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Toxic responses of microorganisms to nickel exposure in farmland soil in the presence of earthworm (*Eisenia fetida*)

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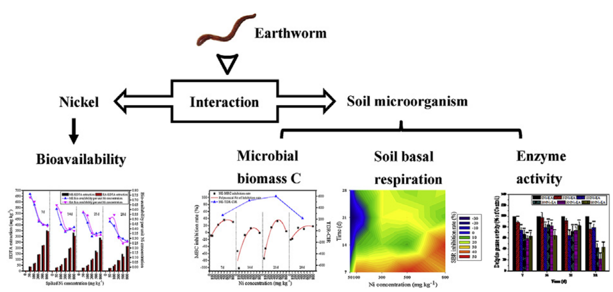
HIGHLIGHTS

- Effects of the presence of earthworm on MBC, SBR, qCO_2, UA and DHA under Ni stress were revealed.
- Variations of MBC/SBR inhibition rate under the conditions of double-factors of time and dose were explored.
- The time-dependent of dose-effect relationship was defined, and its regulations were disclosed.

GRAPHICAL ABSTRACT



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ABSTRACT

Nickel (Ni)-contamination impairs soil ecosystem, threatening human health. A laboratory simulation of Ni-polluted farmland soil study, in the presence or absence of earthworm, was carried out to investigate the toxic responses of soil microorganisms, including microbial biomass C (MBC), soil basal respiration (SBR), metabolic quotient (qCO_2), urease (UA) and dehydrogenase activities (DHA). Additionally, the variations of Ni bioavailability were also explored. Results manifested that MBC and SBR were stimulated at 50 and 100 $mg \cdot kg^{-1}$ of Ni but inhibited by further increasing Ni level, showing a Hormesis effect. Earthworm input delayed the occurrence of a maximum SBR inhibition rate under the combined double-factors of time and dose. No specific effect of Ni concentration on the qCO_2 was observed. UA was significantly suppressed at 800 $mg \cdot kg^{-1}$ Ni ($P < 0.05$ or 0.01), whereas DHA was more sensitive and significantly inhibited throughout all the treatments ($P < 0.01$), indicating a pronounced dose-response relationship. The addition of earthworm facilitated all the biomarkers above. The time-dependent of dose-effect relationship (TDR) on MBC and SBR inhibition rates suggested that the peak responsiveness of microorganisms to Ni stress were approximate on the 21st day. The bioavailable form of per unit Ni concentration declined with time expanded and concentration increased, and the changeable process of the relative amount of bioavailability was mainly controlled by a physicochemical reactions.

1. Introduction

Nickel (Ni) is considered to be a constituent of proteins and enzymes of microorganisms, plants and animals (J.Scott-

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Fordsmand, 1997), and has been defined as an essential element for human health by world health organization (WHO) (Parkin et al., 2005; Celen et al., 2015). However, the toxicity symptoms can occur when too much Ni is taken up. A positive correlation between Ni exposure and incidence of cancer (such as respiratory, lung and prostate cancer) has been reported (Schaumloffel, 2012; Chiou et al., 2015). In modern industry, metallic Ni and its compounds are used widely for unique physical and chemical characteristics. High utilization and consumption of Ni-containing products inevitably cause environmental pollution at all stages of production, use and disposal (Denkhaus and Salnikow, 2002). Heavy metals in soils, including Ni, will adversely affect soil fertility and productivity, and endanger the stability and functions of soil ecosystem (Wang et al., 2007). More serious is that it threatens people's health by transferring from soil to human through food chains (Luo et al., 2011).

Soil microorganisms are crucial to soil ecosystem for their irreplaceable functions in keeping energy flowing, maintaining nutrient cycling and soil structure, decomposing organic matters and restoring the imbalanced terrestrial ecosystem (Verstraete and Top, 1999; Preston et al., 2001; Chodak et al., 2013). Soil microbial biomass, basal respiration and enzymes are the most widely used microbial activity and processes indicators for soil disturbances, especially for heavy metals pollution (Kızılkaya et al., 2004; Wang et al., 2007; Chen et al., 2015a; Zhang et al., 2016). Microbial biomass C accounts for only a small fraction of organic carbon in soil, but it is the most active part of organic matters. A large number of reports have revealed that the microbial biomass C and soil basal respiration would decrease with increasing metal contents (Chen et al., 2014, 2015a), accompanied by the changes of microbial community, abundances and diversities (Li et al., 2014, 2015; Chen et al., 2015b). However, inverse results were also reported that no decrease happened to microbial biomass and respiration as metal contents increased, and microbial community response was also lacked (Schipper and Lee, 2004). In addition, microorganisms would be resistant to heavy metals, displaying a certain degree of stability when metals were re-added (Li et al., 2014). Metabolic quotient has been deemed as a sensitive and valid indicator of soil disturbance, especially for heavy metals (Valentim dos Santos et al., 2016), and it was observed increased with elevated heavy metals (Chen et al., 2015a). Soil enzymes with different sensitivities, responsible for diverse functions, have been used to indicate soil metal contamination. Urease and dehydrogenase were the two most commonly applied enzymes (Chaperon and Sauvé, 2007, 2008; Liu et al., 2014; Xian et al., 2015), and Ni was reported to curb these enzymes (Moreno et al., 2003; Tejada et al., 2008).

It is inappropriate to evaluate the mobility and availability of heavy metals by total metal concentration, since not all chemical speciation could be bio-used and transferred. The bioavailability of heavy metals in soils was defined as the fractions that could be directly or potentially transferred to living organisms (Leleyter et al., 2012). EDTA is one of the most used chelating agents in single leach to assess the potentially available metal fractions (Kim et al., 2016). It is assumed to be able to extract the exchangeable metal fractions, the elements bound to carbonates, element fractions combined with Fe or Mn oxide, and that fixed on organic matter (Rivera et al., 2016).

Earthworm, as a representative soil organism, stimulates soil properties, and their feeding, burrowing and casting activities are beneficial to soil aeration and drainage, affecting the toxicity of heavy metals to microbial populations (Wu et al., 2015; Li et al., 2016). Earthworm could increase microbial catabolic activity, promote hydrocarbon degradation (Schaefer, 2003), and improve soil nutrient availability (Schindler Wessells et al., 1997). Earthworm could also accumulate heavy metals, and their activities were supposed to cause the increase in soil pH and reduction in metal solubility (Karaca et al., 2010). Ramadass et al. (2017) reported that in the presence of earthworm, urease and dehydrogenase activities were significantly increased in diesel-polluted soils.

Numerous laboratory studies have focused on the changes of particular functions or population, community and diversity of soil microbes. However, the impacts and mechanisms of the presence of earthworm on microbial characteristics in Ni-contaminated soil were rarely reported. Toxicity of heavy metals is controlled by time and dose, but their joint effects on microbial processes are lacked and there is no quantitative parameter to reflect the changes of dose-effect over time. The objective of this study is to investigate the responses of soil microorganisms and microbial processes in Ni-treated soil in the case of earthworm addition or no earthworm. Specific indicators contain microbial biomass C (MBC), soil basal respiration (SBR), metabolic quotient (qCO_2), and enzyme activities (urease and dehydrogenase). Taking into consideration the conjunction effects of time and dose, investigate the inhibition rates of microbial actions. The influences of microbes and earthworms on available Ni and transition rules of bioavailability per unit Ni content with time and concentration will also be deeply explored. Furthermore, searching for a useful evaluation indicator to assess the time effect on dose-response manner is another aim. These findings would provide a scientific basis for the ecological risk assessment of Ni-polluted soil.

2. Materials and methods

2.1. Chemicals

Nickel (II) nitrate hexahydrate [$Ni(NO_3)_2 \cdot 6H_2O$], as well as other reagents used in our experiment were all analytical reagents and purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Soil collection and preparation

Top soil (0–20 cm) was collected from a vegetable field at Fengxian district, Shanghai, China. Stones, plants and animals residues and other large debris were removed, and then the remaining soil was air-dried at room temperature, ground and sieved (2-mm). The physicochemical properties of soil were as follows: silty loam consisting of clay 5.17%, silt 63.08% and sand 31.75%, pH of 7.67 (soil:water = 1:2.5 (w:v)), EC of 100.5 μS (soil:water = 1:5 (w:v)), water holding capacity (WHC) of 52.7% and organic matter (OM) of 8.13 $g \cdot kg^{-1}$.

500 g soil (dry weight) and Ni solution of according concentration diluted by stock solution were added to a 600-mL glass beaker, then stirred evenly and the soil moisture content was adjusted to 60% of the WHC with distilled water. The concentrations of the artificially Ni-contaminated soil were as follows: 0 (0Ni), 50 $mg \cdot kg^{-1}$ (50Ni), 100 $mg \cdot kg^{-1}$ (100Ni), 300 $mg \cdot kg^{-1}$ (300Ni), 500 $mg \cdot kg^{-1}$ (500Ni) and 800 $mg \cdot kg^{-1}$ (800Ni) on a dry weight basis. Soil moisture was monitored by weight loss and kept at 60% of the WHC by adding deionized water.

2.3. Earthworm

Earthworm *Eisenia fetida* (*E. fetida*) was obtained from Yonghe earthworm farm, Shanghai, China, and cultured in the mixture of manure and soil at 20 ± 1 °C in the dark for at least 2 weeks before experiment. Mature earthworms weighed 300–600 mg were selected and depurated gut for 24 h previous to adding into soil.