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Individual and combined effects of enrofloxacin and cadmium on soil microbial biomass and the ammonia-oxidizing functional gene

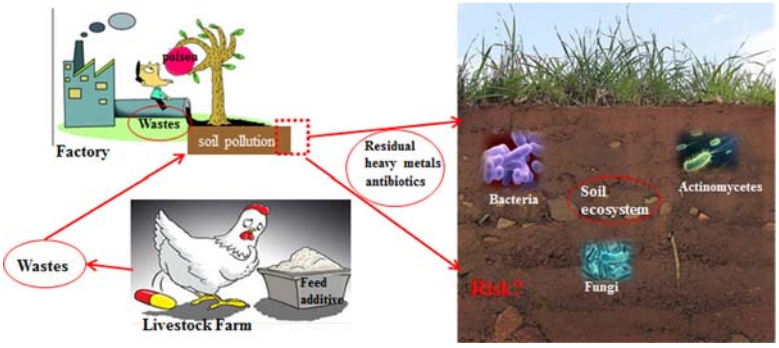
Jinhua Wang *, Lanjun Wang, Lusheng Zhu, Jun Wang

College of Resources and Environment, Key Laboratory of Agricultural Environment in Universities of Shandong, Shandong Agricultural University, Taian 271018, PR China

HIGHLIGHTS

- Soil microbes as indicator to indicate pollution of antibiotics and heavy metals.
- Used amoA gene to research the relative quantities of AOA and AOB in soil.
- Single and combined pollution of ENR and Cd have inhibited the microbial activity.
- There was obvious dose-effect relationship of pollutants on microbes.
- Toxicity of combined ENR and Cd was greater than ENR acting alone.

GRAPHICAL ABSTRACT



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ABSTRACT

The negative effects of residues from antibiotics and heavy metals in agricultural soils are becoming an increasingly frequent concern. To evaluate the toxicity and interaction of antibiotics and heavy metals, enrofloxacin (ENR) and cadmium (Cd) were used as targets to study the individual effects of ENR (0.025, 0.1, 0.4 mmol/kg) and Cd (0.4 mmol/kg) and their combined effects (mole ratios of ENR to Cd of 1: 1, 1: 4 and 1: 16) on soil microbial biomass and function on days 7, 14, 21 and 28 of the study. The results demonstrated that microbial populations, which were counted during 4 sampling periods, were mainly in the order of bacteria > actinomycetes > fungi. The ammonia monooxygenase (*amoA*) gene copies of ammonia-oxidizing archaea (AOA) were more abundant than ammonia-oxidizing bacteria (AOB) on days 14 and 21. Soil bacteria, fungi, and actinomycetes numbers and *amoA* gene abundances of AOB and AOA in soils were inhibited to varying degrees by the single and combined effects of ENR and Cd; the higher the concentration of the treatments, the stronger the inhibition. The combined toxicity of ENR and Cd on soil microbes and AOA- and AOB-*amoA* genes was stronger than when either chemical was used alone; the interaction effects of ENR and Cd were mainly antagonistic. Moreover, the ratios of bacteria/fungi declined significantly on days 14, 21 and 28; the proportions of AOA- and AOB-*amoA* were altered with the addition of ENR and Cd. Thus, ENR and Cd had significant negative effects on the soil microbial community, especially when both contaminants were present.

1. Introduction

Enrofloxacin (ENR), as a kind of animal antibacterial drug, is widely used in the treatment of animal infectious diseases (Anirudhan et al., 2017; Liu et al., 2009; Liu et al., 2015). However, antibiotics are not

* Corresponding author at: College of Resources and Environment, Shandong Agricultural University, 61 Daizong Road, Taian 271018, PR China.
E-mail addresses: wjh@sdau.edu.cn (J. Wang), lushzhu@sdau.edu.cn (L. Zhu), jwang@sdau.edu.cn (J. Wang).

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completely absorbed and metabolized by animals; 25%–75% of them are discharged into the environment with the original drug or metabolites (Leston et al., 2014). In addition, in the process of antibiotic production, the residual antibiotics in the waste water and residue of the pharmaceutical factory will directly or indirectly enter the soil. Hence, substantial uses of ENR in intensive livestock farming and in clinical trials and health care have caused antibiotic contamination in soils (Lillenberg et al., 2010). Additionally, residual cadmium (Cd) in the environment is a contaminant that is highly toxic (Li et al., 2014). Cd pollution enters the soil mainly through the following two routes: geological weathering is the main natural source of cadmium in soil (Khan et al., 2017); industrial waste, wastewater irrigation, mining, the use of municipal solid waste, sludge, heavy metal and pesticide are the main anthropogenic sources of cadmium (Cheng et al., 2014; Lalor, 2008; Wu et al., 2010). High concentrations of Cd can have toxic effects on soil biodiversity that can easily be transferred to vegetation and eventually into the food chain (Pan and Yu, 2011; Rehman et al., 2017). In addition, Cd can cover a large area and has little mobility in soil; it is also not easily leached with water and may pollute crops, agricultural products, surface water and groundwater (Martins et al., 2014). In addition to these impacts, antibiotics and heavy metals often coexist in soil (Zhou et al., 2015; Gao et al., 2015). The interactions of antibiotics and heavy metals in soil ecosystems make their contamination more widespread and complex.

Research on the interactive effects of organic pollutants and heavy metals on environmental organisms has been extensively reported (Bao et al., 2013; Gao et al., 2013). Many studies found that antibiotics and heavy metals individually or in combination can affect soil organism (especially soil microbes) survival and reproduction, metabolism, population, biomass, community structure and biodiversity. Antibiotics and heavy metals have inhibitory or killing effects on microbes, affecting soil nutrient cycling and self-purification abilities (Chibuike and Obiora, 2014; Lotti et al., 2012; Liu et al., 2016; Mertens et al., 2010). Organic pollutants and heavy metals may form a heavy metal-organic complex, which can change the physical and chemical behavior of organic pollutants and heavy metals in the soil (Kong et al., 2006). Hence, it is of great practical significance to study the combined effects of heavy metals and antibiotics on soil ecology.

In soil ecosystems, microbes play important roles in organic matter decomposition, nutrient cycling and plant nutrient utilization (Geisseler et al., 2010). Soil microbes can be indicators of soil fertility, quality and health, which are sensitive to antibiotics and heavy metals (Paul, 2014; Djukic et al., 2013). Meanwhile, ammonia oxidation is the first step in the nitrification process, and it is a key step in the biological cycling of nitrogen; ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) are the main participants in ammonization in the natural environment (Di et al., 2010; Zhang et al., 2012). The ammonia monooxygenase a-subunit (*amoA*) gene, which can be used as molecular marker in ammonia-oxidizing bacteria and archaea, has been detected in many environments (Morimoto et al., 2011; Limpiyakorn et al., 2011). The *amoA* gene can not only reflect the type of species, quantity, and activity of ammonia-oxidizing microbes in the environment but also analyze the distribution of ammonia-oxidized microbes, community structure differences and evolutionary relationships. At present, many scholars are using *amoA* gene copy abundances to study the relative quantities of AOA and AOB in environmental samples (Chen and Gu, 2017; Cotta et al., 2014).

The present study selected ENR and Cd as target pollutants that have high detection rates and large carrying capacities in soil and microbes as the research object to evaluate the effects of single and combined toxicity of antibiotics and heavy metals on soil microbial ecology. Populations of soil bacteria, fungi, and actinomycetes and functional gene expression abundance in soils were studied in this paper. It is hoped that this research will suitably evaluate the ecological toxicity of ENR and Cd to provide important information for evaluating soil environmental quality.

2. Materials and methods

2.1. Chemicals and reagents

Enrofloxacin lactate (99% purity) was obtained from Hetian Biotechnology Co. Ltd. (Zhengzhou, China). $CdCl_2$ (99% purity) was obtained from Yongda Chemical Reagent Co. Ltd. (Tianjin, China). The soil DNA Kit (OMEGA, USA) was obtained from Sangon Biological Engineering Technology and Service Ltd. (Shanghai, China). The SanPrep® quick PCR purification kit, TIAN prep Mini Plasmid Kit and 2 × Pfu Master Mix were obtained from Tiangen Biotech Ltd. (Beijing, China). The Ultra SYBR mixture (with ROX) was obtained from Beijing CW Biotechnology Co. Ltd. (Beijing, China). The PMD18-T carrier and 6 × loading buffer were obtained from Takara Biotechnology Ltd. (Dalian, China). All other chemicals and reagents used in the following experiments were analytical grade and purchased from Beijing Chemical Co. (Beijing, China).

2.2. Soil

The brown soil used in the present experiment was collected from the test field of Shandong Agricultural University (Taian, China). Test soil was sampled from a depth of 1–20 cm, and then it was preincubated for 7 days in a 25 °C incubator. The physical and chemical characteristics of the test soils are as follows: pH 6.50, organic matter 17.6 g/kg, organic nitrogen 132.3 g/kg, available phosphorus 16.5 mg/kg, available potassium 125.7 mg/kg, and maximal water holding capacity of 18.9%, powder 57.2%, clay 10.4%, sand 32.4%.

2.3. Soil treatment with ENR and Cd

Three treatments of single ENR (0.025, 0.1, and 0.4 mmol/kg), one treatment of single Cd (0.4 mmol/kg), three treatments of combined ENR and Cd that were designed with mole ratios of 1: 1 (ENR 0.4: Cd 0.4 mmol/kg), 1: 4 (ENR 0.1: Cd 0.4 mmol/kg), and 1: 16 (ENR 0.025: Cd 0.4 mmol/kg) and a control treatment with equal deionized water were performed in laboratory experiments (Su et al., 2005). When setting the experimental concentration, an increase in the effective concentration due to bioaccumulation was also taken into account.

Then, 10 g of soil was placed in a brown glass bottle. Cd was added to the soil in the form of cadmium chloride solution, ENR was dissolved in deionized water to add into the soil, and the control group was treated with 0.20 mL of deionized water. Each of the treatments for all four of the sampling times (7, 14, 21, 28 days) had three replications. The soil samples were mixed thoroughly and placed in a constant temperature incubator at 25 °C in dark conditions, and the soil moisture was kept at approximately 50 to 60% of the maximum water holding capacity during incubation.

2.4. Soil microbe culture and count

The culture and count of soil microbes were conducted using the plate-count method, and selections of the soil samples from every treatment were collected after 7, 14, 21, and 28 days of incubation. Ten grams of soil was poured into a flask, which included 90 mL of sterile water, the flask was oscillated at 200 RPM for 20 min and then paused for 5 min, and the supernatant was 10^{-1} soil diluent. One milliliter of supernatant of 10^{-1} soil dilution was added to a test tube with 9.00 mL of sterile water, and then, with 1 min vortex oscillation, a 10^{-2} soil dilution as obtained. In addition, a 10^{-4} soil dilution was prepared. Then, soil dilutions (100 μL) were coated on agar plates, and each exposure concentration had three parallels.

Bacteria, fungi, and actinomycetes were cultured and counted in LB medium, PDA medium, and Gao One medium, respectively (Davis et al., 2005). A pre-experiment was performed to determine the best concentration of soil dilution liquid for further use; bacteria and