Belleglade + Manure + 2.5% Al-WTR	255.2 ± 23.2	253.6 ± 27.4
Belleglade + Manure + 5% Al-WTR	268.1 ± 28.1	255.9 ± 13.2

Table 4: Mass balance of TTC in Immokalee and Belleglade soils, and soil amended with manure at high initial TTC concentration (22.5 mg kg^{-1}) rate. The percent recoveries are calculated from TTC remaining in the soils, manure amended soils, TTC in leachates, and TTC accumulation in plants. Values are expressed as mean of two replicates ± one SD

Soil Type	**Soil Treatment**	**Time Zero**		**Time-Final (1 Year)**				
		Initial OTC theoretical (mg)	Initial OTC experimental (mg)	OTC remaining in the soil[a](mg)	OTC in the plant (mg)[b]	OTC in the leachates (mg)	Sum (mg)	Percent recovery
Immokalee	No Al-WTR	90	91.5 ± 0.9	26.8 ± 2.5	0.51 ± 0.1	50.6 ± 2.7	77.6 ± 5.3	86.2 ± 5.8
	25 g kg^{-1} Al-WTR	90	91.8 ± 1.1	51.3 ± 3.2	<MDL†	30.5 ± 2.4	81.8 ± 8	90.8 ± 6.2
	50 g kg^{-1} Al-WTR	90	91.3 ± 1.2	58.9 ± 2.8	<MDL†	22.3 ± 2.5	81.2 ± 5.4	90.2 ± 4.8
Immokalee + Manure	25 g kg^{-1} Al-WTR	90	91.5 ± 1.2	53.4 ± 2.4	<MDL†	25.9 ± 1.8	79.3 ± 5.9	88.1 ± 3.8
	50 g kg^{-1} Al-WTR	90	91.5 ± 1.3	63.4 ± 1.9	<MDL†	17.9 ± 1.5	81.3± 3.2	90.3 ± 3.5
Belleglade	No Al-WTR	90	91.4 ± 1.4	46.7 ± 3.6	0.38 ± 0.02	33.6 ± 2.5	80.7 ± 6.5	89.6 ± 7.2
	25 g kg^{-1} Al-WTR	90	90.8 ± 0.9	59.6 ± 3.1	<MDL†	22.7 ± 3.1	82.3 ± 6.2	91.4 ± 6.8
	50 g kg^{-1} Al-WTR	90	91.2 ± 1.2	65.4 ± 3.2	<MDL†	13.9 ± 2.2	79.3 ± 5.4	88.1 ± 6
Belleglade + Manure	25 g kg^{-1} Al-WTR	90	91.3 ± 1.1	60.5 ± 3.2	<MDL†	21.4 ± 2	81.9 ± 5	91 ± 5.5
	50 g kg^{-1} Al-WTR	90	91 ± 1.2	66.8 ± 1.9	<MDL†	14.1 ± 1.5	80.9± 3.4	88.9 ± 3.7

Table 5: Mass balance of TTC in Immokalee and Belleglade soils, and soil amended with manure at high initial TTC concentration (22.5 mg kg^{-1}) rate. The percent recoveries are calculated from TTC remaining in the soils, manure amended soils, TTC in leachates, and TTC accumulation in plants. Values are expressed as mean of two replicates ± one SD

Soil Type	**Soil Treatment**	**Time Zero**		**Time-Final (1 Year)**				
		Initial TTC –theoretical (mg)	Initial TTC – experimental (mg)	TTC remaining in the soil[a](mg)	TTC in the plant (mg)[b]	TTC in the leachates (mg)	Sum (mg)	Percent recovery
Immokalee	No Al-WTR	90	91.4 ± 1.1	26.5 ± 2.8	0.4 ± 0.1	51.5 ± 3.1	76.5 ±6.0	85 ± 6.6
	25 g kg^{-1} Al-WTR	90	91.5 ± 1.2	50.1 ± 4.7	<MDL†	31.2 ± 3.3	81.3 ± 8	90.3 ± 7.2
	50 g kg^{-1} Al-WTR	90	92.3 ± 1.5	58.3 ± 3.5	<MDL†	24.4 ± 2.4	82.7 ± 5.9	91.8 ± 6.5
Immokalee + Manure	25 g kg^{-1} Al-WTR	90	92.5 ± 1.6	52.3 ± 2.9	<MDL†	27.5 ± 1.7	79.8 ± 5.9	88.6 ± 6.6
	50 g kg^{-1} Al-WTR	90	91.5 ± 1.3	62.9 ± 1.8	<MDL†	18.2 ± 1.4	81.1± 3.2	90.1± 3.5
Belleglade	No Al-WTR	90	91.7 ± 1.2	43.5 ± 3.8	0.34 ± 0.08	34.2 ± 2.6	77.7 ± 6.4	86.3 ± 7.1
	25 g kg^{-1} Al-WTR	90	90.9 ± 1.1	58.3 ± 2.9	<MDL†	23.4 ± 3.3	81.7 ± 6.2	90.7 ± 6.8
	50 g kg^{-1} Al-WTR	90	91.3 ± 1.2	64.3 ± 3.7	<MDL†	14.4 ± 2.1	79.1 ± 5.8	87.4 ± 6.4
Belleglade + Manure	25 g kg^{-1} Al-WTR	90	91.5 ± 1.3	59.4 ± 3.2	<MDL†	22.5 ± 1.8	81.9 ± 5	91 ± 5.5
	50 g kg^{-1} Al-WTR	90	91.6 ± 1.2	65.9 ± 1.9	<MDL†	14.2 ± 1.6	80.1± 3.5	89 ± 3.5