Investigating organic matter properties affecting the binding behavior of heavy metals in the rhizosphere of wetlands

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ABSTRACT

Soil organic matter (SOM) is a crucial factor affecting the immobilization of heavy metal in wetlands. Recent studies have shown that the rhizosphere SOM has great ability to immobilize heavy metals. However, there existed few works on studying molecular characteristics of SOM to explore the mechanisms. Electrospray ionization–Fourier transform ion cyclotron resonance–mass spectrometry (ESI-FTICR-MS) combined with FTIR spectroscopy were applied to investigate the characteristics of SOM in rhizosphere and nonrhizosphere samples and to find out what characteristics the rhizosphere SOM embodies conducive to metal binding in this paper. The rhizosphere contained higher C, P, Mn, and other metal concentrations. The adsorption of Cr on rhizosphere SOM was greater than that on nonrhizosphere SOM. Compared to nonrhizosphere SOM, rhizosphere SOM contained less saturated and more oxidized compounds, greater overall molecular weights (MW), more condensed aromatic structures (56.59% VS 51.56% by peak intensity), less carboxylate and N-containing COO functional groups (25.98% VS 56.63% by peak intensity), more hydrophilicity, and the latter four are conducive to metal binding. This study showed that the rhizosphere SOM had unique compositional and structural characteristics. These results provided evidence for the phytoremediation technologies of heavy metal contaminated wetlands.

1. Introduction

Wetland ecosystems, a significant part of the whole biosphere, are closely related to human activities and have a great ability to purify the environment (Huang et al., 2014; Nie et al., 2018). Due to special ecological environment such as rich vegetation and high humidity, wetlands tend to accumulate high contents of SOM which usually promote the retention of water-borne metals (Huang et al., 2018; Qiu et al., 2011; Tam and Wong, 2000). The variations in SOM properties such as pH, cation exchange capacity (CEC) and SOM contents greatly influence the ability of wetlands to immobilize heavy metal (Wen et al., 2018). Previous studies observed that the increase of SOM content could increase the proportion of HA-metal (Humic acid) complexes among the total immobilized metals (Shuman, 1975; Fan et al., 2016). Hence compared to other factors, SOM serves as an important factor in immobilizing heavy metal in wetlands mainly by adsorption, complexation, or chelation (Fan et al., 2016; Yang et al., 2018).

The rhizosphere is a zone of the root-soil interface with the range from only a few microns to a few millimeters which is closely influenced by plant root and correlative microbial activities (Curl and Truelove, 1986). The low molecular organic acids in the rhizosphere play an important role in promoting metal binding on the rhizospheric soil colloids under different environmental circumstances (He et al., 2009; Park et al., 2011). The effects of plant roots on heavy metal immobilization are obvious in wetlands because of the release of O_2 into the rhizosphere which promotes the growth of microorganisms which could composite molecules and the formation of iron manganese plaque which could bind SOM with metals on it, increasing heavy metal immobilization in the rhizosphere compared to the nonrhizosphere (Huang et al., 2016). Therefore, increasing SOM contents and relatively oxidative states in the rhizosphere of wetlands could benefits the immobilization of metals (Huang et al., 2016; Du Laing et al., 2009).

In the riverside near the mining site or smelting works, soil, especially in the rhizosphere of soil, usually has great impacts on the immobilization of heavy metal (Qiu et al., 2011; Dybowska et al., 2006; Reichman, 2002). In the study of Kaplan et al. (2016), electrospray ionization–Fourier transform ion cyclotron resonance–mass spectrometry (ESI-FTICR-MS) was used to study the specific properties of rhizosphere and nonrhizosphere SOM, and it was the first to explore the probability that the compositional characteristics of SOM in the
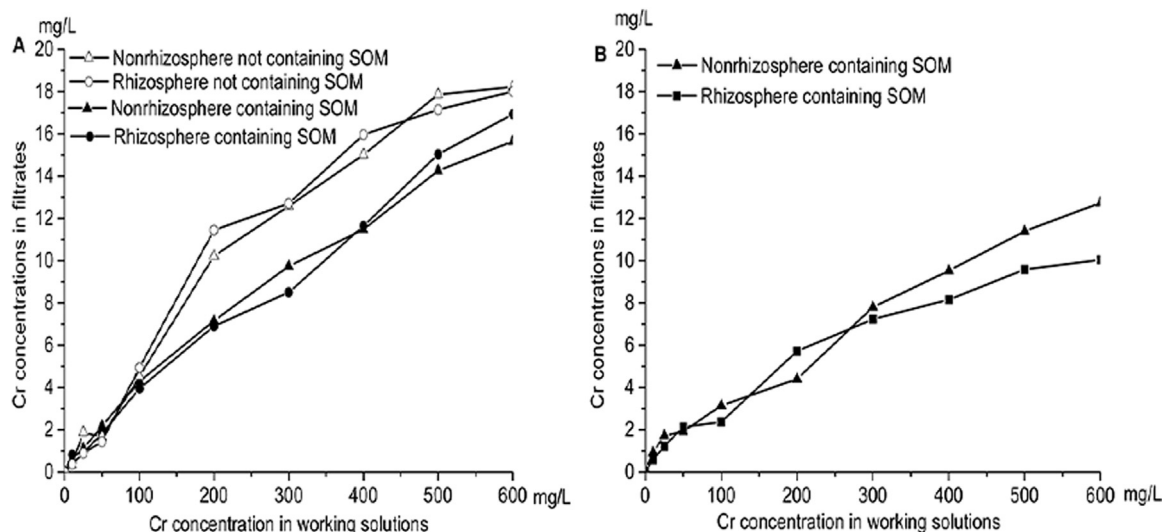
Fig. 1. The results of chromium adsorption experiment shown from the concentrations of Cr in the filtrates of the rhizosphere and nonrhizosphere SOM samples extracted from two replicates ((A) and (B)).

rhizosphere in wetlands may promote the immobilization of metals. However, there existed some limitations in previous studies: (1) ESI-FTICR-MS could only provide the information about molecular formulas while the information about molecular structures was limited. Many molecules have their isomers; hence the results of specific SOM characteristics could not be obtained exclusively from the information about molecular formulas. (2) ESI-FTICR-MS is not a quantitative technique for analyzing SOM in the absence of standards. Therefore, more techniques of quantitative analysis should be applied to complement the data.

Based on these limitations, Fourier transform infrared (FTIR) spectroscopy was adopted into this study to supplement part of the structural information through the detection of functional groups and quantitative analysis by detecting the functional groups and their peak intensity of the samples (Baloglu et al., 2015; Chen et al., 2015; Huang et al., 2017). Furthermore, this study collected different sampling points to obtain more accurate conclusions. The objective of this paper was to study the difference of SOM characteristics between the rhizosphere and the nonrhizosphere of soil in wetlands near the chromium salt factory and to find out what characteristics the rhizosphere SOM embodies conducive to metal binding. Our hypothesis was that the rhizosphere SOM contained more compositional and structural characteristics that were conducive to metal immobilization because of its distance to the roots. Rhizosphere and nonrhizosphere soil samples were separated and analyzed to obtain SOM compositions and structures by ESI-FTICR-MS and FTIR spectroscopy, respectively.

2. Materials and methods

2.1. Sampling and general soil characterization

The study site was located in the washland along the Xiang River in Hunan province (Huang et al., 2015); there existed a chromium salt factory. The study site was presented in Fig. S1 (see Supporting information (SI)). This river received depleted chromium from the chromium salt factory with the shortest distance only ~ 75 m. An average of about 15 cm deep soil samples were obtained in March 2017 from the washland including 9 points from upper reaches to lower reaches of the factory. The 9 points were divided into three groups with each group had a distance about 500 m. Each group covered three points, and they were about 10 m from each other. Each point ~ 500 g sample containing undisturbed roots (from reed) and soil was dug up by a shovel. The plant species selected is thought to be one of many influencing

factors on the rhizosphere composition (Hou et al., 2017). However, this factor is out of our present study and will be considered fully in the next projects. Once dug out, the samples were divided into rhizosphere and nonrhizosphere subsamples. The rhizosphere subsamples were manually classed as soil near the plant root generally within 1–10 mm distance from the root and they were separated from the roots with a trowel. The remaining soil was collected as nonrhizosphere subsamples. Each subsample was then placed in a valve bag and stored in the approximately vacuum environment by manually cramping out the air. The plants and roots of each sample were also stored in the independent valve bag. All of these samples were transported instantly to the laboratory where they were stored in a moist state at 4 °C.

About 200 g of each soil sample was collected, and the sample we choose to have a test was No.4 (Fig. S1) because of its representative features. The soil sample was used to analyze soil general properties such as triplicate pH, particle size, and total element content. Furthermore, triplicate chromium adsorption experiments, FTIR spectroscopy analyses, and duplicate ESI-FTICR-MS analyses were then conducted. The pH values set at 1:4 solid: liquid suspensions (soil in moisture) were measured after equilibration for 24 h. The soil particle size was tested using the Beaker method and the total element contents without total P (TP) and total organic carbon (TOC) were obtained through inductively coupled plasma–optical emission spectrometry (ICP–OES) (Optima 5300 DV) after fully digested (Kettler et al., 2001; Makonnen and Beauchemin, 2015). The TP and TOC were determined according to *Soil Agro-chemical Analysis* (Gardi, 2001).

2.2. Chromium adsorption experiment

A 1.0 g of each rhizosphere and nonrhizosphere soil sample was weighed into a 50 ml polyethylene centrifuge tube. The sample was added with a control experiment (samples with SOM extracted with methanol, defined as "not containing SOM" in Fig. 1), in which rhizosphere and nonrhizosphere SOM were depleted with 5 ml 5% of methanol in water (This comparison test designed to correspond with the ESI-FTICR-MS analysis). All samples were added with 20 ml $Cr(NO_3)_3$ solution with different concentration levels (0, 10, 25, 50, 100, 200, 300, 400, 500, 600 mg/L) (the concentration of working solutions diluted from 1000 mg/L $Cr(NO_3)_3$ solution) and 2 ml 0.01 M $NaNO_3$ solution serving as background strength. Each sample was adjusted to the initial soil pH values. Then the soil suspensions were shaken on a reciprocal shaker at 240 rpm for 4 h at 25 °C and then equilibrated in the box for 12 h. The tubes were then centrifuged at $5000 \times g$ for 5 min and