Supplementary Materials

Supporting information S1 Monensin and Oxytetracycline Antibiotic Residue Analysis Details

The kits, consisting of 96-well plates coated with the respective antibodies, were designed mainly for testing residues in foods and feed. The manufacturer's protocol was modified for use with collected plant tissue, soil and manure samples based on discussions with the manufacturers and published methodology [1] which found the first kit to be highly sensitive for MON assessments when testing environmental samples with a 1.5 μg L-1 detection limit and a 3.0 μg L-1 quantification limit. Reagen LLC stated high sensitivity and low detection limit for their kit as well (0.15 μg L-1). Antibiotic residue was quantified with a microplate reader at manufacturer-recommended wavelengths of 405 nm for MON and 450 nm for OTC. As shown in Table. S1, both ELISA kits displayed high R2 values for linear equations based upon average absorbance. The average absorbance values were determined from the standards (0, 1, 5, and 25

ng mL-1) in the MON kits and standards (0, 0.15, 0.375, 0.75, 1.5 and 4.5 ng mL-1) in the OTC kits. From the average absorbance, a standard curve was determined for each kit which was used to quantify antibiotic content in the sample extraction solution, and ultimately in the sample itself.

Supporting information S2 Conversion of antibiotic ELISA concentration to soil, plant tissue, and manure content

To convert ELISA concentrations (ng mL^{-1}) to soil, plant tissue, and manure content (µg kg^{-1}) the moisture content of each sample type was used (11.8% for soil, 30% for plant and 16% for manure), calculated based on the sample weight (1 g for plant tissue and 2 g for soil and manure). This moisture amount was added to the buffer extraction amount used (4 mL) to give the total solution used for the antibiotic extraction (mL). Next, the weight of sample (g) per volume of total extraction solution (mL) was calculated by dividing the weight of the sample by the total extraction solution (g sample/ (mL) extraction solution). This number was then multiplied by 1000 to convert to (g) sample $(L)^{-1}$ extraction solution. Finally, the ELISA concentration was converted to µg of the antibiotic $(L)^{-1}$ of solution and this concentration was divided by the (g) sample $(L)^{-1}$ extraction solution, giving the µg antibiotic $(g)^{-1}$ of sample. This answer was then multiplied by 1000 to convert it to µg antibiotic $(kg)^{-1}$ of sample.

Supporting information S3 Oxytetracycline ELISA Testing Protocol

To develop an OTC standard curve, 75-µL of each standard was added in duplicate (from low content to high) to the bottom of different wells. Similarly, 75-µL of each plant and soil sample was added into different sample wells. Then, 100-µL antibiotic antibody #1 was added and mixed thoroughly by rocking the plate manually for 1 min., followed by incubating the plate for 50 min. at room temperature. The plate was washed three times with 250-µL 1X Wash Solution. Following the last wash, the plate was inverted and gently tapped dry on paper towels. Immediately after,

150-µL 1X antibody #2 solution was added and the plate was incubated for 20 min. at room temperature. Next, the plate was washed three times with 250-µL 1X wash solution. Again, after the last wash, the plate was inverted and gently tapped dry on paper. Then, 100 µL of TMB substrate was added and, immediately afterwards, an incubation time of 15 min. at room temperature was started. During the first minute of this reaction time, the solution was mixed by rocking the plate manually. Following this incubation, 100-µL Stop Buffer was added to stop the enzyme reaction. Immediately afterward, the plate was read using a spectrophotometer on a plate reader with 450-nm wavelength.

A standard curve was constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its content in ng mL-1 on a logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100$$

The mean relative absorbance values for each sample were used to determine the corresponding content of the tested sample from the standard curve.

Table S1. Linear equations for ELISA kits

Kit	***Linear Equation***	***R^2***
Monensin Plate 1	y = 21.935ln(x) + 4.3422	0.9999
Monensin Plate 2	y = 18.625ln(x) + 9.64	1
Oxytetracycline Plate 1	y = -16.07ln(x) + 47.037	1
Oxytetracycline Plate 2	y = -16.07ln(x) + 47.037	1

Table S2. Antibiotic concentration in plant tissue extraction solutions (n=4).

Treatment	Stem/leaf Monensin (ng mL^{-1})	Root Monensin (ng mL^{-1})	Stem/leaf Oxytetracycline (ng mL^{-1})	Root Oxytetracycline (ng mL^{-1})
Incorporated unspiked manure treatment	2.2 ± 0.3	4.1 ± 1.0	1.7 ± 0.41	1.2 ± 0.4
Surface applied unspiked manure	1.4 ± 0.6	5.4 ± 3.1	1.73 ± 0.41	1.72 ± 0.25
Incorporated antibiotic-spiked manure	7.0 ± 1.8	31.1 ± 14.5	2.0 ± 0.62	8.85 ± 4.89
Surface applied antibiotic-spiked manure	5.9 ± 2.5	28.3 ± 4.5	11.07 ± 4.26	25.77 ± 4.97
No manure/antibiotic control	3.7 ± 1.3	3.2 ± 1.3	1.42 ± 0.12	2.17 ± 0.80
Antibiotic-spiked soil	19.8 ± 1.4	34.7 ± 2.9	8.92 ± 3.66	27.81 ± 12.76

Table S3. Antibiotic concentration in soil sample extraction solutions (n=4).

Treatment	Soil with T85 Monensin content (ng mL^{-1})	Soil without T85 Monensin content (ng mL^{-1})	Soil with T85 Oxytetracycline content (ng mL^{-1})	Soil without T85 Oxytetracycline content (ng mL^{-1})
Incorporated unspiked manure treatment	13.4 ± 2.3	37.9 ± 14.9	1.1 ± 0.4	1.1 ± 0.2
Surface applied unspiked manure	3.0 ± 0.9	70.4 ± 35.4	0.9 ± 0.3	1.9 ± 1.7
Incorporated antibiotic-spiked manure	101 ± 25.4	140.9 ± 8.9	6.2 ± 3.0	8.3 ± 4.2
Surface applied antibiotic-spiked manure	60.8 ± 36.5	169.3 ± 10.5	6.3 ± 2.0	6.3 ± 2.0
No manure/antibiotic control	2.4 ± 1.6	1.2 ± 0.5	0.6 ± 0.1	0.5 ± 0.2
Antibiotic-spiked soil	174.5 ± 7.9	170.6 ± 4.4	3.8 ± 2.7	1.7 ± 0.8

Table S4. Antibiotic content per kg of plant tissue (n=4).

Treatment	Stem/leaf Monensin (µg kg^{-1})	Root Monensin (µg kg^{-1})	Stem/leaf Oxytetracycline (µg kg^{-1})	Root Oxytetracycline (µg kg^{-1})
Incorporated unspiked manure treatment	9.6 ± 1.6	17.8 ± 5.1	7.3 ± 2.0	5.3 ± 2.1
Surface applied unspiked manure	6.0 ± 3.0	23.0 ± 13.2	7.4 ± 2.0	7.4 ± 1.2
Incorporated antibiotic-spiked manure	29.9 ± 8.9	133.6 ± 71.8	8.5 ± 3.1	38.1 ± 21.0
Surface applied antibiotic-spiked manure	25.5 ± 10.7	145.5 ± 51.6	47.6 ± 21.1	110.8 ± 21.4
No manure/antibiotic control	15.8 ± 6.6	13.7 ± 6.3	6.1 ± 0.6	9.3 ± 4.0
Antibiotic-spiked soil	85.3 ± 7.1	149.1 ± 12.5	38.4 ± 15.7	119.6 ± 54.9

Table S5. Antibiotic content per kg of soil (n=4).

Treatment	Soil with T85 Monensin content (µg kg^{-1})	Soil without T85 Monensin content (µg kg^{-1})	Soil with T85 Oxytetracycline content (µg kg^{-1})	Soil without T85 Oxytetracycline content µg kg^{-1})
Incorporated unspiked manure treatment	33.6 ± 10.2	80.2 ± 25.8	3.0 ± 1.0	2.4 ± 0.4
Surface applied unspiked manure	7.1 ± 3.7	149 ± 75.1	2.0 ± 0.6	4.0 ± 3.0
Incorporated antibiotic-spiked manure	214 ± 53.9	298.5 ± 15.3	13.1 ± 5.2	17.6 ± 8.8
Surface applied antibiotic-spiked manure	128.8 ± 77.3	358.6 ± 18.1	13.3 ± 3.4	13.3 ± 4.3
No manure/antibiotic control	5.2 ± 2.8	2.5 ± 1.0	1.3 ± 0.3	1.0 ± 0.5
Antibiotic-spiked soil	369.5 ± 16.8	361.4 ± 9.4	8.1 ± 5.8	3.6 ± 1.4

References

1. Dolliver, H.; Kumar, K.; Gupta, S.; Singh, A. Application of enzyme-linked immunosorbent assay analysis for determination of monensin in environmental samples. *J.*

Environ. Qual. **2008**, *37*, 1220–6.