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# Health risk to residents and stimulation to inherent bacteria of various heavy metals in soil



### Juan Zhang <sup>a,b</sup>, Li-Hong Wang <sup>c</sup>, Jun-Cheng Yang <sup>d</sup>, Hui Liu <sup>a</sup>, Jiu-Lan Dai <sup>a,\*</sup>

<sup>a</sup> Environmental Research Institute, Shandong University, Jinan 250100, China

<sup>b</sup> School of Metallurgical and Ecological Engineering, University of Science and Technology Beijing, Beijing 100083, China

<sup>c</sup> Shandong Analysis and Test Center, Shandong Academy of Sciences, Jinan 250014, China

<sup>d</sup> Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China

#### HIGHLIGHTS

• Health risks of Mn and As were higher than other metals and metalloids.

• Bacteria species richness was determined by Mn, As, Pb, and OM.

• Bacterial species evenness was determined by Mn, pH, N, C, Cd, and Pb.

• Specific bacteria were identified in the soil with high or highest risks.

• Mechanisms of pollutant resistance and element cycling were discussed.

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#### ABSTRACT

The toxicities and effects of various metals and metalloids would be misunderstood by health risks based on their concentrations, when their effects on bacterial and ecological functions in soil are disregarded. This study investigated the concentrations and health risks of heavy metals, soil properties, and bacterial 16S rRNA gene in soil around the largest fresh water lake in North China. The health risks posed by Mn and As were higher than those of other heavy metals and metalloids. Mn, As, and C were significantly correlated with the bacterial species richness indices. According to canonical correspondence analysis, species richness was mainly affected by Mn, Pb, As, and organic matter, while species evenness was mainly affected by Mn, pH, N, C, Cd, and Pb. Covariable analysis confirmed that most effects of metals on bacterial diversity were attributed to the combined effects of metals and soil properties rather than single metals. Most bacteria detected in (almost) all soil were identified as *Gammaproteobacteria*. Specific bacteria belonging to *Proteobacteria* (*Gamma*, *Alpha*, *Epsilon*, and *Beta*), *Firmicutes*, *Actinobacteria*, *Cyanobacterium*, *Nitrospirae*, and *Fusobacterium* were only identified in soil with high concentrations of Mn, Pb, and As, indicating their remediation potency. Bacterial abilities and mechanisms in pollutant resistance and element cycling in the region were also discussed.

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#### 1. Introduction

Various sources such as wastewater irrigation, solid waste disposal, sludge application, vehicular exhaust and atmospheric deposition result in wide-spread heavy metal contamination in soil (Wang et al., 2005). Depending on their concentration, heavy metals in soil can result in a wide range of toxic effects on humans, plants, animals, and microbes (Caliza et al., 2012; Qu et al., 2012). The hazard quotients (HQ) of the US Environmental Protection Agency (EPA) are extensively used to characterize the noncarcinogenic health effects of heavy metals by comparison of their effects from exposure to a reference dose (RfD) (Qu

\* Corresponding author. *E-mail address:* daijiulan@sdu.edu.cn (J.-L. Dai). et al., 2012). And the RfD values were from various published references based on various epidemiological investigation and rat chronic study (Connor et al., 2007). Furthermore, it is important to combine both biological responses and chemical analysis for integrated environmental assessment because of limitation of chemical monitoring (Blasco and Picó, 2009).

Bacterial communities present important functions in soil because of their contribution in nutrient cycling, plant symbioses, decomposition, and other ecosystem processes. The effects of heavy metals on bacterial growth, morphology, biomass, and activity have been reported (Fliessbach et al., 1994; Rajapaksha et al., 2004). Molecular methods have been applied to analyze the effects of heavy metals on the total bacterial community structure (Sandaa et al., 2001). The 16S rRNA gene cloning approach, an extensively used molecular method could provide new insights into bacterial diversity and dynamics in environments (Yakimov et al., 2005). The similarities and dissimilarities of bacterial community composition, as well as the effect of heavy metals and irrigation on bacterial community have been studied using CANOCO 4.5 (Frey et al., 2006; Nguyen-Viet et al., 2007). The results from the multivariate statistical analysis of an entire dataset are more robust than those from the analysis of diversity indices. However, previous studies only focused on species richness and did not cover the effects of heavy metals on species evenness or link the bacterial diversity to material and energy circling. Moreover, how health risks of various heavy metals in soil are correlated with the effects of heavy metals on soil bacterial diversity is yet to be reported.

Nansi Lake in North China is one of the largest inland freshwater lakes in the east route of the National South-to-North Water Transfer Project. The economy of the Nansi Lake region has been rapidly developing since the 1970s, and this rapid progress has led to an excessive release of pollutants into the lake. The sediments of Nansi Lake and its main inflow rivers have been polluted by heavy metals (Li et al., 2011; Yang et al., 2004). Therefore, more attention should be directed to the heavy metals in the agricultural soil irrigated with water from the lake. This study aims to explore the main influence factors on the bacterial richness and evenness in the soil in the region, to study the sensibility of bacterial diversity to heavy metals with various health risks, to reveal the mechanisms underlying microbial resistance to heavy metals, and to link the effects of heavy metals on bacterial diversity through material and energy circling using various statistical analyses, risk-based corrective action (RBCA) tool kit for chemical releases, and community characterization based on 16S rRNA gene analysis.

#### 2. Material and methods

#### 2.1. Sampling

In October 2012, surface soil (0 cm to 20 cm) was collected in the Nansi Lake region, which is located in the southwest of Shandong Province, China. The region has 720,000 ha under cultivation and experiences a typical temperate monsoon climate. The annual mean temperature in the region is 13.7 °C, and the annual mean precipitation is 750 mm. The principal soil type includes clay loamy alluvial soil, and rice paddy fields were one of the dominant uses to which the land was put. The irrigation water in this area was from the heavily contaminated Nansi Lake. Five sampling areas (SZ, JX, TF, XZ, and WS) were selected along the direction of lake flow (from north to south). Three soil units distant from the roads were randomly selected in each sampling area (Fig. 1). Five diagonal subsamples were sampled, homogeneously mixed to obtain one sample, and then placed in a sterile plastic bag in each unit. The 15 samples (5 areas  $\times$  3 units) were transported to the laboratory at 4 °C. A portion of each sample was ground to 2 mm and kept frozen at -80 °C for bacterial community structure analysis. The other portions were air-dried and ground for basic soil properties and heavy metal content analyses.

2.2. Determination of concentration and analyses of health risk of heavy metals

The concentrations of metals and metalloids (Cd, Pb, Cr, Cu, Mn, Ni, Zn, Hg, and As) in soil were measured after their digestion in a mixture

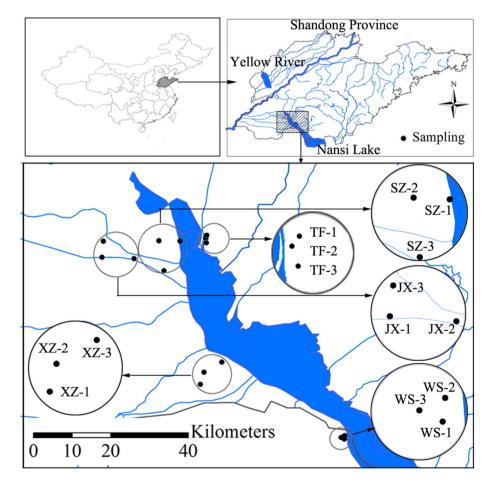


Fig. 1. Location of sampling sites in the Nansi Lake region.

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