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Hormetic effects of Cd on alkaline phosphatase in soils across particle–size fractions in a typical coastal wetland

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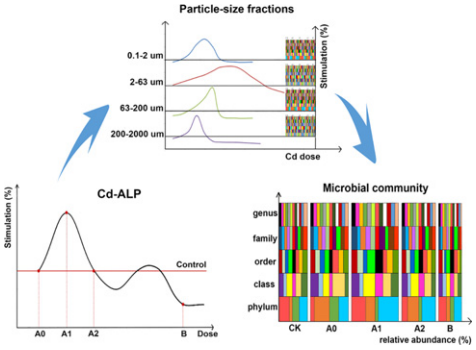
HIGHLIGHTS

- The hormetic effects of Cd on alkaline phosphatase (ALP) were studied.
- The activity of soil ALP in the 63–200 μm was obviously higher than in other fractions.
- The 2–63 μm and 63–200 μm fractions were the major or only expression vectors of the hormesis.
- *Gillisia* and *Pontibacter* possibly contribute to the hormetic responses of soil ALP when low contents of Cd present in soils.

GRAPHICAL ABSTRACT



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ABSTRACT

Hormetic responses in soil ecosystem are increasingly reported recently. Soil enzymes are involved in almost all biochemical reactions, but insufficient investigations were conducted to define its hormetic responses. The objective of this study is to investigate the hormetic responses across soil particle–size fractions with cadmium (Cd) as a stressor and alkaline phosphatase (ALP) as a potential endpoint. Soils were treated by adding $CdCl_2 \cdot 2.5H_2O$ solution with 0, 0.003, 0.03, 0.3, 3.0 and 30.0 mg·kg^{-1} of Cd, respectively. A low–power ultrasonic method was used to separate the bulk soil into 0.1–2, 2–63, 63–200 and 200–2000 μm fractions. In 2–63 μm, ALP activity at doses of 0.3–3.0 mg·kg^{-1} of Cd was significantly higher than that of CK (0.0 mg·kg^{-1} of Cd), showing a typical U–shaped dose–response with the amplitude of 72.3–118.6%. Similarly, ALP activity at 0.003–0.3 mg·kg^{-1} of Cd was 36.4–66.1% higher than that of CK in 63–200 μm. However, no similar phenomenon was observed in 0.1–2 and 200–2000 μm fractions. This suggested that low doses of Cd induced the hormetic responses of soil ALP, particularly in 2–63 and 63–200 μm. In addition, analysis of the microbial community structure and diversity indicates that, at genus level, the relative abundance (RA) of *Gillisia* at 0.03–0.3 mg·kg^{-1} of Cd was significantly higher than that of CK with the amplitude of 3.7–37.5% in 2–63 μm. The similar responses were observed that the RA of *Pontibacter* at 0.003–0.03 mg·kg^{-1} of Cd was 4.0–85.4% higher than that of CK in 63–200 μm. This showed that *Gillisia* and *Pontibacter* possibly contribute to the hormetic responses of soil ALP when low contents of Cd presented in soils. This study will provide a good insight into the hormetic phenomenon at soil ecosystem scales.

1. Introduction

Hormesis referred to a biphasic dose–response to an environmental agent, which was characterized by low doses stimulation and high

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doses inhibition (Calabrese and Baldwin, 2000, 2003; Calabrese and Blain, 2009, 2011). The hormetic effects were widely reported in various experimental models, such as animals, plants, microbes, protozoa, phytoplankton, viruses and so on (Calabrese, 2002; Calabrese et al., 2012; Iavicoli et al., 2014). Recently, increasing investigations were conducted in soil ecosystem. Soil nitrification rates were induced to express a hormetic response by low dose of Ag (0.5–1.6 $mg \cdot kg^{-1}$) in soils (Ramadassa et al., 2017). Petcu et al. (2015) found that herbicides at low dose in soils led to hormesis of soil respiration. It should be noted that soil enzyme, one of the most important soil components, catalyzes almost all biochemical reactions in soils (Marx et al., 2001), however, whether it can be stimulated to present a hormetic dose–response relationship is unclear yet.

Heavy metals are the typical stressors which generally induced hormetic effects. Xu and Wang (2014) reported that decabromodiphenyl ether (BDE–209) degradation was stimulated by low concentrations of Cu and Pb whereas inhibited at high doses. Petr (2003) suggested that low doses of Cd, Mn or Zn were beneficial to the growth of white–rot fungi, but inhibited at high doses. Lin et al. (2017) investigated the effects of Cd on the hepatopancreas of the crab *Eriocheir sinensis*. Results showed that the activities of superoxide dismutase, catalase, glutathione peroxidases increased at 2.52–10.07 $mg \cdot L^{-1}$ Cd and subsequently decreased with increasing Cd concentrations, and malondialdehyde (MDA) and H_2O_2 in *E. sinensis* were enhanced by lower Cd concentrations (1.26–5.04 $mg \cdot L^{-1}$). Kowalczyk-Peckaa et al. (2017) evaluated the effects of Cu micro–supplementation on fatty acid profiles in snails *Helix pomatia* (*Gastropoda Pulmonata*). They found that the low doses of Cu induced an evidently positive stimulation of metabolic transformations of fatty acids in the snails. Therefore, exploring the potential hormetic responses of heavy metals on soil enzymes will lead to new understanding of hormesis at soil ecosystem scales.

On the other hand, it should be noted that the activity and stability of soil enzymes were very heterogenous across soil particle–size fractions. (Tisdall, 1994; Mikha and Rice, 2004). Stemmer et al. (1998) classified soil into five fractions (>200, 200–63, 63–2, 2–0.1 and <0.1 μm) by using the low–power ultrasonic method to evaluate the distribution of soil enzymes in different particle–size fractions. They found that the highest activity of invertase was determined in 2–63 μm, but xylanase activity in >200 μm was evidently higher than other fractions. However, Zhang et al. (2015) found the highest activities of β–glucosidase, β–cellobiosidase, α–glucosidase, aminopeptidase, phenol oxidase and peroxidase were observed in 63–200 μm, while the highest activities of phosphatase and sulfatase were determined in the 0.1–2 μm. Meanwhile, Qin et al. (2010) reported that the highest activities of alkaline phosphomonoesterase, protease and urease were determined in 0.1–2 μm, but *N*–acetyl–β–glucosaminidase and β–glucosidase were mainly distributed in 63–200 μm. Moreover, carbohydrase was found to be predominated in >200 μm, but phosphatase and leucine–aminopeptidase were determined in 0.1–2 μm (Marx et al., 2005). Therefore, it is very necessary to evaluate the contributions of soil particle–size fractions when exploring the hormetic response of soil enzymes.

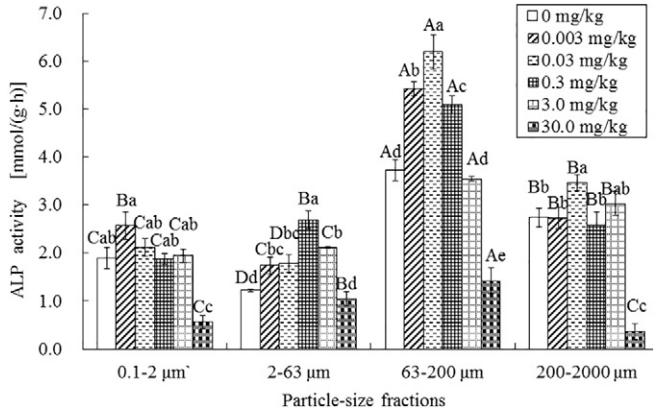

Fig. 1. Changes of ALP activity in soil particle-size fractions. Different capital letters indicate significant differences across different particle-size fractions; and lower-case letters present significant differences ($P < 0.05$).

2. Materials and methods

2.1. Site description

The Chongming Dongtan wetland (E: 121°50′–122°05′, N: 31°25′–31°38′) is the largest tidal mudflat of the Yangtze River estuary. It is located in the eastern of Shanghai, China, with the altitude ranging from 3.21 to 4.20 m. Chongming Island is a subtropical monsoon climate with mean annual air temperature of 15.3 °C and mean annual rainfall of 1025 mm. Due to influence of the East Asian monsoon, the study area is mild and humid, abundant rainfall and relative humidity maintained at 80% throughout the year (Wang, 2012). Reed (*Phragmites australis*) is the dominant vegetation of the wetland.

2.2. Soil samples collection and processing

Three study zones (3 × 5 m) with an inter–space of 50 m were selected from the reed marshes in Chongming Dongtan on 15 December 2014. Each zone was further divided into three small sampling areas (1 × 5 m) surrounded by bamboo fences. The latitude and longitude of the sampling areas were determined by using GPS. Before sampling, the reed stalks and roots were removed from the soil surface. Then, 0.3 × 0.3 × 0.3 m^3 of soil samples were collected, air dried, ground and sieved through a 2–mm nylon sieve. The air dried soil samples were then stored for microbiological and biochemical analyses.

2.3. Experimental design and treatment

A total of 20.0 g air–dried soil was placed into a 100 mL flask. 8.0 mL ultrapure water was uniformly added and the flasks were then sealed with breathable membrane and incubated at 30 °C for 24 h. After 1 day, 4.0 mL of $CdCl_2$ solutions with different concentrations were added to the soil in each flask to obtain Cd concentrations of 0, 0.003, 0.03, 0.3, 3.0 and 30.0 $mg \cdot kg^{-1}$ dry soil (3 replicates), respectively. These flasks were then sealed and incubated again at 30 °C for 12 h before conducting the soil particle–size separation.

2.4. Soil particle–size separation

The soil samples from the incubation experiment were treated for particle–size separation according to Stemmer et al. (1998). Briefly, 80 mL ultrapure water was added into 20 g of soil samples. After sufficient mixing and allowing standing for 2 min, the samples were placed into an ultrasonic cell crushing apparatus. The device was setup to 45 $J \cdot s^{-1}$ output energy for 3 min, with an ultrasonic time of 1 s and an intermittent time of 2 s. After then, the ultrasonic probe was inserted into suspension liquid with a distance of 1–2 cm away from the liquid level. The suspension was then filtered through a 200–μm sieve. The particles left on the sieve were washed repeatedly until the flushing liquid became clear, after which they were backwashed into a 1 L beaker and washed repeatedly. After backwashing, the suspension liquid was placed into 100–mL centrifuge tubes and centrifuged at 150g for 5 min to obtain the size range of 200–2000 μm. The suspension liquid was then filtered through a 63–μm sieve, washed, backwashed and centrifuged as above to obtain 63–200 μm fraction. This material was then