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Differences in the response of soil dehydrogenase activity to Cd contamination are determined by the different substrates used for its determination

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HIGHLIGHTS

- Different responses of soil DHA-TTC and DHA-INT to external Cd stress.
- DHA-INT is better than DHA-TTC to indicate and assess Cd contaminated soil.
- TOC content is major factor to influence Cd toxicity to DHA.

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ABSTRACT

Dehydrogenase activity (DHA) is an important indicator of heavy metal toxicity in contaminated soils. Different instances of DHA were determined using various substrates and which could affect the description of heavy metal toxicity. Currently, too few investigations have been done on selecting appropriate substrates. This study employed indoor simulation to determine soil DHA and its response to external cadmium (Cd) using two substrates (TTC and INT). Hormesis for DHA obtained using the TTC method (DHA-TTC) in low Cd concentration was observed which was quickly inhibited in high Cd concentration. While DHA obtained using the INT method (DHA-INT) decreased slowly when Cd concentration increased. The DHA-TTC and DHA-INT in soils at Cd concentration of 500 mg kg^{-1} decreased 86% and 53%, respectively, compared to the control. The dose-response relationship of Cd to DHA can be well simulated using the logistic model ($p < 0.01$), which indicated DHA could be used to indicate soil Cd toxicity. Multiple stepwise regression analysis revealed that total organic matter (TOC) is the major factor influencing the toxicity of Cd to DHA-TTC, while TOC, pH and cation exchange capacity (CEC) are major factors influencing the toxicity of Cd to DHA-INT. The different responses of soil DHA-TTC and DHA-INT to Cd are due to the differences in electron transport chain characteristics between TTC and INT, as well as the influence of soil properties. Although both DHA-TTC and DHA-INT can monitor soil Cd contamination, DHA-INT is recommended as a superior bio-indicator to indicate and assess contamination of Cd in soil.

1. Introduction

Soil is essential for food security and a key resource in human activity such as agriculture. However, intensive human activities have resulted in over-accumulation of heavy metals in soils causing environmental pollution (Luo et al., 2009). At present, 10%–20% of soil in China is contaminated by heavy metals (Ye et al., 2014), and Cd contamination is reported to be one of the most serious issues in arable land according to the Bulletin on National Survey of Soil Contamination, jointly issued in 2014 by the Ministry of Environmental Protection of China and Ministry of Land Resources of China.

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This increases the risk of adverse effects on human health via direct contact or accumulation in the food chain (Das et al., 2011). Meanwhile, elevated Cd concentrations in soil can have strong adverse effects on soil microorganisms and microbial functions (Smolders et al., 2009). An accurate assessment of the toxicity of Cd contamination on soil biochemical processes is helpful for identifying their environmental exposure risks and providing important information for protecting their ecological functions against environmental contamination (Chen et al., 2015).

Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in soil systems (Burns et al., 2013; Dick and Gupta, 1994). They respond rapidly to the natural and human disturbance of soil (Dick and Gupta, 1994). Dehydrogenase is one type of essential enzyme in living cells which participates in redox reactions, promotes dehydrogenation of soil organic matter, and transmits energy to hydrogen acceptors via the respiratory chain (Visser and Parkinson, 1992). Dehydrogenase activity (DHA) has been widely used to assess the general condition of microorganisms in soil and activated sludge, and DHA has been considered a good indicator of microbial oxidative activities (Wolińska and Stępniewska, 2012). The determination of soil DHA usually involves using 2, 3, 5-triphenyltetrazolium Chloride (TTC) and 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium Chloride (INT) as hydrogen acceptors. Compared to TTC, INT is reported to be a better substrate for determining soil DHA given its lower toxicity to cells, easier to eliminate and less interference from other oxidizers (Friedel et al., 1994; Gong, 1997; Mersi and Schinner, 1991). But it may underestimate DHA in high organic matter content soil (Friedel et al., 1994), and it has relative poorer solubility than TTC.

Many research reported that soil DHA could be considered an early and sensitive indicator of wastes or heavy metal stress (Araujo et al., 2015; Santos et al., 2011). However, TTC is more widely applied than INT to determine the response of soil DHA to environment stress (such as Cd contamination) (Antunes et al., 2011; Fernández-Calviño et al., 2010; Manzano et al., 2014; Moreno et al., 2001; Welp, 1999). It is reported that DHA-INT is less sensitive than DHA-TTC for determining heavy metals toxicity from sewage sludge, as the half maximal inhibitory concentration (IC_{50}) obtained from DHA-INT is higher than that from DHA-TTC (Yin et al., 2005). However, others reported that DHA-INT may be more sensitive to heavy metals stress than DHA-TTC (Mersi and Schinner, 1991; Welp, 1999), or both DHA-INT and DHA-TTC are imperfect (Gong, 1997). Because they have different oxidation reduction potential (ORP), which gives rise to INT higher electron affinity (+90 mV) compared to TTC (+490 mV). Michaelis constant (K_m) can be used to determine optimal substrate through measuring affinities of enzymes to substrates. The higher the K_m value is, the lower will be the affinities of enzymes to substrates. It was reported that the optimal K_m for DHA-TTC and DHA-INT ranged from 25 to 69 mM and from 4.0 to 7.9 mM, respectively (Masciandaro et al., 2000; Zhang et al., 2009). Moreover, it is difficult for TTC to enter microbial cells in order to participate in the respiratory chain of the electron transport system (Yin et al., 2005). Furthermore its reductive products are toxic to microorganisms (Friedel et al., 1994; Gong, 1997; Mersi and Schinner, 1991). These different characteristics of TTC and INT may be determined through the response of DHA to heavy metals toxicity.

Most of the literature focuses on DHA in sewage sludge, and there is a lack of experiment evidence for screening the best methods for investigating the soil DHA in response to heavy metals stress. We hypothesis that the response of DHA to Cd toxicity is due to the different substrates used for its determination. In this study, 18 farming soils were collected from different locations in China representing 14 different agriculture soil types, which were used to investigate the influence of Cd on DHA and the dose-response relationship using TTC and INT as subtracts. The aims of this study were to explore: 1) the mechanisms of DHA response to external Cd in various soils using the two substrates; and 2) the influence of different subtracts to soil DHA.

2. Material and methods

2.1. Soils sampling and preparation

A total of 18 soils covering a wide range of soil properties were sampled from primary production sites in China (Fig. 1), which were representative of the major soil types through China varying in soil pH and organic matter content in agricultural land. Each topsoil sample (0–20 cm depth) was taken from 5 subsites of an uncontaminated piece of farmland using a stainless steel spade and then mixed thoroughly. The coordinates of the sampling sites were recorded with GPS (GARMIN GPS72, Taiwan, China). All soils were air-dried at room temperature, homogenized, passed through a 1-mm sieve to remove plant debris and large stones, and then kept in sealed sample bags for further use (Trofymow et al., 1983).

The main physicochemical properties of the soils are showed in Table 1. Soil pH was measured by a glass electrode pH meter (PHS-3C, Leici, Shanghai, China) at a soil: water ratio of 1:1 (g mL^{-1}) (Mclean, 1982) which ranged from 4.90 to 8.80. The total organic carbon (TOC) content for these soils ranged from 4.92 to 27.38 mg kg^{-1} and averaged at 12.63 mg kg^{-1} which was determined based on an oil bath heating method (Nelson and Sommers, 1996). The cation exchange capacity (CEC) ranged from 8.12 to 31.11 cmol kg^{-1} was assessed via a 1 M ammonium acetate leaching method (pH = 7) (Gillman et al., 1983). Clay content was evaluated via the standard pipette method (Kettler et al., 2001) ranged from 6.66% to 45.94%. Calcium carbonate ($CaCO_3$) determined by a gasometric method (Leo, 1963) showed alkaline soils (pH > 7.5) had free $CaCO_3$, with the mean of 15.07 mg kg^{-1}. Total soil Cd was determined using ICP-MS (X-7, Thermo-Elemental, USA) after mixed acid digestion (HNO_3-$HClO_4$-HF) (Zarcinas et al., 1996). Background Cd concentrations ranged from 0.11 to 0.26 to mg kg^{-1}, which all are less than the class II Soil Environmental Quality standard in China (0.3 mg kg^{-1}, GB 15618–1995).

2.2. Experimental protocol

Heavy metal reactions with enzyme units are rapid processes and they last for a few hours (Matyja et al., 2016). Furthermore, the changes in proliferation of microbial cells and therefore enzyme synthesis can lead to misinterpretation of the toxicity measured over a long period of exposure to heavy metal. Matyja et al. (2016) suggested a shorter reaction time (e.g., 30 min) was suitable for determining the toxicity of Cd to soil DHA. In this study we used the TTC and INT methods for determination of DHA respectively proposed by Burns (1978) and Camiña et al. (1998).

Soil contamination with Cd and DHA-TTC determination: add 3 g dry soil into a 50 mL centrifuge tube, followed by adding 3 mL various concentrations of Cd^{2+} ($3(CdSO_4)\cdot 8H_2O$, AR, Xilong, China) solution to give the final soil Cd^{2+} concentration as 0, 0.6, 5, 25, 50, 100, 200, 300, and 500 mg kg^{-1} dry soil. After being mixed thoroughly, the tubes were left standing for 30 min at room temperature. After adding 0.03 g $CaCO_3$ (AR, Xilong, China) in all tubes and mixing them thoroughly again, 0.5 mL 3% TTC (BR, Duly, China) solution was added to the tubes which were then capped and static incubated at a constant temperature incubator for 24 h (30 °C, relative humidity of 70%). Then 10 mL methanol was added and all tubes were shaken for 5 min followed by centrifugation at 4000 r min^{-1} for 5 min. The supernatant was obtained and analyzed using