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Effects of heavy metals and soil physicochemical properties on wetland soil microbial biomass and bacterial community structure

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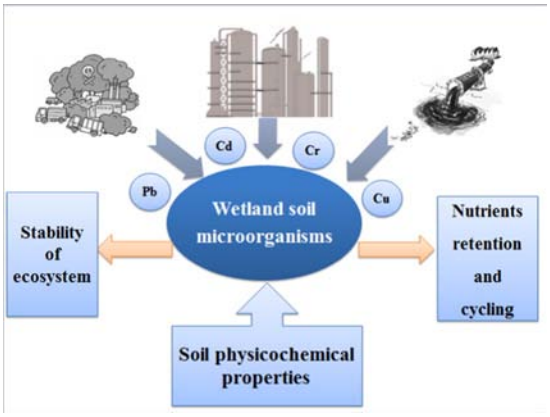
HIGHLIGHTS

- Soil microbial biomass declined with the increase of polluted level.
- Shannon–Weiner diversity index showed some difference but DGGE bands number did not.
- Cr, Cd, Pb and Cu explained 43.4% of the variation in bacterial DGGE profiles.
- Cr and Cd were the major factors to bacterial community structure change.

GRAPHICAL ABSTRACT



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ABSTRACT

Heavy metals (HMs) contamination is a serious environmental issue in wetland soil. Understanding the micro ecological characteristic of HMs polluted wetland soil has become a public concern. The goal of this study was to identify the effects of HMs and soil physicochemical properties on soil microorganisms and prioritize some parameters that contributed significantly to soil microbial biomass (SMB) and bacterial community structure. Bacterial community structure was analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Relationships between soil environment and microorganisms were analyzed by correlation analysis and redundancy analysis (RDA). The result indicated relationship between SMB and HMs was weaker than SMB and physicochemical properties. The RDA showed all eight parameters explained 74.9% of the variation in the bacterial DGGE profiles. 43.4% (contain the variation shared by Cr, Cd, Pb and Cu) of the variation for bacteria was explained by the four kinds of HMs, demonstrating HMs contamination had a significant influence on the changes of bacterial community structure. Cr solely explained 19.4% ($p < 0.05$) of the variation for bacterial community structure, and Cd explained 17.5% ($p < 0.05$), indicating Cr and Cd were the major factors related to bacterial community structure changes.

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1. Introduction

Wetland is one of the three ecosystems in the world. It plays a significant role in nutrients (carbon, nitrogen and phosphorous) retaining and cycling (Moreau et al., 2013). As the aggravation of population growing, agricultural fertilizer leaching and industrial wastewater discharging, wetland ecosystem has been increasingly influenced by heavy metals (HMs). Contamination and risk assessment of HMs in wetland soil have attracted worldwide attention for HMs' persistence, toxicity and non-biodegradable properties (Cheng et al., 2015; Liang et al., 2014). Microorganisms are always recognized as sensors towards disturbance in soil ecosystem for it is far more sensitive than macroorganisms to environmental stress (Giller et al., 2009; Khan et al., 2010). Substantial research efforts have been performed to evaluate the impacts of HMs on soil microbial biomass (SMB) and microbial community structure (Shentu et al., 2014; Sun et al., 2010; Xu et al., 2012). In most cases, accumulation of HMs had an adverse effect on microorganisms (Jiang et al., 2010; Wang et al., 2010; Zhang et al., 2015). However, some other studies reported no obvious relationship between HMs pollution and SMB or bacterial community structure (Grandlic et al., 2006; Zhu et al., 2013). For example, Gillan et al. (2005) reported that the biomass was not correlated with metal level, and the microbial diversity was not affected by the contamination. Grandlic et al. (2006) studied the indigenous sediment bacteria in Rush Lake responded to lead pollution and the result indicated that lead did not appear to affect bacterial community structure. Those conflicting reports clouded our understanding on the relationship between microorganisms and HMs. Moreover, many researchers indicated that soil physicochemical properties (soil organic matter, moisture, pH, soil type and so on) could influence HMs toxicity. The shifts in microbial community structure depend not only on HMs concentrations but also on those parameters (Boivin et al., 2006; Kenarova et al., 2014; Stemmer et al., 2007). So far, the effects of HMs and soil physicochemical properties on wetland soil microorganisms have not been evaluated simultaneously. It is of interest to perform such research to determine the effects of HMs and soil physicochemical properties on wetland soil microorganism.

In recent decades, the molecular biological technique polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) has allowed for a more complete analysis of microorganisms at a molecular level (Chodak et al., 2013; Epelde et al., 2012; Jousset et al., 2010). Correlation analysis and redundancy analysis (RDA) have been widely used to provide statistical tests for the correlation between environmental characteristics and microbes (Wu et al., 2015). Nowadays, combining of that molecular biological technique and analysis technique have been widely applied in dealing with microbes changes in HMs polluted soil (Machado et al., 2012; Zhu et al., 2013).

East Dongting Lake (EDT) wetland locates in the middle of Yangtze River (the third-longest river in the world). It plays a critical role in health and stability of global ecosystem. In our study, soil texture (clay was used as a representative for soil texture), moisture content (MC), soil organic matter (SOM), and pH with four kinds of HMs (Cd, Cr, Pb and Cu) were used as variables in analysis model. The main objectives of the study were: (1) to identify the effects of HMs and soil physicochemical properties on microorganisms and to find out parameter(s) which would be the pivotal one(s) affect the SMB and bacterial community structure; (2) to quantify the relationship between parameters and bacterial community structure. This investigation would lead to a deeper understanding of wetland soil micro environment.

2. Materials and methods

2.1. Study area

Dongting Lake (EDT is a part of Dongting Lake) is the second largest fresh water lake in China (Yang et al., 2011). EDT, at latitudes 28°59′N to 29°38′N and longitudes 112°43′ to 113°15′E, lies in the subtropical monsoon climate zone (Liang et al., 2015; Yuan et al., 2014). The annual precipitation is about 1100–1400 mm and average annual temperature is 16.4–17.0 °C. Wet season starts from May to October and dry season from November to March. According to previous researches three areas with different degrees of HMs were sampled in our study (Li et al., 2013; Liang et al., 2014). The first study area was Caisang Lake (CS). The next study area was Junshan Island (JS). And the third one was LuJiao Port (LJ). Those areas can be classified as (1) protected area (CS); (2) those with high human impact (JS) and (3) those with high industry impact (LJ). In order to reveal the HMs concentration as accurate as possible we selected three sites in each study area. The locations of the sample sites are shown in Fig. 1.

2.2. Sample collection

Nine soil samples were collected in March 2014. Samples were stored at a temperature of 4 °C, and then carried backed to laboratory within 4 h. Those soil samples were divided into two parts, one part was for molecular genome experiment (stored at −20 °C) (Wang et al., 2007). And the other part was for physicochemical properties and HMs experiment (air-dried and processed with a 2 mm sieve to remove stone and root fragments, stored at 4 °C).

2.3. Soil physicochemical properties and HMs

MC of soils was dried at 105 °C for 24 h. SOM was measured by loss-on-ignition method, ashing at 550 °C for 4 h (Wright et al., 2008). The pH was tested in deionized water at a soil/solution ratio of 1:2.5 using HI 3221 pH meter (Hanna instruments Inc., USA). Particle size distribution (soil texture) of soil was measured using a hydrometer method (Patrick, 1958). The total content of HMs was determined by TAS-990 flame atom adsorption spectrophotometer (Persee Inc., China).

2.4. Soil microbial Biomass

Our study used microbial biomass carbon (MBC) to express SMB. MBC was determined by chloroform fumigation-extraction method. The amount of carbon was extracted by K_2SO_4 (0.5 M, 20 ml) and measured by Shimadzu TOC-V CPH total organic carbon analyzer. The conversion factor between fumigation and un-fumigation is 0.37 (Ye and Wright, 2010).

2.5. Soil bacterial community structure (DNA extraction and PCR-DGGE)

Genomic DNA was extracted from 0.5 g soil using a soil DNA kit (OMEGA, USA). The extracted DNA was purified and then dissolved in 200 μl TE buffer and preserved at −20 °C before used.

The 16S rDNA genes were amplified with universal primer 338F/518R. (F338:5′CCTACGGGAGGCAGC3′ and R518:5′ATTACCGCGGCTGG3′) (Rölleke et al., 1999). The PCR mixture was composed of 1 μl of template DNA, 1 μl of each primer, 25 μl of 2 × Power Taq PCR Master Mix and adjusted to a final volume of 50 μl with sterile water. The PCR amplification was carried out as the follow steps: initial denaturing at 94 °C for 5 min, and then followed by 35 cycles consisting of denaturing (at 94 °C for 45 s), annealing (at 55 °C for 44 s), elongation (at 72 °C for 40 s), single elongation (at 72 °C for 5 min), and end at 4 °C. (Wu et al., 2013).

DGGE was performed using the Dcode™ Universal Mutation Detection System according to instruction (Bio-Rad, USA). The purified PCR products were loaded onto the 1-mm-thick 8% (w/v) polyacrylamide gels in 1 × TAE buffer using a denaturing gradient ranging from 30% to 70%. Electrophoreses were carried out at 60 °C for 12 h at voltage of 120 V (Zhang et al., 2011). Gels were stained with SYBR Green I nucleic

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