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Soil properties influence kinetics of soil acid phosphatase in response to arsenic toxicity

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ABSTRACT

Soil phosphatase, which plays an important role in phosphorus cycling, is strongly inhibited by Arsenic (As). However, the inhibition mechanism in kinetics is not adequately investigated. In this study, we investigated the kinetic characteristics of soil acid phosphatase (ACP) in 14 soils with varied properties, and also explored how kinetic properties of soil ACP changed with different spiked As concentrations. The results showed that the Michaelis constant (K_m) and maximum reaction velocity (V_{max}) values of soil ACP ranged from 1.18 to 3.77 mM and 0.025–0.133 mM h^{-1} in uncontaminated soils. The kinetic parameters of soil ACP in different soils changed differently with As contamination. The K_m remained unchanged and V_{max} decreased with increase of As concentration in most acid and neutral soils, indicating a noncompetitive inhibition mechanism. However, in alkaline soils, the K_m increased linearly and V_{max} decreased with increase of As concentration, indicating a mixed inhibition mechanism that include competitive and noncompetitive. The competitive inhibition constant (K_{ic}) and noncompetitive inhibition constant (K_{iu}) varied among soils and ranged from 0.38 to 3.65 mM and 0.84–7.43 mM respectively. The inhibitory effect of As on soil ACP was mostly affected by soil organic matter and cation exchange capacity. Those factors influenced the combination of As with enzyme, which resulted in a difference of As toxicity to soil ACP. Catalytic efficiency (V_{max}/K_m) of soil ACP was a sensitive kinetic parameter to assess the ecological risks of soil As contamination.

1. Introduction

Arsenic (As) is a metalloid placed in the same family with phosphorus (P) in the periodic table of elements. It exists mainly as inorganic forms with pentavalent and trivalent state dominating in oxidizing and reducing environment, respectively (Bissen and Frimmel, 2003; Duker et al., 2005). The anthropogenic activities, including mining, application of fertilizer and pesticides, burning of fossil fuels, have caused the release of As into the environment, which leads to As pollution worldwide and causes environmental incidents (Mandal and Suzuki, 2002; Singh et al., 2015). Arsenical compounds not only threat indirectly human being and animal health, but also have very toxic effects on microorganisms and soil enzymes through replacing phosphorus in molecular and/or interacting with sulfhydryl groups of proteins (Duker et al., 2005). Knowing the mechanisms of As pollution on soil biochemical processes is useful for identifying its environmental exposure and providing important information for the remediation of contaminated soils (Koo et al., 2012; Lyubun et al., 2013).

Soil enzyme participates in important ecosystem processes in soils, such as the decomposition of organic matter, the formation of soil humus, and the cycling of nutrients (Burns et al., 2013). Meanwhile, soil enzyme is known as a sensitive indicator of natural and anthropogenic changes in ecosystems, which is used to assess the impact of various pollutants including heavy metals contaminated in the soil (Ciarkowska, 2015; Rao et al., 2014). Heavy metals inhibited enzyme activity in several ways by masking catalytically active groups, denaturing the protein conformation or competing with metal ions (Karaca et al., 2010). Soil enzyme kinetics can depict the relationship between enzyme activity and substrate concentration, and provide a rapid approach for assessing the relative amounts of an enzyme and the catalytic characteristics in soils (Dick, 2011). The Michaelis-Menten equation was used to describe quantitatively this relationship. In the equation, the maximal catalytic reaction rate (V_{max}) at saturated substrate concentration and enzyme affinity (K_m) for its substrate could

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provide information of enzyme intrinsic properties. Exploring the kinetic characteristics of soil enzymes can provide a mechanistic understanding of how various biological processes occur and are regulated in heavy metal contaminated soils (Dick, 2011). For instance, after studying the kinetics of soil enzyme in loquat orchards polluted by Cu pollution, Fu et al. (2009) found that urease V_{max} and V_{max}/K_m were negatively correlated while invertase K_m was positively correlated with Cu concentration, indicating smaller amount of soil urease and lower affinity of invertase for substrate in Cu polluted soil than in uncontaminated soil.

Soil phosphorus cycling is one of the most important biological processes in soil environment. Soil phosphatase catalyzes the hydrolysis of ester–phosphate bonds, breaking down the organic phosphorus into inorganic form which is easily taken up by plants or microorganisms (Nannipieri et al., 2011). Therefore, phosphatase plays an important role in phosphorus cycling. Soil phosphatases are classified in acid phosphatase, neutral phosphatase and alkaline phosphatase according to the optimum pH (Nannipieri et al., 2011). Phosphatases are sensitive to heavy metals (Nannipieri et al., 2011). Many studies have suggested that As has inhibitory effect on soil phosphatase activity (Bhattacharyya et al., 2008; Das et al., 2013; Speir et al., 1999; Wang et al., 2017). Phosphatase activity then has been used as a bio-indicator to assess As toxicity (Nannipieri et al., 2011; Speir et al., 1999). However, these studies mainly focused on enzyme activities, the processes of As combination with enzyme and inhibition mechanism have not been researched thoroughly. Enzyme kinetics reflects the process of a reaction. Identification of changes for kinetic properties of soil phosphatase under As pollution could help in understanding how As effect on soil phosphatase and clarifying its environmental exposure risks (Dick, 2011). Furthermore, the toxicity based on enzyme activity may vary with the substrate type and concentration used to measure the enzyme activity (Tan et al., 2017a, 2017b). In addition, soil properties can influence As toxicity to soil phosphatase (Speir et al., 1999). The complex soil properties may affect the inhibition kinetics and reaction process resulting in different toxicity of As to soil phosphatase. To our knowledge, the kinetic characteristics of soil phospahtase-catalyzed reaction in response to As pollution and the influence of soil properties on As toxicity have not been characterized clearly. Thus, it is of importance to clarify the mechanism and reaction process of As inhibition on soil phosphatase to get an better understanding of soil quality and phosphorus cycling in As contaminated soils.

Therefore, this study has focused on soil acid phosphatase (ACP, EC 3.1.3.2) because of its wide spread presence in acid and neutral soils and in a certain amounts of alkaline soils from decomposition of plant debris (Nannipieri et al., 2011). We investigated the kinetic properties of soil ACP under exogenous As stress using soil samples representing 14 different agriculture soil types in China. The objectives of this study were to 1) investigate the reaction process and mechanism of As inhibition on soil acid phosphatase among various soils, and 2) determine the feasibility of using kinetic parameters to assess As toxicity and 3) reveal how soil properties affect As toxicity to soil ACP through enzyme kinetic study.

2. Materials and methods

2.1. Soil sampling and physicochemical analysis

Fourteen uncontaminated farmland soils with varied properties were sampled from different provinces in China (Fig. 1). The soils were selected to be representative of the major soil types and the distribution of soil pH of agricultural soils in China. Soil samples were taken from 0 ~ 20 cm depth and air-dried in the laboratory. Soils were ground and passed through a 1 mm nylon sieve before use. Soil physical chemical properties were determined using methods illustrated in Bao (2000) and presented in Table 1. Briefly, soil pH was determined by a pH electrode with a water: soil ratio of 2.5:1. Soil texture was measured using the pipette method (Kettler et al., 2001). Soil organic matter (SOM) was determined by potassium dichromate digesting and ferrous sulfate titration method (Walkley and Black, 1934). Total P was digested with melting sodium hydroxide and determined using molybdenum blue colorimetric method (Bao, 2000). Cation exchange capacity (CEC) was determined with ammonium acetate pH 7.0 (Sumner and Miller, 1996). Amorphous Fe oxide was extracted by ammonium oxalate and determined by flame atomic absorption spectrometry (Bao, 2000). The soil samples were digested using nitrohydrochloric acid and background total arsenic was determined by atomic fluorescence spectroscopy method (AFS) (GB/T, 22105.2, 2008).

2.2. Experimental design

Heavy metal reactions with enzyme are rapid processes. The changes in proliferation and enzyme synthesis by microbes can mask the toxicity if measured over a long period of exposure to heavy metal. Thus a shorter exposure time (e.g., 30 min) was suitable for determining the toxicity of heavy metal to soil enzyme (Matyja et al., 2016).

Three gram of air-dried soils were spiked with 3 ml As solutions ($Na_3AsO_4{\cdot}12H_2O$, AR, Sinopharm Chemical Reagent Co., Ltd, Shanghai China) to obtain final soil As concentrations of 0, 10, 25, 50, 100, 200, 400 and 600 mg kg^{-1} soil (*Weight*(soil): *Volume*(As) = 1:1) in a 50 ml flask then the flask were shaken to homogenize the soils and solutions. After 30 min of equilibration, the soil ACP activity was measured. Triplicate samples were prepared for each treatment.

2.3. Determination of soil ACP activity

Soil ACP activity was determined by the following procedure (Guan, 1986; Zavišić et al., 2016): 20 ml disodium phenyl phosphate (1, 2.5, 5, 10 mM) prepared in pH 5.0 acetate buffer were added to the soil mixtures that treated with As in the flasks, then the flasks were capped and incubated at 37 °C for 4 h. After incubation, the released phenol was determined colorimetrically at 510 nm, using 4-aminoantipyrine and potassium ferricyanide as coloring reagents. A substrate control (without soil) and an optional abiotic control (without substrate) were simultaneously incubated. The concentration of phenol was calculated using a standard curve (Guan, 1986).

2.4. Data analysis

2.4.1. Soil enzyme kinetics without As treatment

The two kinetic parameters, the Michaelis constant (K_m) and maximum reaction velocity (V_{max}) were determined by nonlinear regression of Michaelis-Menton equation:

$$V = V_{max}S/(K_m + S) \quad (1)$$

Where V is soil ACP activity, S is substrate concentration.

When K_m and V_{max} were determined, the catalytic efficiency was calculated by the ratio of V_{max}/K_m.

2.4.2. Soil enzyme kinetics with As treatment

Kinetic parameters will change at the presence of heavy metal inhibitors. Inhibitors of single substrate soil enzyme-catalyzed reactions can often be grouped as either simple competitive (unchanged V_{max} but increased K_m), simple noncompetitive (decreased V_{max} but unchanged K_m) inhibitors (Dick, 2011), and a linear mixed (decreased V_{max} and increased K_m) inhibitors (Cornish-Bowden, 2012). The competitive inhibition constant (K_{ic}) can be estimated by a linear plot of ${}^{app}K_m/{}^{app}V_{max}$ against As concentration (Eq. (2)), thus K_{ic} = intercept/slope (Cornish-Bowden, 2012).

$${}^{app}K_m/{}^{app}V_{max} = K_m/V_{max} + K_m/V_{max}{*}I/K_{ic} \quad (2)$$

where ${}^{app}K_m$ and ${}^{app}V_{max}$ are the apparent Michaelis constant and the