The Bio-availability of Sulfomethoxazole and its Effects on Soil Functions with Cu co-addition in soil

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ABSTRACT

Antibiotics and heavy metals are often co-occurrence in animal feces/manures, and then are detected to coexist in soil and organic fertilizer samples. To evaluate the interactive effects of antibiotics and heavy metals on soil microbial ecology, the present study investigated the effects of sulfomethoxazole singly or in combination with Copper (Cu) on soil function and its bio-availability in soil. Results showed that there was a higher bioavailability at the initial time of test, and it was lithely affected by Cu addition in the whole investigation. The inhibition of SMX on urease and soil nitrification increased with the increase of SMX spiking levels without Cu co-addition or not. When applied together, the negative effects on urease activity were lighter compared to that in the SMX single treatments.However,co-occurrence of SMX and Cu had more serious inhibition than SMX single treatments did on soil potential nitrification rate.

Keywords

Cu, Sulfomethoxazole, Co-pollution, soil function.

1.INTRODUCTION

Modern animal feeds often include nutritional mineral supplements (eg: Cu, Zn) and antibiotics (eg: sulfonamides) to control bacterial infective diseases and promote the growth of domestic animals. It was reported that up to 90% of antibiotics that are fed to livestock are excreted with manures and feces [4] and the amount of Cu eliminated through the animal feces corresponds to 70~80% of the amount ingested [14]. Thus, application of contaminated manure is supposed to be the main source of heavy metals and antibiotic co-contamination in agricultural soil. However, there was only few studies that reported the interactive influence of antibiotics and heavy metals on soil microbial communities[2,10].

Sulfonamideswas one of the old types of antibiotics and widely used since 1930s. They are still among the most commonly used antibiotics both for human and animals, with 2.5 million tons being used in China annually [17]. Sulfonamides are considered to be bacteriostatic, inhibiting the synthesis of folic acid. A number of different sulfonamides have recently been the subject of several soil ecotoxicological studies, and effects including soil microbial community structure alteration, ecological function and resistance expansion[1]. However, till now, researches about the interactive effects of sulfonamides and heavy metals on soil ecological functions are still limited.

The objective of this study was to evaluate the interactive effects of antibiotics and heavy metals on soil function. Sulfomethoxazole (SMX) and Cu were selected representatively to investigate (1) the effects of Cu addition on the bioavailability of SMX in soil microbial activities, and (2) the interactive effects of SMX and Cu on soil function of N transformation.[1]

2. MATERIALS AND METHODS

2.1 Soil Sampling, Preparation, and Properties

The soil was collected from an agricultural field of Zibo, China. Soil was sampled from the plowed topsoil (0 – 15 cm deep). After the removal of large pieces of plant materials and soil animals, soil was air-dried to about 20% of maximum water-holding capacity (MWHC) in laboratory. Then, it was milled with a gavel, passed through a 1mm sieve, and stored in a dark location at 4 °C until use. The soil characteristics were listed in Table 1.

Table 1. The selected physico-chemical properties of the soils

Soil properties	pH	OC ($g\cdot kg^{-1}$)	CEC ($cmol\cdot kg^{-1}$)	Clay (%)	Sand (%)	Silt (%)
Mean (SD)	7.96(0.11)	16.88(1.49)	35.88(0.14)	45.08(2.34)	38.12(2.05)	14.22(1.83)

2.2 Experiment Design

Thirty portions of 300 g soil were weighed and put into each beaker. Soil moisture was adjusted to 40% of MWHC by adding distilled water to the desired weight. The beakers were sealed with plastic film and incubated in the dark at 25±1 °C for 7 days.

After 7 days, SMX / $CuCl_2$ solutions were spiked into the pre-incubated soils. For SMX spiking treatments, five treatments were spiked with concentrations of 0, 5, 25, 50, and 100 $mg\cdot kg^{-1}$ dry soil, respectively. For the combination contamination treatments, $CuCl_2$ solutions in the rate of 100mg kg^{-1} for Cu^{2+} were straightly added to the soil samples pretreated with different concentrations of SMX, and then, appropriate pure water was used to adjust the soil moisture to 60% MWHC. The samples, received no SMX and Cu, were selected as control treatment, but an equaled amount of distilled water were added at the same time. Soil moisture was kept constant at 60 of % of the maximum water holding capacity and was controlled gravimetrically throughout the experiment. The beakers were sealed with plastic film and kept in the dark at 25±1℃. The analyses of microbial activities and structure were mostly focused on the sampling dates 14 and 21 days.

2.3 Bioavailability of SMX in Soil

The SMX concentration of the soil samples was extracted using 0.01 M $CaCl_2$ (soil-solution ratio 1:2), which extract is operationally defined to represent the mobile and bioavailable fraction [6]. Samples were shaken for 24 h before centrifugation for 20 min at 3000g and filtration.. The extracted samples were determined by high-performance liquid chromatography (HPLC) (Agilent LC1200 with a diode-array detector) with the ultraviolet wavelength (UV) of 270 nm for SMX according to Liu et al. [12,13], which was analyzed by the state key lab of Food and Ceramics analysis and inspection.

2.4 Microbiological Analysis

Urease activity in the soil was determined using a modification of the method proposed by Kandeler and Gerber[8]. Soil potential nitrification was determined according to the ISO method described by Rusk et al. [15]. The extracted (NO_3^- + NO_2^-)-N (referred to hereafter as NOx) was analyzed by a colorimetric assay. The production of NOx over the duration of the test was calculated by subtracting the concentration of NOx at day 0 from that at day. The incubation of soil without addition of $(NH_4)_2SO_4$ was used as control.

2.6 Statistical Analyses

All measurements were made in triplicate and reported as mean values with standard error(SD). Statistical analyses were carried out using SPSS 11.0 (SPSS Inc., USA). For multiple comparisons, it was assumed that the data were from two independent groups. Paired t tests were employed on comparable treatments.

3.RESULTS AND DISSCUSSION

3.1 Bioavailability of SMXin Soils

Sulfomethoxazole, a sulfonamide drug, is ionizable in soil environment, and hence its behavior and biological activity depend on soil physic-chemical properties, such as pH, organic carbon and other ions in soil solution. In this study, the effect of Cu on bioavailability of SMX was determined in order to investigate the interactive effects of SMX and Cu on soil microbial communities. Results were shown in Figure 1 and Figure 2.

The extractability of SMX rapidly declined after addition to soil. Only minor were extractable at day 1. For example, the extractable SMX in Cu absent and present soil were 22.8 ug·L^{-1} and 36.8 ug·L^{-1} respectively, for spiking levels at 5 mg·kg-1 soil treatment; and they were almost 250 ug·L^{-1} for SMX spiking level at 100 mg·kg^{-1} soil treatment. After 14 days incubation, For SMX spiking levels at 100, 50, 25, and 5 mg·kg^{-1} soil treatments, the extractable fractions were only 76.31, 50.4, 26.91 and 6.54 ug·L^{-1}, respectively, in Cu absent soils; and were 74.01, 51.12, 25.75 and 12.06 ug·L^{-1}, respectively, in Cu present soils.

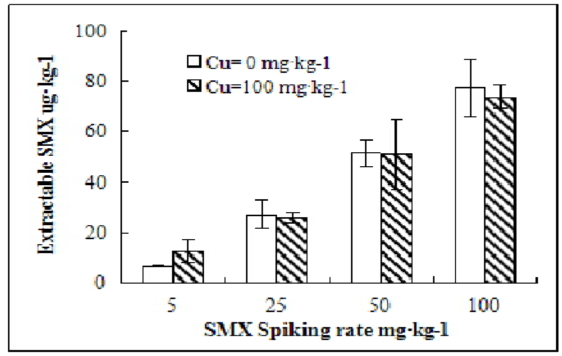

Figure 1. The extracted concentration of SMX on 1 day

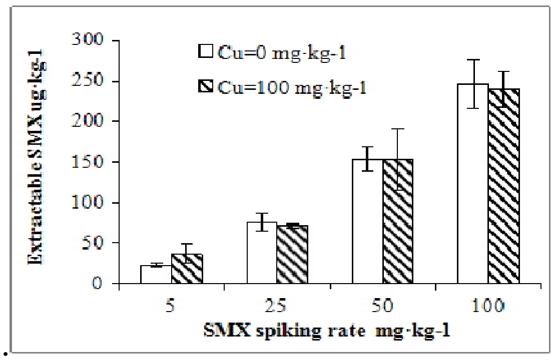

Figure 2.The extracted concentration of SMX on day 14.

All above results might indicate that bioavailability of SMX is reduced by an increased sorption of sulfonamides with time[7], by primary degradation (deactivation), or by the formation of non-extractable residues [5]. However, there was a higher bioavailability at higher initial concentration for SMX (Figure 1 and 2), which is similar to sulfadiazine in soil [11]. However, there was no significant difference in the extractable SMX without Cu absent or present soil treatments, except of SMX spiking level at 5 mg·kg^{-1} (Figure 1 and 2). This might suggest that Cu addition has little effect on the bioavailability of SMX when it was spiked at relatively higher levels.

3.2 Effects of SMX and Cu on Soil Function

In order to draw a clearer picture of the interactive effect of SMX and Cu on soil function, Urease activity and soil potential nitrification were determined in the different soils treatments. The results were shown in Figure 5 and 6.

As shown in Figure 3, soil urease activity was reduced with increasing SMX spiking level without it singly or in combination with Cu; but it was always relatively higher in Cu co-exist soil treatments than that in SMX singly treatment, which was similar to the effects of SMX and Cu on soil microbial biomass (Figure 3). The similar effects of SMX (singly or in combination) on urease activity and soil microbial biomass are in agreement with results by Klose and Tabatabai [9] who reported a correlation of microbial biomass with urease activity.

As shown in Figure 4, soil potential nitrification of the control sample was 3.17 NO_3^--N mg·kg^{-1}dm day^{-1}. The factors SMX and Cu influenced the soil potential nitrification. For the SMX singly soil treatments, the activation of soil potential nitrification was pronounced in 5 mg kg^{-1} spiking level soil treatment, but the decrease of soil potential nitrification was still found with the increase of SMX concentrations in comparison to the control treatment. It indicated that the high concentration of SMX addition led to a reduction of the potential nitrification rates in the soils, which is similar to the effect of Sulfadiazine on soil nitrification [11]. Soil nitrification was very sensitive to Cu contamination, and soil spiked with Cu revealed lower nitrification rate than control and SMX singly treatments. The factors SMX and Cu show a synergetic effect on soil potential nitrification, that is the inhibiting effects were more serious in soil treatments with SMX and Cu together than that in soil treatments with SMX singly.

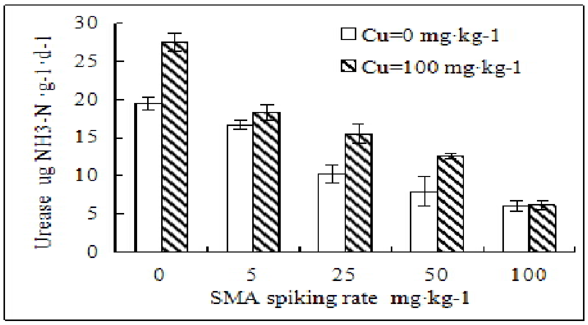

Figure3.Effects of SMX and Cu on Urease activity.

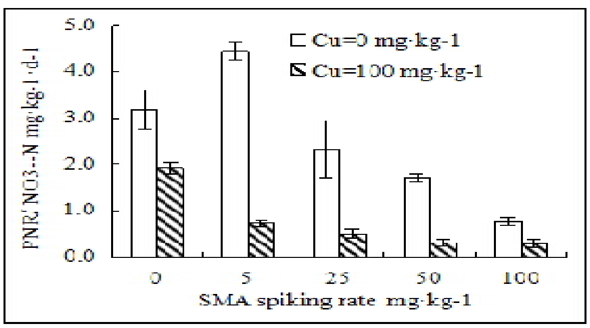

Figure4.Effects of SMX and Cu on soil nitrification.

The inhibiting or stimulating effects of Cu on soil nitrification were investigated popularly in recent years, indicating that nitrification bacteria is sensitive in first then be tolerant to Cu contamination. Furthermore, some studies also indicated that sulfonamides could inhibited nitrifying bacteria in soil, and then reduced the soil nitrification rates [11,16]. However, When comparing the results of our experiments to previous studies, it should be taken into account that the soil used here was no history of antibiotics or heavy metals contamination and the incubated time was relatively shorter while most previous studies used soils with a long history of antibiotics' application or pre-exposure with Cu[2].

4.CONCLUSION

In the current study, Cu addition had little effects on the extractable SMX in the soil sample. However, Cu could reduce the inhibition of SMX on urease activity, and improved the negative effects of SMX on soil potential nitrification rate. Therefore, it is needed to put more research on the interactive influence of antibiotics and heavy metals on soil microbial ecology in the future.

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