Accepted Manuscript

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PII: S0045-6535(18)30686-6

DOI: 10.1016/j.chemosphere.2018.04.046

Reference: CHEM 21194

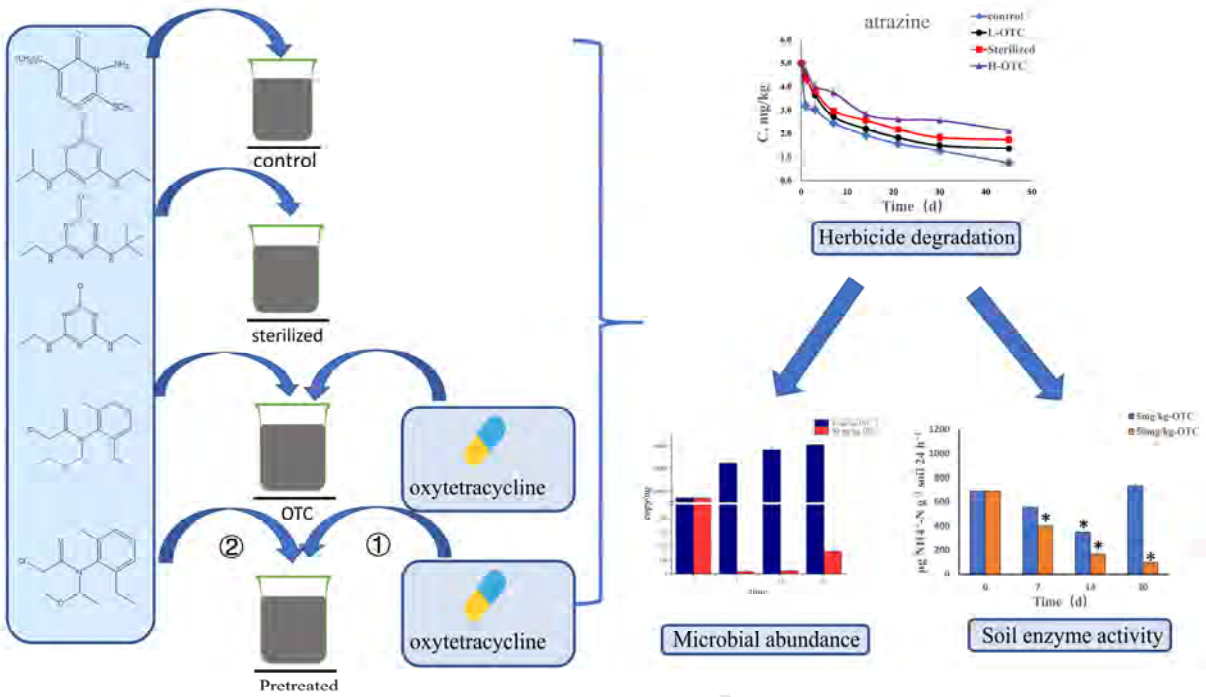
To appear in: *ECSN*

Received Date: 12 January 2018

Revised Date: 1 April 2018

Accepted Date: 8 April 2018

Please cite this article as: Jiang, W., Gao, J., Cheng, Z., Wang, P., Zhou, Z., Liu, D., The effect of antibiotics on the persistence of herbicides in soil under the combined pollution, *Chemosphere* (2018), doi: 10.1016/j.chemosphere.2018.04.046.



The effect of antibiotics on the persistence of herbicides in soil under the combined pollution

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1. Introduction

Antibiotics are widely used in public health and animal husbandry in the treatment and prevention of bacterial infections. The US Food and Drug Administration (FDA) reported that 14.6 million kilograms of annual domestic sales of antimicrobial drugs were used in food-producing animal husbandry in 2014 (FDA. 2014). Particularly, tetracyclines accounted for 71% of these sales. In many countries, the use of antibiotics both for animal disease treatment and growth promotion is unsupervised, which often leads to abuse of antibiotics. Most of veterinary antibiotics were poorly absorbed within the gastrointestinal tract of livestock, causing the presence of biologically active antibiotics in animal waste. It was not unusual to detect high concentrations of antibiotic residues in animal manures (Qiao et al., 2012; Pan et al., 2011;Nordenholt et al., 2016). In an agricultural field at Hannover, the tetracycline (TTC) level in the dried manure from the soil surface was 4 mg/kg (Gerd Hamscher, 2002). Similarly, Martinez analyzed pig manure and turkey dung samples from livestock farms in Austria, Vienna. They revealed that the content

of chlortetracycline (CTC), oxytetracycline (OTC), and TTC up to 46 mg/kg, 29 mg/kg and 23 mg/kg respectively (Martinez-Carballo et al., 2007). In China, the antibiotic residues in animal feces are also commonly detected. In a research work, tetracycline antibiotics in feces of livestock breeding farms were investigated in seven provinces. The average content of OTC, TTC and CTC was 5.9 mg/kg, 2.6 mg/kg and 1.3 mg/kg, respectively (ZHANG et al., 2005). Besides, the occurrence of veterinary antibiotics in organic vegetable bases in northern China were analyzed. Strikingly, the highest concentration of antibiotics was up to 183.5 mg/kg for OTC (Hu et al., 2010).

With the expanding of animal husbandry, tons of livestock manure were produced. China produces an estimated 618 billion kilograms of swine manure annually (Wang et al., 2006). Most of them were spread into farmland as fertilizer, which were desired practices for recycling nutrients and waste disposal. However, manure is a major source of antibiotic pollution in the environment. Farm land application of manure as a soil fertility amendment can introduce antibiotics into the environment directly (Brown D et al., 2015). tetracyclines were detected in the soil form protected vegetable fields in Shandong province and the detectable rate was 100%. The total concentration of tetracyclines ranged from 0.27 to 1.01 mg/kg (YIN et al., 2012). Besides, it was reported that the concentrations of tetracyclines ranged from 4.54 to 24.66 mg/kg in the soils collected from multiple feedlots in Shanghai (Ji et al., 2012). Repeated use of antibiotic containing manure in farmland resulted in accumulation of antibiotic residues in the soil.

Although pesticides play an important role in agricultural production, they also lead to environmental contamination. Generally, rational farming application will minimize unintended environmental impacts of pesticide (Asare, 2000). Pesticide dissipation includes hydrolysis,

photolysis, dealkylation, dehalogenation and other chemical and biological processes (Fantke and Juraske, 2013). Environmental factors such as temperature, moisture, illumination intensity and pH value influence the dissipation of pesticides. Biodegradation is an important pathway and it can be influenced by antibiotics. Therefore, antibiotic–pesticide combined pollution complicated the situation. In previous reports, Ostrofsky found that antibiotics and their metabolites could become carbon or nitrogen sources of microorganisms and promote soil microorganisms growing, subsequently enhancing herbicide dissipation percentages in soil (Ellen B. Ostrofsky, 1997). On the contrast, Accinelli found that the sulfamethazine (SMZ) in sandy loam soil had no significant influence on metolachlor dissipation at level of micrograms per kilogram (Accinelli et al., 2006). Furthermore, Kim found that in the sand which was pretreated with monensin, narasin, and salinomycin, atrazine concentrations and its half-lives were conspicuously greater (Kim et al., 2010). More research was needed to get a clear understanding of antibiotic–pesticide interactions.

Chloroacetanilide herbicides and triazine herbicides were widely used in weed control. Nevertheless, ecotoxicological data have suggested that some chloroacetanilide herbicides can cause DNA damage and tumor induction in fish, rat and human cells (Ateeq et al., 2005). Besides, triazine herbicides such as atrazine, simazine, metribuzin and terbumeton were listed as potential environmental endocrine disruptors by the United States Environmental Protection Agency (EPA) in 2012 (EPA, 2012). Oxytetracycline (OTC) is a typical kind of tetracyclines interfering with the syntheses of proteins of microorganisms, which has been widely detected in farm animal manure, water and soil (Chen et al., 2014). Some dissipation products of tetracyclines are toxic to environmentally relevant bacteria (Halling-Sorensen et al., 2002). Pesticides and antibiotics coexist in agricultural environment commonly owing to manure fertilizer application or

wastewater irrigation. It is necessary to evaluate the fate of herbicides under the combined pollution circumstance.

In this study, OTC was used as a model antibiotic to examine the effect on dissipation of herbicides coexisting with antibiotic. Besides, the dissipation in the soils pretreated with OTC for 30 days were detected to estimate the persistence of inhibiting effect. Additionally, the soil enzyme activity and soil microbe were investigated to figure out the soil property changes caused by antibiotics. We hope to provide a better understanding of environmental behavior of herbicides under the combined pollution circumstance.

2. Material and method

2.1 Chemicals and materials

Oxytetracycline standard (98%) were purchased from Sigma−Aldrich Corp. (St. Louis, MO), Atrazine, simazine, metribuzin, terbuthylazine, acetochlor, metolachlor were obtained from Institute for Control of Agrichemicals, Ministry of Agriculture of China (Beijing, China). Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, USA). Water was purified with a Millipore purification system. All the mobile phase eluents were used after being filtrated and deaerated. All the other chemicals and solvents were analytical grade and purchased from Beijing Chemical Reagent Company Limited (Beijing, China). We collected soil from Baiwang Mountain Forest Park (Beijing, China) at depth of 10 cm on the surface (clay, 54.2%; silt, 22.6%; sand, 23.2%; and pH, 8.1), which belong to histosols (FAO World Reference Base, USDA Soil Taxonomy). Soil total organic carbon (TOC) content was determined by dichromate oxidation. The TOC was 36.60 g/kg. Baiwang Mountain Forest Park (Beijing, China) is far away from agricultural areas and we detected the contamination of the soil. It was no relevant pesticide

residue. Soil was air-dried at room temperature and sieved (2 mm) before use.

2.2 Incubation experiment

Air-dried and sieved soil samples were incubated in 500-mL beakers under laboratory conditions with six treatments, and the experimental design was shown in Table S1. An incubator was used to keep the incubation at 25°C under a light/dark regime of 12:12. An aliquot of 500 g of soil was added with the mixed herbicide standard solution (5000 mg/L, acetone, including atrazine, simazine, metribuzin, terbuthylazine, acetochlor, and metolachlor) to achieve a final concentration of each one herbicides of 5 mg/kg. For the sterilized treatments, soil was autoclaved at 120 °C for 60 mins and sterile water was used to adjust moisture at 25%, before and during the incubation period. For L-OTC and H-OTC treatments, soil was added with OTC and herbicides simultaneously. For Pre-L-OTC and Pre-H-OTC treatments, soil was added with OTC at first, after 30 days the herbicides were added. The concentrations of OTC were tested at 5 mg/kg and 50 mg/kg, representing actual level in a field with manure application and relatively high level in the land with a higher frequency of manure application.

After the reagents were added, the soil samples were stirred evenly. Purified water was used to adjust and maintain soil moisture at 25%, before and during the incubation period. Three repetitions were conducted. The samples were collected at 1,3,7,10,14,21,30,45 days and stored at -20°C until further analysis. The analysis of herbicide, soil enzyme and microbial were conducted in the same soil incubation pots.

2.3. Extraction and analysis

2.3.1 Extraction of the herbicides

The soil samples were subjected to homogenization, and 5 mL of acetonitrile and 2 mL of

111 purified water were added to 2 g of soil in a 15-mL polypropylene centrifuge tube. The sample
112 was vortexed vigorously for five minutes and then centrifuged at 3500 rpm for 5 mins. Collect the
113 supernatant and the same extraction step was repeated once. The combined supernatant was
114 concentrated to dryness under the dry nitrogen flow, then dissolved in 1 mL of acetonitrile and
115 passed through a 0.22 mm filter. Dilute the extract 10 times for analysis by HPLC-MS/MS as
116 described below.

117 2.3.2 Extraction of OTC

118 Extraction of OTC referred to a previous work (Gerd Hamscher, 2002). Briefly, 5 mL of ethyl
119 acetate and 1.2 mL of EDTA-Mcllvaine (pH=4) were added to 1 g of sample in a 15-mL
120 polypropylene centrifuge tube. The sample was vortexed and centrifuged. The supernatant was
121 collected and the same extraction step was repeated once. The combined supernatant was
122 concentrated and redissolved by acetonitrile, then passed through a 0.22 mm filter and analyzed by
123 HPLC-MS/MS.

124 2.4 Instrument conditions

125 The herbicides and oxytetracycline were determined using UHPLC UltiMate 3000 system
126 coupled to a TSQ Quantum Access Max (Thermo Scientific, San Jose, CA, USA) in the selected
127 reaction monitoring (SRM) scan mode. The following conditions were used: spray voltage at
128 3500V for positive polarity, capillary temperature at 350 °C, vaporizer temperature at 300 °C,
129 sheath gas pressure at 40 psi, aux valve flow at 3.3 L/min, Q_2 collision gas pressure at 1.5 mTorr.
130 Quantification ion pair and other optimized conditions of HPLC/MS-MS were shown in Table S2.
131 HPLC/MS-MS chromatograms of herbicides in select reaction monitoring mode were shown in
132 Figure S1.

Herbicides were separated with Eclipse XDB-C_{18} column (4.6 x 150 mm, 5 μm) at 25°C. A binary mobile phase in isocratic elution mode composed of acetonitrile/water 90/10 (v/v). The flow rate was 0.5 mL min^{-1} and injection volume was 5 μL. OTC was analyzed with Hypersil GOLD AQ-C_{18} (150×4.6 mm, 5 um). Mobile phase was acetonitrile/water (10/90).

2.5 **Method validation**

Blank soil spiked with the herbicides at 0.5, 1.0, and 5.0 mg/kg were used for recovery test. The extraction steps are the same as described above. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on a signal-to-noise ratio of 3:1 and 10:1, respectively. Linear curve was generated by plotting peak area versus the concentration over the range of 20–1000 μg L^{-1} in the matrix standard solution. The precision of the method for all the chemicals was measured by three replicates.

The details were shown in the table S3. The recoveries of all the target analytes ranged from 72.3% to 113.0%. Relative standard deviation (RSD) ranged from 2.0% to 11.2%. Good linearities were obtained in 20-1000 μg/L in the matrix standard solution. Correlation coefficients were between 0.9902-0.9966. The limit of detection (LOD) was 4.3-71.4 ng/kg, and the limit of quantification (LOQ) was 14.3-238.1 ng/kg.

2.6 Soil enzyme activity

Dehydrogenase activity was determined by the reduction of triphenyltetrazolium chloride (TTC) to triphenylformazone (TPF), expressed as μg TPF g^{-1} soil 24 h^{-1} (Xie et al., 2017). Urea was used as the substrate to detect the activity of urease and activity was expressed as μg NH_4^+-N g^{-1} soil 24 h^{-1} (Wei et al., 2017). To analyze the activity of catalase, hydrogen peroxide was used as the substrate to detect the activity of catalase, reacting with the soil for 20 mins. Then measure

the absorbance changes and calculate catalase activity, expressed as μmol $H_2O_2 \cdot g^{-1}$ soil • 24 h^{-1} (Xu et al., 2018).

2.7 Real-time fluorescence quantitative PCR detection of microbial biomass in soil

Total DNA of soil was extracted with a SoilGen DNA Kit (cwbiotech, Beijing, China) from an aliquot of 0.3 g dry weight frozen samples (-80℃). The soil sample DNA concentration was obtained by reading the plate with Nanodrop 2000 (Thermo Fisher Scientific, USA). Purified DNA samples were stored at -20℃ for further use (Cao et al., 2017). To determine the microbial biomass, fungal 18S rRNA genes and bacterial 16S rRNA genes were measured. Bacteria forward primer was Bac331 and reverse primer was Bac797. Forward and reverse primer of fungus was ITS1f and 5.8s. (Supporting Information table S6). Reactions were run in triplicate. The thermal cycling condition was set as 1 cycle of 95 °C for 10 mins for denaturation, 40 cycles of 95 °C for 15 s and 60 °C for 1 mins for annealing and extension, respectively.

2.8 Data analysis

Dissipation data of the herbicides were fitted by first-order kinetic equation ($C= C_0e^{-kt}$). The half-life was calculated by the equation $T_{1/2}$=ln2/k. Summary of kinetic formulas of herbicides dissipation showed in Table 1. In the process, SPSS 21.0 was used to conduct statistical evaluations of the half-life. Each experiment was repeated three times and the values of half-life were the means over them. The significant differences between groups were evaluated by the one-way analysis of variance (ANOVA, $p < 0.05$) in half-life values of three repetitions. The comparison was based on the method of Tukey's range test.

3.Results and Discussion

3.1 Dissipation of the herbicides in presence with OTC

Figure 1 represented the influences of OTC on the dissipation of the six herbicides. Among the trazine herbicides, metribuzin dissipated fastest in the soil. The half-life of metribuzin was 9.5 days in control soil, while it was prolonged to 14.4 days and 43.3 days with the presence of OTC at 5 mg/kg and 50 mg/kg respectively. The half-life increased more than 4 times affected by 50 mg/kg of OTC. In the sterilized soil the half-life was 21.6 days. Atrazine, simazine and terbuthylazine, with similar chemical structures, had longer persistence with half-lives of 19.8, 23.9 and 23.9 days in control soil. In sterilized soil, half-lives of atrazine, simazine and terbuthylazine were 30.1, 27.7 and 30.1 days respectively. With the presence of OTC at 5 mg/kg, small increase in the half-lives was found, which were 24.7, 26.6 and 27.7 days for atrazine, simazine and terbuthylazine. When the level of OTC was 50 mg/kg, the half-lives of atrazine, simazine, and terbuthylazine increased to 38.5, 34.6 and 34.6 days, which indicated that the dissipation was significantly inhibited. Those results were similar with that Kim reported. In the sand pretreated with antibiotic, persistence of atrazine was greater (Kim et al., 2010).

Compared with triazine herbicides, chloroacetanilide herbicides had shorter half-lives. In control group, the half-lives of acetochlor and metolachlor were 6.9 days and 11.7 days respectively, and those were 10.8 days and 21.0 days in sterilized soil. With the presence of OTC at 5 mg/kg, the half-life of acetochlor was 7.2 days, which almost had no change, while the half-life of metolachlor was increased significantly to 18.2 days. The half-lives of acetochlor and metolachlor were 21.6 days and 43.3 days in the soil with OTC at 50 mg/kg, showing the dissipation was dramatically inhibited. In a previous research, Accinelli found that at level of

197 micrograms per kilogram of antibiotic had no significant influence on metolachlor dissipation
198 (Accinelli et al., 2006). Therefore, the inhibiting effect depended on concentration. With the
199 combined pollution, especially at high concentration, the persistence of the herbicides was
200 significantly prolonged, indicating potential increasing risks.
201
202 The dissipation percentages of the herbicides after 45 days were shown in figure 2. In control
203 group, the dissipation percentages of all the tested herbicides were over 75%. Residual
204 concentrations of chloroacetanilide herbicides were much lower than triazine herbicides in general.
205 When OTC were spiked at 5 mg/kg, the dissipation percentages of atrazine, simazine,
206 terbuthylazine, and metolachlor decreased significantly. But the influence on metribuzin and
207 acetochlor could be neglected. With the presence of OTC at 50 mg/kg, the dissipation percentages
208 of all the tested herbicides decreased significantly. After the 45-day exposure, 94.8% of metribuzin
209 was dissipated, while in the soil with 50 mg/kg OTC only 53.8% was dissipated. And the
210 dissipation percentages of atrazine, metribuzin, acetochlor, and metolachlor were even lower than
211 those in sterilized group. The final residues of atrazine, simazine, and terbuthylazine in the soil
212 with 50 mg/kg OTC was 2.12 mg/kg, 1.70 mg/kg and 1.72 mg/kg, which were significantly higher
213 than the corresponding part in control soil. High concentration of antibiotic pollution may lead to
214 high terminal residue of the herbicides. Those results showed the dissipation of the herbicides was
215 inhibited with the presence of OTC, especially with high concentration of OTC.
216 **3.2 Dissipation of the herbicides in soil pretreated with OTC**
217 Repeated use of manure in farmland may cause the “pseudo persistence” of antibiotics. In
218 order to study the durability of the inhibitory effects of OTC on the herbicide dissipation described

above, the soils were pretreated with OTC for 30 days, then the dissipation of the herbicides was monitored. The dissipation of OTC in the soil was also investigated, and the dissipation kinetic equation was $y = 3.5044e^{-0.039x}$, (R^2=0.9812) with the half-life of 17.7 days (Figure S2). The dissipation kinetic curves of herbicides were shown in Figure 3 and the dissipation percentages after 45 days were shown in Figure 4. In the soils pretreated with 5 mg/kg OTC for 30 days, the half-life of metribuzin was 12.6 days. There was no significant influence compared to 11.2 days in control group. Similarly, the half-life of the other selected herbicides was almost uninfluenced. And dissipation percentages of the herbicides did not change significantly（Figure 4.）. However, in the soils pretreated with 50 mg/kg of OTC for 30 days, the dissipation of the selected herbicides was significantly inhibited, and the dissipation percentages decreased. Especially, the half-life of metolachlor increased from 11.7 days to 21.7 days, and the dissipation percentage decreased from 93.1% to 79.4% after 45 days. Those results showed there was durability of the inhibitory effects to some extent. Once high concentration of OTC polluted farm land, the inhibitory effects on the herbicide dissipation may last for a month at least. Conversely, the relatively fast dissipation of OTC resulted in no effects at 5 mg/kg OTC, suggesting that any inhibition of herbicide dissipation was short-lived and only high concentrations of OTC would cause a persistent effect.

3.3 The effects of OTC on soil

3.3.1 Effects of OTC on soil enzyme activity

Soil enzyme activity has been widely used as an indicator to measure the ecological health of terrestrial ecosystems which may be influenced by xenobiotic contaminants. Soil samples were conducted from group L-OTC and H-OTC. Figure 5 showed the impact of OTC on soil enzymes.

With the presence of OTC at 5 mg/kg, soil urease and catalase were affected. Soil urease activity was inhibited and touched the bottom after two weeks. After that, it recovered gradually. Finally, urease activity fully recovered after 30 days. Similarly, soil catalase activity went all the way down to the lowest point at the 7th day then recovered. However, soil dehydrogenase activity was unaffected. In a previous work, researchers found 3.6 mg/kg of OTC had no discernible effect on the dehydrogenase activity within 28 days. Urease activity decreased at the 14th day then recovered gradually, which was similar to our result (Ma et al., 2016). When the concentration of OTC increases to 50 mg/kg, the activity of urease, dehydrogenase, and catalase decreased significantly at the 7th day. The negative effect was stronger. Throughout the trial period, the urease activity reduced continuously. The catalase activity kept at a lower level. But dehydrogenase activity recovered soon after 14 days and showed an activation of enzyme activity at the 30th day.

3.3.2 Effects of OTC on the abundance of total bacteria and fungi

The influence of OTC on the abundance of total bacteria and total fungi in soil was shown in Figure 6. Soil samples were conducted from group L-OTC and H-OTC. The abundance of total bacteria was relatively insensitive to OTC application. With the presence of OTC at 5 mg/kg, the copy number of soil bacteria in monitoring points increased gradually. While the copy number of soil fungi decreased significantly at the 7th day then recovered gradually. Soil fungi were more sensitive to OTC than bacteria. Gao reported similar result. They investigated the interactive effect of oxytetracycline on soil microbial biomass and found that at the same level of OTC contamination, inhibition rate of bacteria was -12.48% while inhibition rate of fungi was -82.99%

(Gao et al., 2013). With the increasing concentration of OTC, the negative effect on soil microbial became obvious. With the presence of OTC at 50 mg/kg, the copy number of soil bacteria and fungi decreased rapidly at the 7th day. During the 30-day period, bacteria and fungi did not recover and restrained at a significant low level. Those results indicated that OTC addition could disturb the soil microbe. OTC may promote soil bacterial growth at the relative low level. When the concentration increased, the inhibiting effect became greater. Nevertheless, soil fungi were sensitive to OTC. A low concentration of OTC would reduce the abundance of soil fungi.

4. Conclusion

The impacts of OTC on the dissipation of triazine and chloroacetanilide herbicides under combined pollution were investigated. High concentrations of OTC inhibited the dissipation rate of the herbicides significantly, increasing the persistence in soil, and the inhibition effects still existed after 30 days in the OTC pretreated soil. OTC affected the activity of soil urease, catalase, and dehydrogenase especially at high concentration. In terms of soil microorganism, bacteria and fungi were also influenced by OTC, and fungi were more sensitive. When biodegradation was the predominant contributors to dissipation of pesticide, OTC influenced their dissipation and the impacts increased with OTC concentration level. For those the dissipation included the other processes like chemical dissipation or photodegradation, and did not mainly depend on microbial dissipation process, the effects of antibiotics on the dissipation were less affected. OTC might decrease the dissipation rate of herbicide through inhibiting soil enzyme and reducing microbial abundance, leading to high terminal residues of herbicides. These results indicate that for herbicides degraded by soil microorganisms, the presence of antibiotics may increase their

285 persistence, but the effects are temporary and concentrations dependent. This could result in
286 greater losses of herbicides to water resources or cause carry over problems for the next year's
287 crop. The issue of co-application of antibiotics and herbicides on cropland is a significant concern
288 and the methods that reduce antibiotic in land-applied manure would help reduce these risks.
289

290 ## Acknowledgement

291 This work was supported by the National Natural Science Foundation of China
292 (Contract Grants: 21337005, 21677175) and by Chinese Universities Scientific Fund
293 2017LX001.
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Figure captions

Figure 1. The dissipation curves of the herbicides in the soils with OTC combined pollution

Figure 2. The dissipation percentages of the herbicides after 45 days

(The asterisk indicate that the means are significantly different from the means of CK soil)

Figure 3. The dissipation of the herbicides in the soils pretreated with OTC for 30 days

Figure 4. The dissipation percentages of the herbicides after 45 days. (The asterisk indicates that the means are significantly different from those in CK soil.)

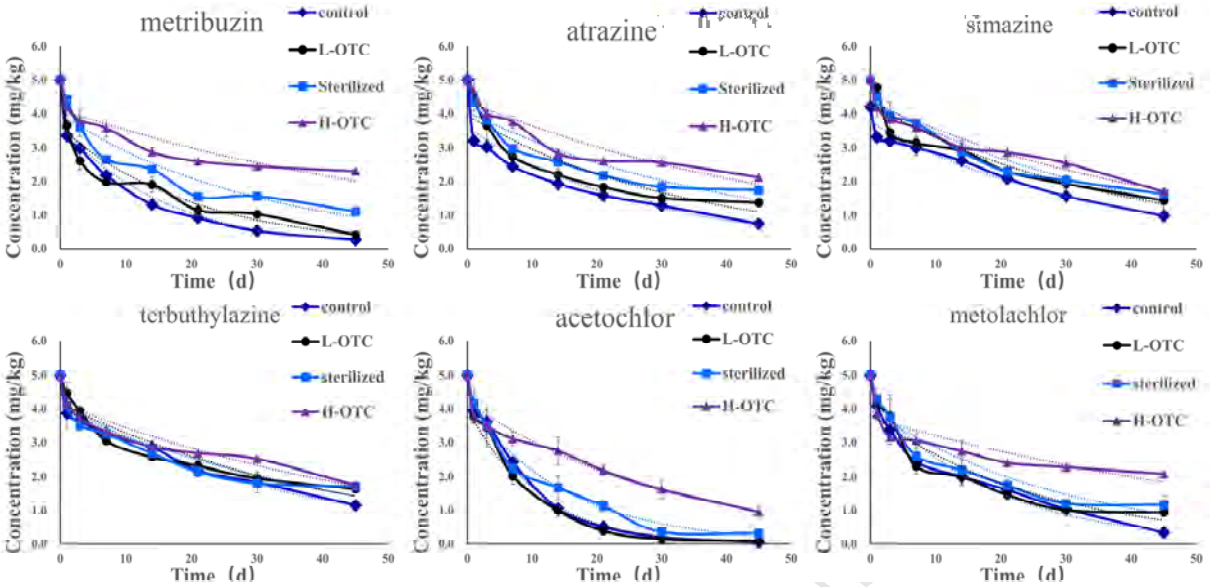
Figure 5. Effects of OTC on soil enzyme activity. (A) Urease, (B) Catalase, (C) Dehydrogenase

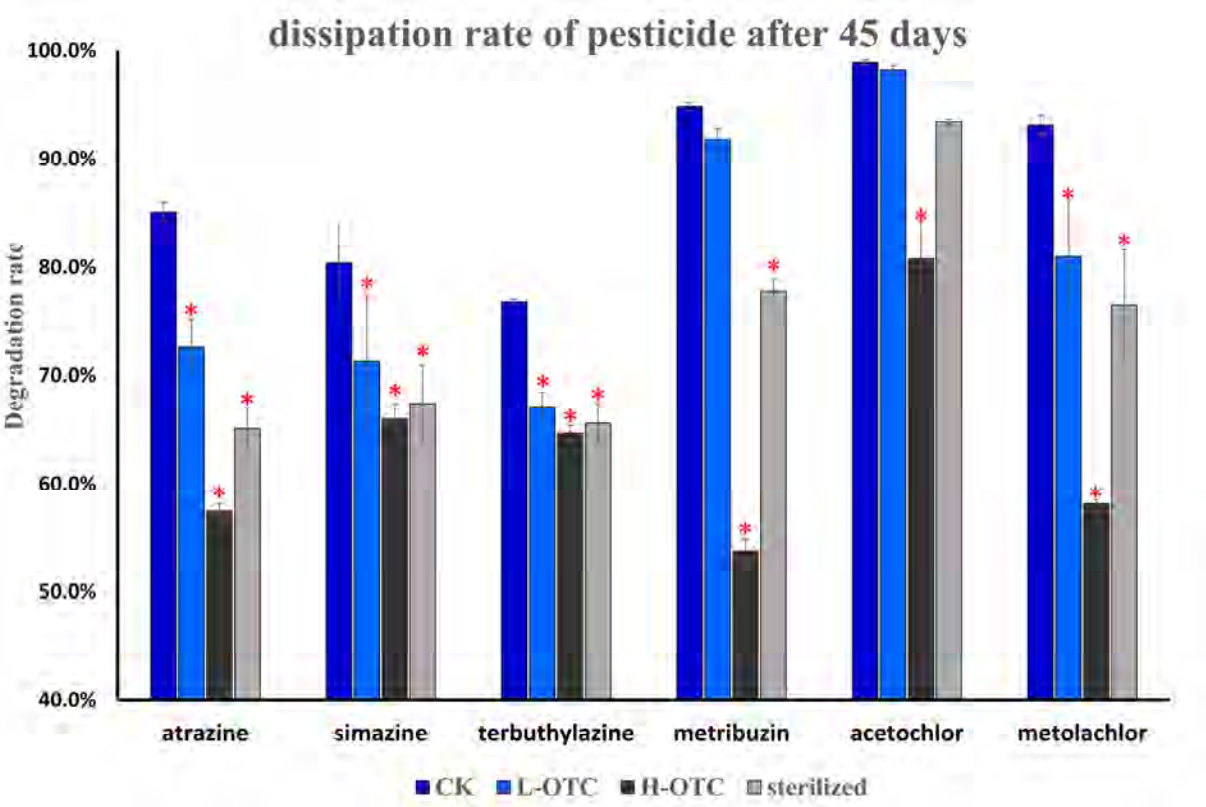
Figure 6. Effects of OTC on soil microbial quantity. (A.bacterial abundance, B. fungal abundance)

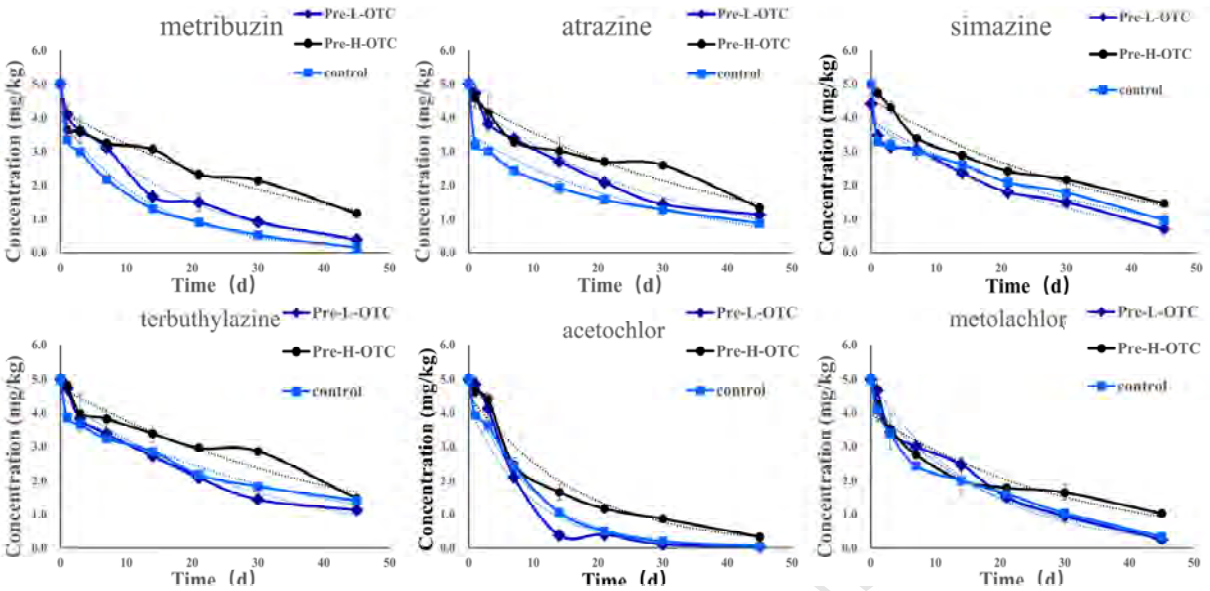
(The asterisk indicates that the means are significantly different from those in initial soil.)

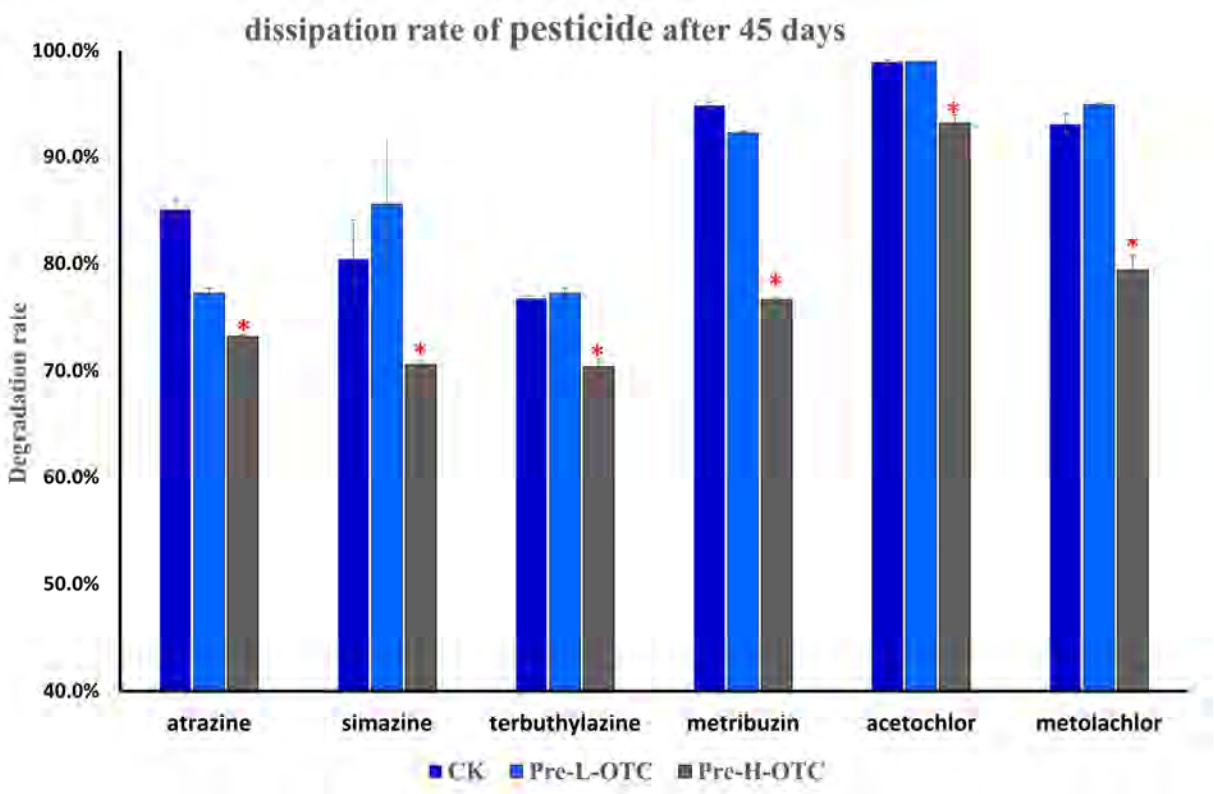
Table 1. Summary of kinetic formulas of herbicides degradation

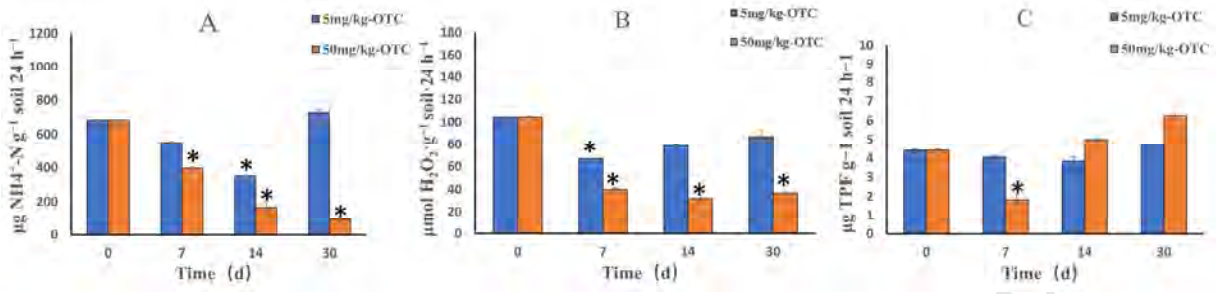
Group \ Herbicide	atrazine	simazine	terbuthylazine	metribuzin	acetochlor	metolachlor
CK	$y = 3.4711e^{-0.035x}$	$y = 3.744e^{-0.029x}$	$y = 4.2176e^{-0.029x}$	$y = 3.6843e^{-0.062x}$	$y = 4.7266e^{-0.102x}$	$y = 4.5378e^{-0.059x}$
	$R^2 = 0.9625$	$R^2 = 0.9794$	$R^2 = 0.9714$	$R^2 = 0.9769$	$R^2 = 0.9969$	$R^2 = 0.9558$
L-OTC	$y = 3.9372e^{-0.028x}$	$y = 4.2649e^{-0.026x}$	$y = 4.2068e^{-0.025x}$	$y = 3.5787e^{-0.048x}$	$y = 4.0203e^{-0.096x}$	$y = 3.8444e^{-0.038x}$
	$R^2 = 0.8637$	$R^2 = 0.9293$	$R^2 = 0.9209$	$R^2 = 0.9328$	$R^2 = 0.9645$	$R^2 = 0.8867$
H-OTC	$y = 4.3541e^{-0.018x}$	$y = 4.3412e^{-0.02x}$	$y = 4.1877e^{-0.02x}$	$y = 4.1152e^{-0.016x}$	$y = 4.2029e^{-0.032x}$	$y = 3.7493e^{-0.016x}$
	$R^2 = 0.8752$	$R^2 = 0.9488$	$R^2 = 0.916$	$R^2 = 0.8239$	$R^2 = 0.9734$	$R^2 = 0.7484$
sterilized	$y = 4.0325e^{-0.023x}$	$y = 4.4049e^{-0.025x}$	$y = 4.1503e^{-0.023x}$	$y = 3.9907e^{-0.032x}$	$y = 4.1854e^{-0.064x}$	$y = 3.9655e^{-0.033x}$
	$R^2 = 0.8568$	$R^2 = 0.9497$	$R^2 = 0.8869$	$R^2 = 0.8985$	$R^2 = 0.9407$	$R^2 = 0.8861$
Pre-L-OTC	$y = 4.686e^{-0.039x}$	$y = 3.8877e^{-0.036x}$	$y = 4.686e^{-0.039x}$	$y = 4.4075e^{-0.054x}$	$y = 4.0851e^{-0.109x}$	$y = 4.9667e^{-0.062x}$
	$R^2 = 0.9856$	$R^2 = 0.9769$	$R^2 = 0.9856$	$R^2 = 0.9865$	$R^2 = 0.9452$	$R^2 = 0.9771$
Pre-H-OTC	$y = 4.5665e^{-0.025x}$	$y = 4.4759e^{-0.026x}$	$y = 4.5665e^{-0.025x}$	$y = 4.2086e^{-0.027x}$	$y = 4.6143e^{-0.061x}$	$y = 3.9641e^{-0.032x}$
	$R^2 = 0.932$	$R^2 = 0.942$	$R^2 = 0.932$	$R^2 = 0.9401$	$R^2 = 0.9562$	$R^2 = 0.916$

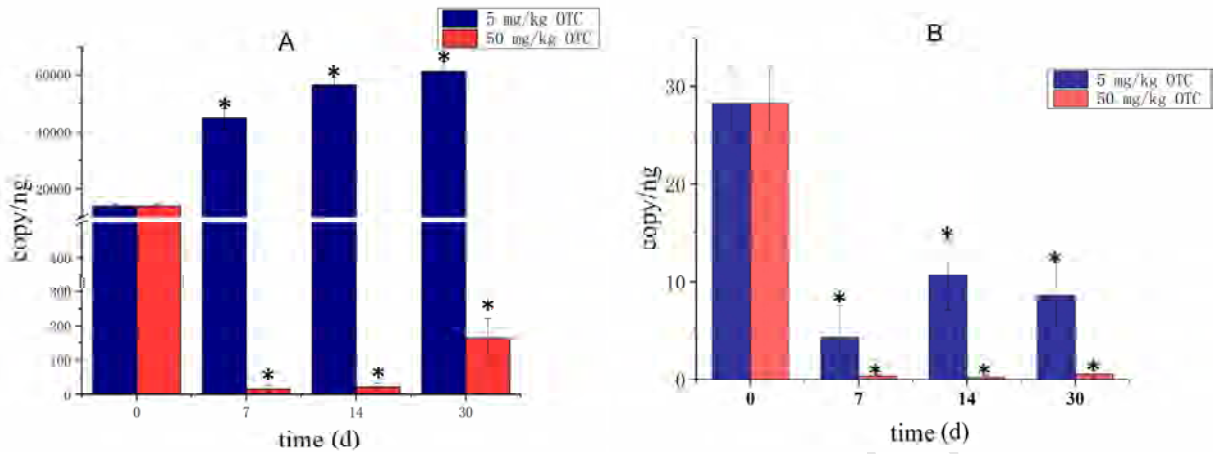












Highlights:

1. Co-application of herbicides and antibiotic containing manure resulted in greater herbicide persistence.
2. Oxytetracycline reduced the abundance of total bacteria and fungi in soil.
3. Oxytetracycline may decelerate the degradation of the herbicides through affecting soil microorganism and enzyme.