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Interactive effect of oxytetracycline and lead on soil enzymatic activity and microbial biomass

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ABSTRACT

Interactive effect of oxytetracyline (OTC) and lead on soil enzymatic activity and population of microbes was studied in the paper. The results showed effect of pollutants on bacteria, actinomycetes and enzymatic activity increased in the order: (OTC + Pb) > Pb > OTC, (OTC + Pb) > Pb > OTC and (OTC + Pb) > OTC > Pb, respectively. However, impact of pollutants on fungi decreased in the order: (OTC + Pb) < Pb < OTC. The analysis of X-ray photoelectron spectroscopy (XPS) showed that binding energy and atomic percentage of Al, Fe, C, O, N, Si, Mg and Ca altered after the amended with OTC or Pb. The decrease of oxygen atom density and increase of binding energy can be associated to the charge transfer, resulting from oxygen and carbon atoms coordinated with metal ions, other chemicals or partial decomposition. Thus, the findings of this study can provide a better insight into the interaction of both pollutants and their impacts on soil quality.

1. Introduction

Pharmaceutical antibiotics are widely applied to humans and animals for preventing or treating infectious diseases, or used as growth promoters in animal production. Antibiotics have been frequently identified in agricultural land, which was irrigated with reclaimed wastewater (Gulkowska et al., 2008; Yang et al., 2005; Kemper, 2008.) or amended with antibiotic-contaminated sewage sludge and manure (Díaz-Cruz et al., 2006; Brambilla et al., 2007; Hammesfahr et al., 2008). For example, the concentration of tetracycline (one of widely used pharmaceutical antibiotics) has been reported to be 86.2 $\mu g\,kg^{-1}$, 198.7 $\mu g\,kg^{-1}$, and 171.7 $\mu g\,kg^{-1}$ in the three top agricultural soil layers (0–10 cm, 10–20 cm and 20–30 cm) in an area with intensive livestock farm in northern Germany (Hamscher et al., 2002). Oxytetracycline (OTC) is one of tetracycline antibiotics, which is generally used for treating infectious diseases of animals in livestock production. Bao (2008) found that the concentration of OTC was up to 200 $mg\,kg^{-1}$ in the soil and 285 $mg\,kg^{-1}$ in sediment in some places in China. OTC may remain in the soils for 40 days if the concentration of OTC was greater than 25 $\mu g\,ml^{-1}$ (Hou et al., 2009). Tetracyclines, including OTC and other veterinary antibiotics may lead to the antibiotic resistance of microorganisms, which is potentially hazardous to non-target organisms in the terrestrial environment (Auerbach et al., 2007; Kong et al., 2006; Agersø et al., 2006; Thiele-Bruhn and Beck, 2005; Jia et al., 2008).

Much attention has paid to lead (Pb) pollution in agricultural land that is caused by irrigation of industrial waste water and sewage slurry and precipitation of industrial and traffic waste gases (Li et al., 2006). The concentration of Pb is usually from 2 $mg\,kg^{-1}$ to 200 $mg\,kg^{-1}$ in soil. It has been reported

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that the concentration of lead varies from 51.84 mg kg^{-1} to 1185 mg kg^{-1} in polluted soil in China (Tzu-Hsing et al., 2007; Wu et al., 2008). Increasing availability of lead can cause microbial biomass reduction and change microbial community structure in soils (Suhadolc et al., 2004). Moreover, excessive lead intake can inhibit the reproductive function and immunity of the human's body; retard the intelligence development of children, resulting in various physical abnormalities (Chen and Zhu, 2004).

Soil microorganisms are important component of ecosystem. Soil microbial properties and activity, such as community functional diversity, microbial biomass, soil respiration and enzymatic activity, are usually employed as indicators of soil quality (Kong et al., 2006; Kulshrestha et al., 2004; Li et al., 2006; Ute et al., 2008; Zielezny et al., 2006). Soil respiration can reflect mineralization rate of soil organic carbon and microbial activity, which is closely relevant to soil quality (Landi et al., 2000). Soil respiration increased with the increasing concentration of heavy metal (Barajas-Aceves, 2005; Chander and Joergensen, 2001; Yang et al., 2006). It can be explained: microorganisms in high level of metal soils need to consume more energy to survive, resulting in more evolved CO_2. Soil enzymatic activity is a sensitive indicator that can reflect the heavy metal on soil microorganisms. Soil enzymatic activity decreases with an increase of heavy metal content. The mechanism of heavy metal on enzymatic activity may associate to the formation of stable complexes that derive from the combination of heavy metal with active sites of enzyme (e.g., sulfydryl and iminazole groups). In addition, heavy metal can inhibit soil microbial growth and reproductions, eventually lead to the decrease of soil enzyme activity.

Interactive effects of heavy metal and organic pollutants on environmental organism have also been reported extensively (Schmitt et al., 2005; Gu and Karthikeyan, 2005; de Souza et al., 2006). Antibiotics and heavy metal are often detected to coexist in soil and organic fertilizer samples. Interactive effect of antibiotics and heavy metal is pronounced on soil microbes (Lao, 1988; Kong et al., 2006). Many studies showed that interactive effect of antibiotics and heavy metals was potentially hazardous to bacteria and other organisms in the environment. Kong et al. (2006) reported the antibiotic (OTC) and Cu had significantly negative effects on soil microbial community function, particularly when both pollutants presented in the environment. However, the study about effect and mechanism of antibiotics and heavy metal on soil microorganisms is scarce.

The objectives of this study were (1) to investigate the effect of OTC and lead on the activity of alkaline phosphatase (ALP) and sucrase; (2) to investigate the effect of OTC and lead on the quantity of bacteria, fungi and actinomycetes; (3) to explore interaction mechanisms of OTC and lead in polluted soil based on the results from X-ray photoelectron spectroscopy (XPS).

2. Materials and methods

2.1. Soil samples and chemicals

The surface soils (0–30 cm) were sampled from a suburban farmland of Tianjin, China. No chemicals were applied prior to the collection of soil sample at this site. The samples were air-dried and passed through a 2-mm sieve, then stored in containers at 4 °C. Selected soil characteristics were analyzed according to the method descried in Lao (1988). The soil had a medium loam texture with a pH value of 8.24, the organic matter content of 1.61%, water holding capacity of 41.98%, sand of 10.58%, silt of 50.12%, clay of 29.30%, and cation exchange capacity of 10.86 cmol kg^{-1}. The soil background content of OTC and Pb was 0 mg kg^{-1} and 8.15 mg kg^{-1}, respectively.

Oxytetracycline (98.0% purity) was purchased from HuBei Dragon Hall Biotechnology Development Company Ltd. Lead nitrate, salts and acids used in this study were the analytical grade and purchased from Chemical Reagent Factory (Tianjin, China).

2.2. Sample preparation and incubation

Twelve portions of 300 g soil were weighed and put into each beaker. Soil moisture was adjusted to 40% of the water holding capacity 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, oxytetracycline and $PbNO_3$ were applied into the preincubated soils of each beaker. Both concentrations of OTC and $PbNO_3$ used in the experiments were 100 mg kg^{-1}; the combination of the two pollutants was 100 mg kg^{-1} soil. Each treatment was conducted in triplicates. The samples were mixed thoroughly in order to insure a uniform distribution of OTC and lead applied. All treated samples were then adjusted to 60% of the water holding capacity. The beakers were sealed with plastic film and kept in the dark at 25 ± 1 °C. Ten grams of soil were removed from each treatment at 1, 7, 15, 30, 45, 75 and 105 days. Soil moisture levels were maintained by adding distilled water every 7 days.

2.3. Sample analysis

Soil enzymatic activities and microbial biomass were determined in different intervals during the incubation. Alkaline phosphatase was assayed as follows: 5 g soil was placed in 50 ml triangular flask, and then 5 drops toluene and 20 ml of 0.5% disodium phenyl phosphate substrate were added into triangular flask. The samples were incubated at 37 °C for 2 h. One milliliter filtrate of each sample was assayed by spectrophotometer (SP-756, China). Sucrase was determined by using sodium thiosulfate titration as follows: 10 g soil was placed in 100 ml triangular flask, 1.5 ml toluene was added for 15 min. Then 10 ml of 20% sucrose and 0.1 M acetate–phosphate buffer (pH 5.5) were added and incubated at 37 °C for 24 h. Fifty milliliters distilled water was added after incubation. The samples were shaken and filtrated. Twenty milliliters filtrate and 10 ml fiilin solution were placed in triangular flask. Then it was heated for 10 min at boiling water bath. Three milliliter of 33% KI and 4 ml H_2SO_4 (1:3) were added to triangular flask after cooling. The samples were titrated by 0.1 N sodium thiosulfate. The methods were described by Guan (1986).

The number of microorganism was assayed using pour plate method. 0.5 grams soil was placed in triangular flask including 49.5 ml sterile distilled water, then shaken for