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Interactive effects between earthworms and maize plants on the accumulation and toxicity of soil cadmium

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ABSTRACT

Multi-species tests are preferable in ecotoxicological studies as they allow the investigation of the influence of ecological interactions on mobility, bioavailability, and toxicity of contaminants. We performed monospecific and combined culture experiments in a soil-maize-earthworm testing system to determine the interactive effects between earthworms and maize plants on the accumulation and toxicity of cadmium (Cd) to each respective species. It was found that the co-presence of maize plants and earthworms in soil promoted the mobility and availability of Cd, but reduced Cd accumulation in earthworms and maize roots and showed little effects on cadmium's accumulation in maize stems and leaves. The presence of plants reduced Cd toxicity to earthworm cocoon output and body mass development, while the presence of earthworms showed no significant effects on Cd toxicity to maize shoot and root biomass. Meanwhile, the presence of earthworms reversed the effects of Cd on soil microbial community and stimulated and increased maize rhizosphere microbial species. It was concluded that the increase of metal mobility and availability in a multi-species testing system does not necessarily indicate a corresponding increase of ecotoxicity, and the interspecific effects between species belonging to different trophic levels on the accumulation and toxicity of metals should be taken into account in ecotoxicological risk assessment.

1. Introduction

Ecotoxicological data are essentially important for evaluating hazards, setting priorities, assessing risks, and establishing environmental quality criteria for contaminants. In soil ecotoxicology, a majority of ecotoxicological data is generated from single-species-based ecotoxicity tests by which standard approaches in guidelines of Organisation for Economic Co-operation and Development (OECD) and International Organization for Standardization (ISO) were generally followed. Single-species tests bear many advantages regarding the simplicity, practicability, controllability, low variability, reproducibility, and the interpretability of the test results (Breitholtz et al., 2006). However, these tests are less ecologically relevant as the interspecific interactions are generally ignored and the test outcomes usually differ from that of the multi-species tests (Edwards, 2002; De Laender et al., 2009). Ecotoxicological risk assessment based on single-species tests is no longer a solution to understand the complex relationships between toxicants and ecosystem structure and function (Artigas et al., 2012). There is, hence, an increasing appreciation for the use of ecologically more relevant multi-species tests to investigate the influence of ecological interactions on the mobility, bioavailability and therefore toxicity of contaminants (Alonso et al., 2006; Carbonell et al., 2009).

Many soil processes are mediated by soil invertebrates, of which earthworms form the highest biomass in many temperate and tropical soils (Lee, 1985). Earthworms fundamentally determine soil structure and fertility and stimulate numerous biological

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processes and interactions (Edwards and Bohlen, 1996). Generally, earthworms appear to influence the mobility and availability of metals in contaminated soils (Wen et al., 2006; Fritsch et al., 2008; Sizmur and Hodson, 2009), but this influence varied with different earthworm species, different soil components, and different types and forms of metals (Sizmur and Hodson, 2009; Sizmur et al., 2011). The precise mechanisms for the impacts of earthworms on metals mobility and bioavailability are unclear and, particularly, the role of earthworms in altering metals bioavailability in relation to ecotoxicity (e.g., inhibition of plant growth) is little understood.

Of all the toxic metals, cadmium (Cd) is perhaps the metal which has attracted most attention in soil environmental science (McLaughlin and Singh, 1999). Cadmium is a highly toxic metal with no known biological function and its risks to terrestrial organisms have been well established (McGrath, 1999; Lock and Janssen, 2001; Benavides et al., 2005). However, most previous studies have been carried out in single-species testing systems and the effects of ecological interactions on the accumulation and toxicity of Cd remain relatively ignored. In order to properly assess Cd risks in environment, it is necessary to have a more thorough understanding of the effects of ecological interactions on cadmium's mobility, availability, and therefore ecotoxicity. We hypothesized that the co-presence of plants and earthworms in a soil ecosystem may increase metals mobility and availability and consequently increase metals accumulation and toxicity to both plants and earthworms. Experiments were therefore carried out to investigate the interactive effects between plants and earthworms on cadmium's accumulation and ecotoxicity in a soil-maize-earthworm testing system. The objective of the study was to investigate the effects of the co-presence of plants and earthworms on the mobility and availability of Cd in soil and to determine the interactive effects between maize plants and earthworms on the accumulation and toxicity of Cd for each respective species. Meanwhile, soil microorganisms are known to mediate many interactions between earthworms and plants (Jusselme et al., 2012). The involvement of important microbes in ecotoxicity studies has been strongly advocated (Megharaj et al., 2003). Microorganisms associated with plant-root are known to be major drivers of metal bioavailability (Abou-Shanab et al., 2003), and there is an appeal for more research on changes in species diversity and microbial adaptation in relation to Cd pollution in rhizosphere soils (Wang et al., 2004). The effects of Cd on maize rhizosphere microbial community in the presence of both maize plants and earthworms were therefore also investigated.

2. Materials and methods

2.1. Chemical and soil

Analytical-grade Cd ($CdCl_2 \cdot 5/2H_2O$) with more than 98% purity was obtained from Sinopharm Chemical Reagent Company (Shanghai, China). Fluvo-aquic soil, a typical soil in north of China, was collected from the upper layer soil (0–20 cm) of a local orchard at Beijing, China. No pesticides and fertilizers had been used in the past decades in the orchard, and the soils were considered to be relatively "clean" as no prior contamination had occurred. The soils were air-dried and screened through a 2-mm mesh sieve to remove rocks and plant residues, and then stored at room temperature in plastic drums until use (less than one month). The soil had the following particle size distribution: sand 73.3%, fine silt 15.1%, and clay 11.6%. The content of organic matter was 1.13%, and the soil pH, density, and the total cation exchange capacity was 7.28, 2.96 kg dm^{-3}, and 7.2 cmol kg^{-1}, respectively. The baseline concentrations of As, Hg, Cu, Cd, Cr, Zn, Ni, and Pb in the soil were 7.3, 0.09, 19.0, 0.30, 56.5, 78.5, 32.6, and 26.0 mg kg^{-1}, respectively.

2.2. Test organisms

The earthworm *Eisenia fetida*, a model organism for ecotoxicological studies, was chosen to study its effects on cadmium's bioavailability and toxicity to maize plants based on the potential that *E. fetida* might have interactions with maize plants as this earthworm species was found to occur in maize-planted fields (Cao et al., 2004). Earthworms were commercially obtained from a local earthworm breeding farm (Tongzhou Shuangqiao Farm, Beijing, China), and have been acclimated and maintained for more than two years in laboratory before used for experiments. Earthworms were cultured in a temperature-controlled chamber on a mixture medium of cow manure, sphagnum peat and pear leaves at an ambient temperature of 20 ± 3 °C and natural light cycle (approximately 12:12 h light:dark). Adult (visible clitellum) earthworms ranged from 300 to 500 mg were used. Seeds of maize (*Zea mays*) cultivar "Zhengdan 958" were purchased from the Chinese Academy of Agricultural Sciences, and visually the health and equal-sized seeds were chosen and used for experiments.

2.3. Soil spiking and bioassay

Pot experiments were carried out in greenhouse to investigate the interactive effects of maize plants and earthworms on the accumulation and toxicity of Cd in each respective species. Also, the effects of earthworms on cadmium's toxicity to rhizosphere microbes of maize seedlings were studied. Cadmium (added as $CdCl_2$ in deionized water, 100 ml kg^{-1}) was spiked into the soil substrates at eight concentrations in a logarithmic series with four concentrations per order of magnitude, i.e. 0, 10, 22, 46, 100, 220, 460, and 1000 mg Cd kg^{-1} dry wt. After spiking, the substrates were thoroughly mixed by hand on a plastic sheet and the moisture content was raised to 20% by adding deionized water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). The same volume of deionized water was added to the controls. An amount equivalent to 2 kg dry soil was put into a plastic pot (21 cm upper diameter, 17 cm lower diameter, 13 cm high, with mesh cover drainage holes), and the soil was equilibrated for 7 d prior to the start of the experiments.

The bioassays fall into three categories: tests with maize plants, without earthworms; tests with earthworms, without maize plants; and tests with maize plants plus earthworms. Each test category contained eight Cd-treatment levels, and each treatment level had four replicates. Maize seeds were firstly soaked overnight (12 h) in deionized water, and then 10 seeds after imbibitions were sown (2 cm deep) in each pot. Fifteen adult earthworms were or were not introduced into each pot at the start of the experiment, and five grams of finely ground cow manure serving as food for earthworms were spread onto the soil surface. Food sources for earthworms were renewed weekly, and totally about 10 g of cow manure was added to each pot, which accounted for about 0.5% of the test soil in each pot. To be consistent, pots with maize plants alone (without earthworms) were also applied with the same amount of cow manure. Overhanging adhesive tape (48 mm width) was attached to the rim of each pot to prevent earthworms from escaping. After inoculation of maize seeds and/or earthworms, a dish was placed below each pot and all pots were placed into a greenhouse. Pots were arranged in a randomized design and maintained under natural light cycle, ambient temperature 22–28 °C, and RH 60%. The pots were watered daily and applied with 1/2 Hoagland nutrient solution once every week to give N, P, K nutrients.

After five days of initiation of experiment maize seedlings were thinned to 4 plants per pot, and at the 28th d of the experiment plants and earthworms were harvested. Plants were divided into roots and shoots, and the length of shoots and the dry mass of both shoots and roots were determined. For the determination of shoot and root dry