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Long-term effects of untreated wastewater on soil bacterial communities

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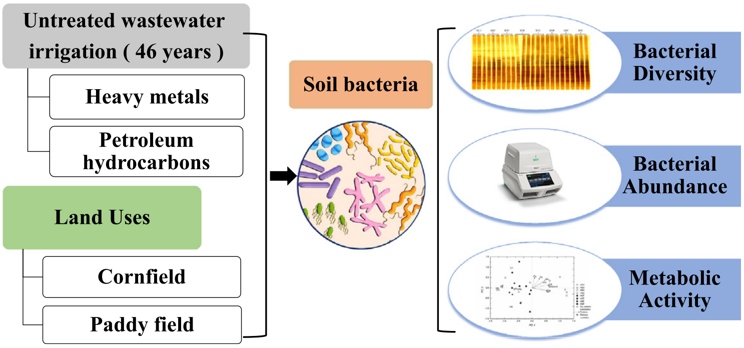
HIGHLIGHTS

- Assessing heavy metal and petroleum hydrocarbon toxicity in paddy and cornfields.
- Responses of bacteria in agricultural soil with untreated wastewater.
- Heavy metals in cornfield soils were more serious than that of paddy soils.
- The impact of petroleum hydrocarbons focused on soil bacterial composition.
- Different heavy metals disturbed microbial communities in different ways.

GRAPHICAL ABSTRACT



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ABSTRACT

For 46 years (1957–2002), irrigation with wastewater has increased the amount of heavy metal and organic contaminants in soils and altered bacterial communities in Shenyang, northeastern China. There has been characterization of the different heavy metal and petroleum contaminants in two types of land uses (cornfields and paddy fields). The Nemerow composite indices of heavy metal contaminants have been higher in cornfields (1.17–4.73) than those in paddy fields (0.57–1.64). Molecular-based techniques and biochemical-based techniques were used to analyze soil microbial diversity in our study. The metabolic activity of soil microbe communities was higher in paddy sites than that in cornfields. Organic pollutants such as saturated and polycyclic aromatic hydrocarbons have significantly affected soil bacterial compositions. Heavy metals differed in how they disturbed the microbial communities. Arsenic (As) and lead (Pb) shifted the community composition and decreased microbial diversity; copper (Cu) reduced bacterial abundance in soil; and cadmium (Cd) and chromium (Cr) lowered the metabolic capabilities of bacteria.

1. Introduction

Soil contamination is of great concern in croplands because of potential effects on food safety and soil ecosystems, especially microbial communities (Muhammad et al., 2005; Chen et al., 2015; Mekki et al., 2017).

The structure, abundance, and activity of soil microbial communities greatly affect the growth and health of plants and animals. The disturbance of soil microbes may reduce soil fertility, which may affect the quality of produce (Tecon and Or, 2017). Bååth et al. (E. Bååth, 1989; Fritze and Bååth, 1993; Erland Bååth et al., 1998; Erland Bååth et al., 2005; Fernández-Calviño et al., 2010) developed methods to investigate the responses of bacterial communities to different types of contamination under laboratory conditions. These methods have been used to

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determine the effects of soil Hg (Harris-Hellal et al., 2009), Cu, Cr, and Zn (Xie et al., 2009), total petroleum hydrocarbons (Nie et al., 2009), polycyclic aromatic hydrocarbons (Muhammad et al., 2005; Zhou et al., 2009) and other substances on soil microorganisms. Although the authors successfully applied monitoring techniques to assess the health of soil bacteria, the assay was complicated and not suited for field use (Liu et al., 2012). Thus, experiments involving field samples are warranted to help elucidate the in situ risks from these pollutants. Recently, comprehensive methods have been reported for in situ evaluation of the responses of soil microbial communities to pollutants (Yao et al., 2003; Burges et al., 2015; Liu et al., 2015; Zhang et al., 2016). Molecular-based techniques and biochemical-based techniques have been the two main categories of methods for analyzing microbial diversity in situ. Our study used denaturing gradient gel electrophoresis (DGGE) and real-time polymerase chain reaction (PCR) as molecular techniques to determine the composition and abundance of soil bacterial species, and we utilized Biolog EcoPlates as biochemical techniques to measure the metabolic capabilities of soil bacteria. These two techniques had different limitations. Additionally, there has been disagreement in the field regarding whether to rely on genetic diversity calculated by molecular techniques or functional diversity determined by biochemical techniques (Kirk et al., 2004; Liu et al., 2006; Paul, 2014). Based on current knowledge, the best way to study soil microorganisms is to use a variety of methods to obtain the broadest information on the soil microbial community (Philippot et al., 2012). The data obtained by both molecular-based and biochemical-based techniques were high – dimensional and involved multiple response variables. Calculating the relationships between these large sets of variables and the environmental factors present was complex. Therefore, the application of multivariate statistical analyses such as ordination approaches to gather information on the variables was better than 'simple' descriptive analyses in soil microbial ecology (Thioulouse et al., 2012; Buttigieg and Ramette, 2014). Using ordination approaches such as principal coordinates analysis (PCoA), principal components analysis (PCA), detrended correspondence analysis (DCA), redundancy analysis (RDA) and canonical correspondence analysis (CCA) could represent the relationships between sample and variables as faithfully as possible in a low-dimensional space, arranging the dozens or hundreds of variables along gradients and analyzing the effects of multiple environmental factors on variables simultaneously to obtain a good evaluation of soil quality and a good description of soil microbiological indicators (Šmilauer and Lepš, 2014).

Contaminants in cropland soils mainly originate from atmospheric deposition, wastewater irrigation, pesticide spraying, and fertilization (Luo et al., 2009; Xie et al., 2009). Wu et al. (2010) estimated that nearly one-fifth of Chinese farmland area was contaminated with heavy metals (Cheng, 2003). Application of wastewater for irrigation occurs worldwide to enhance crop yields using readily available water that is premixed with carbon, nitrogen, phosphorus, and major and micronutrients (Yadav et al., 2002; Sridhara Chary et al., 2008). However, wastewater may contain toxic pollutants that contaminate the soil and groundwater (Liu et al., 2005; Rattan et al., 2005). Hence, long-term wastewater irrigation can introduce toxic substances into the food chain (Sridhara Chary et al., 2008; Lu et al., 2015) and affect soil microbial communities (Yan, 2008; Zhang et al., 2008). The Hunpu area in our research was irrigated by wastewater from 1957 to 2002. The source of this wastewater was the Xihe River, which has high levels of contaminants in both the river water and its sediments (Table 2). Previous studies (Guo et al., 2011; Zhang et al., 2013a) have indicated that irrigated water was the major source of surface soil pollution in this area based on multivariate statistical analysis and isotope analysis. The sediments also cause a high potential toxicity for soils and groundwater near the river (Pang et al., 2003; Guo et al., 2011; Liu, 2011; Yang et al., 2011).

The effects of land uses on soil contamination and degradation were different (Liu et al., 2010; Zhao et al., 2012). In our study area, the cultivation of rice was sensitive to water shortages in this area. The soil had to be flooded in the transplanting, panicle formation and flowering of rice growth stages. The fields for corn do not require flooding in all growth stages. Previous studies (Zhang et al., 2012b) using geochemical parameter analysis have indicated that there was more serious organic pollution in the cornfields than there was in the paddy fields in the Hunpu area.

The objective of the present study was to assess the characterization of the heavy metal and petroleum contaminants in different land use types after wastewater irrigation and to determine the effects of pollution from long-term wastewater irrigation on bacterial communities including the composition and abundance of bacterial species and the metabolic capabilities of soil bacteria. Our hypothesis was that there would be decreasing concentrations of inorganic and organic contaminants as one moved further away from the pollution source, the Xihe River and that this gradient would have diverse effects on soil bacterial communities.

2. Materials and methods

2.1. Study site and sampling procedures

This study was conducted in an area irrigated with wastewater at Hunpu, Liaoning Province, in northeastern China (Fig. 1). The study site was an old industrial area and wastewater-irrigated area that was established in 1957. Liaoning Province has a temperate, continental monsoon climate with four distinct seasons. The mean annual precipitation and temperatures are 734.4 mm and of 7.8 °C, respectively (China N. M. I. C. o, 1981–2018). Rice paddies (often flooded) and cornfields (not flooded) are the predominant land uses in this region. The soil sampling area was located between the Hunhe and Puhe rivers, spanned 60 km from east to west and 20 km from north to south, and included 410,000 ha^2 (41° 30′ 15.6″ to 41° 39′ 56.2″ N; 122° 49′ 43.3″ to 123° 4′ 10.6″ E). The region receives low precipitation but is well supplied with wastewater rich in nitrogen (N), phosphorus (P), potassium (K), and other nutrients. Hence, the wastewater has been widely used to irrigate croplands in the area since 1957 (W.-h. Liu et al., 2005; Rattan et al., 2005). Irrigation water originated from the Xihe River (wastewater), the Hunhe River (clean water), and the Dahuofang Reservoir, with the latter having ratios of wastewater to clean water between 1:10 and 1:12 from 1957 until 2002. Initially, the Xihe River was mainly supplied with residential wastewater and then, with increasing industrial development in the 1980s, mostly industrial wastewater (Wu et al., 2012). >0.4 million tons per day of combined domestic and industrial wastewater, including sewage, were disposed in the Xihe River. This wastewater originated from Shenyang from the 78 large- and mid-size enterprises located along the river (Song et al., 2006; Yan, 2008). The high levels of hazardous and toxic material in untreated wastewater and other environmental concerns led to a prohibition by the local government in 2002 on using the water for irrigation (Xiao et al., 2008). This area is presently irrigated with water from the Hunhe River and shallow groundwater. Many wastewater treatment plants have been established along the Xihe River, but the sediment in this river contributes to potential toxicity of the soil and groundwater near the river (Table 2).

Soil samples were collected from four sites in a cornfield (H1U, H2U, H3U and H4U) and four sites in a rice paddy (H1P, H2P, H3P, and H4P) in May 2008 after harvesting the crops. Based on the preliminary tests, there was no success in finding a control site because all tested sites that possessed the same soil type were found to be contaminated after 46 years of wastewater irrigation (Data not show). At each location, three 2 × 2 m sample plots were randomly chosen, and within each plot, five samples were randomly taken. Each soil sample was collected 2–20 cm deep; then, the five samples from each site were combined to form one replicate. Soil samples were collected using a stainless steel shovel, placed in stainless steel containers, and then stored in an ice