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Toxicity of sulfadiazine and copper and their interaction to wheat (*Triticum aestivum* L.) seedlings

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ABSTRACT

A pot experiment was carried out to investigate the single and combined effect of different concentrations of sulfadiazine (SDZ) (1 and 10 mg kg^{-1}) and copper (Cu) (20 and 200 mg kg^{-1}) stresses on growth, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), antioxidant enzyme activities of wheat seedlings and their accumulation. High SDZ or Cu level significantly inhibited the growth of wheat seedlings, but the emergence rate was only inhibited by high SDZ level. The presence of Cu reduced the accumulation of SDZ, whereas the effect of SDZ on the accumulation of Cu depended on their concentrations. Low Cu level significantly increased the chlorophyll content, while high Cu level or both SDZ concentrations resulted in a significant decrease in the chlorophyll content as compared to the control. Additionally, H_2O_2 and MDA contents increased with the elevated SDZ or Cu level. The activities of superoxide dismutase, peroxidase and catalase were also stimulated by SDZ or Cu except for the aerial part treated by low Cu level and root treated by high SDZ level. The joint toxicity data showed that the toxicity of SDZ to wheat seedlings was generally alleviated by the presence of Cu, whereas the combined toxicity of SDZ and Cu was larger than equivalent Cu alone.

1. Introduction

Extensive antibiotics are not only widely used to treat or prevent infective diseases in human and animals but also used as growth promoters in livestock and fish farming. However, since antibiotics are poorly adsorbed by the bodies of animals, most of them are excreted in feces and urine, either unchanged or as metabolites, and subsequently enter into the environment (Boxall et al., 2004; Sarmah et al., 2006). Soil is a prominent sink for antibiotics because most of them are strongly sorbed by soils and are not readily degraded. High levels of antibiotics are usually detected in agricultural soils, which reach up to the order of μg kg^{-1} or mg kg^{-1} (Golet et al., 2002; Martínez-Carballo et al., 2007; Aust et al., 2008; Dalkmann et al., 2012; Shi et al., 2012). Recently, many researchers have demonstrated that plants could uptake antibiotics from solution or soil media (Dolliver et al., 2007; Herklotz et al., 2010; Eggen et al., 2011), and in some cases they could produce phytotoxic effects (Adomas et al., 2013; Michelini et al., 2013; Pan and Chu, 2016).

The coexistence of antibiotics and heavy metals commonly occurs in soil environment because they can enter into soils through the same pathway, e.g. the application livestock manure or sludge and wastewater irrigation (Tamtam et al., 2011; Ji et al., 2012; Xu et al., 2013, 2015a, 2015b). Many antibiotics with various functional groups can complex with heavy metal ions (Kremer et al., 2006; Ftouni et al., 2012), which would alter their individual speciation and consequent environmental behavior. For example, some recent reports showed that the presence of Cu obviously enhanced the sorption of fluoroquinolones on organic matters (Pan et al., 2012), montmorillonite and kaolinite (Pei et al., 2009) and soils (Graouer-Bacart et al., 2013) through the formation of ternary surface complexes. Therefore, it is expected that these change of speciation and environmental behavior may influence their bioavailability. However, it still lacks for knowledge about the joint effect of antibiotic and heavy metal on plant until now.

In the present study, sulfadiazine (SDZ) and copper (Cu) were selected as representatives of antibiotic and heavy metal, respectively, due to their extensive use in livestock farming. In this study, wheat was selected as a mode plant to investigate the single and joint effect of SDZ and Cu on the growth parameters, chlorophyll, H_2O_2, MDA, antioxidative enzymes activities (superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)), and accumulation of them. The aim of this study was to understand the mechanism of SDZ and/or Cu toxicity to wheat seedlings.

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2. Materials and methods

2.1. Materials

SDZ was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) with a purity > 98%. $CuSO_4 \cdot 5H_2O$ was obtained from Sinopharm Chemical Reagent Co (Shanghai, China). All reagents were of analytical or HPLC grade. Wheat (*Tritium aestivum* L), cv. Liaochun-10 seeds were purchased from a seed company in Shilihe Town, Shenyang City, China. The soil used in the experiment was taken from the surface layer (0–20 cm depth) of an uncontaminated field in Shenyang, China. The general physicochemical properties of the soil are listed as follows: pH (1:2.5 water) 6.24, cation exchange capacity 5.38 cmol kg^{-1}, organic matter 3.56%, total nitrogen 1.57 g kg^{-1}, total phosphorus 0.76 g kg^{-1}, sand 58.4%, silt 21.7%, clay 19.9%. The background contents of $CaCl_2$-extracted SDZ and total Cu in the tested soil were below the limit of detection and 20.15 mg kg^{-1}, respectively.

2.2. Experimental design and treatments

The seeds were surface sterilized by soaking in 5% (w:w) sodium hypochlorite solution for 10 min and then rinsed three times in sterilize deionized water. 20 seeds were sowed in a white plastic pot. Three different SDZ concentrations (0, 1, 10 mg kg^{-1} soil) and three different Cu concentrations (0, 20, 200 mg kg^{-1} soil) were single and combined as additions to soil, resulting in nine treatments (Control, SDZ1, SDZ10, Cu20, Cu200, SDZ1 + Cu20, SDZ1 + Cu200, SDZ10 + Cu20, and SDZ10 + Cu200), and each treatment had three replicates. The pots were put into an illumination incubator at a constant temperature of 25 ± 1 °C and a photoperiod of 12 h. Soil moisture was maintained at 24% (w:w) by watering with distilled water. After growing for 14 days, plants were harvested, washed thoroughly with water. Roots and aerial parts were separated and used for analysis The emergence rate of wheat seedlings was also calculated.

2.3. Determination of SDZ and Cu concentrations

SDZ in plant sample was extracted according to a procedure outlined by Dolliver et al. (2007). The SDZ concentration was measured by a high performance liquid chromatography (HPLC) system (Waters2695, Milford, MA, USA) with a Supercol C18 reverse-phase column (5 μm, 4.6 mm × 150 mm) and a diode array detector operating at 260 nm. Mobile phase was composed of 0.1% formic acid and methanol at a flow rate of 0.2 mL min^{-1}.

0.50 g crushed plant sample was digested with a mixture of HNO_3/$HClO_4$ (4:1) at 210 °C. Cu concentration was quantified by a flame-atomic absorption spectrophotometer (FAAS, PerkinElmer AA800, USA).

2.4. Measurement of chlorophyll, H_2O_2 and MDA contents

Chlorophyll content of wheat leaves was determined following the method of Lichtenthaler (1987).

The H_2O_2 content was determined by monitoring the A415 of titanium peroxide complex according to the methods described by Patterson et al. (1984).

The MDA content was determined by thiobarbituric acid (TBA) reaction according to the method of Heath and Packer (1968).

2.5. Determination of antioxidant enzyme activities

Fresh plant samples were ground with chilled 50 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.8) containing 0.2% Triton X-100% and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000*g* for 30 min, and the supernatant was collected for the protein assay and the determination of SOD, CAT and POD activities.

Protein level were measured by the method of Bradford (1976) using bovine serum albumin as a standard.

SOD activity was measured by the method described previously (Beyer and Fridovich, 1987). The assay was based on inhibiting reduction of nitroblue tetrazolium (NBT) to form formazan by superoxide. The activity was expressed as U mg^{-1} protein. One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction at 560 nm.

POD activity was assayed following the method described by Lagrimini (1991). In the presence of H_2O_2, POD catalyzes the transformation of guaiacol to tetraguaiacol. This reaction can be recorded at 470 nm. The activity was expressed as U mg^{-1} protein, where one unit of peroxidase converts one μmol of H_2O_2 per min.

CAT activity was assayed according to the method of Beers and Sizer (1952). The principle of the method was based on the hydrolysis of H_2O_2 and decreasing absorbance at 240 nm.

2.6. Statistical analysis

Statistical analyses were carried out using SPSS13.0 software (SPSS Inc., Chicago, IL, USA). Least significant difference (LSD) was applied to test for significance at $p < 0.05$ among treatments.

3. Result

3.1. SDZ and Cu concentrations in plant

As shown in Figs. 1 and 2, the SDZ and Cu concentrations in wheat seedlings increased with increasing their levels in soil, and root showed higher SDZ/Cu concentration than aerial part. The presence of Cu reduced SDZ concentration in wheat seedlings, and 200 mg kg^{-1} Cu resulted in a larger decrease than 20 mg kg^{-1} Cu. The concentrations of Cu in SDZ1 and Cu20 + SDZ1 significantly increased compared to the control and Cu20, respectively, while the presence of 10 mg kg^{-1} SDZ

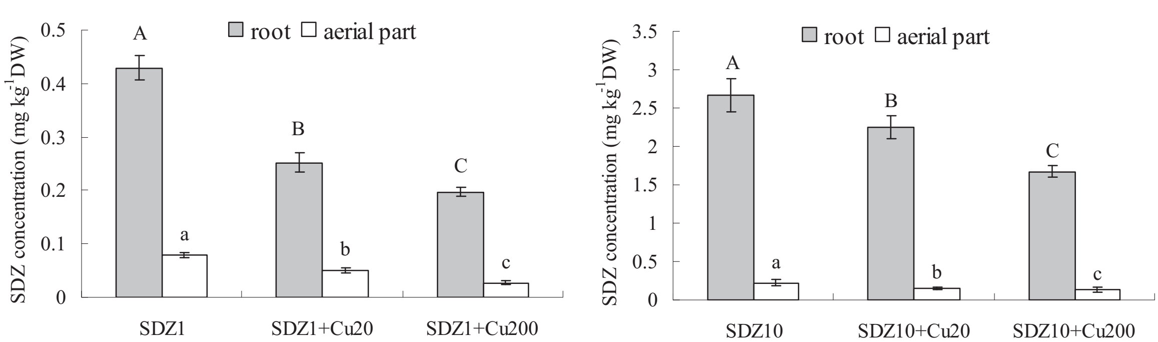

Fig. 1. Accumulation of SDZ in different parts of wheat seedlings.