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THE METABOLIC DIVERSITY OF SOIL MICROBIAL COMMUNITY IN THE PETROLEUM EXPLOITATION AREA OF NORTHERN SHAANXI, CHINA

La diversidad metabólica de la comunidad microbiana en el área de explotación petrolera de Shaanxi del Norte, China

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Key words: petroleum pollution, soil microorganisms, carbon source, metabolic activity, Northern Shaanxi.

ABSTRACT

In order to explore the changes of soil chemical properties and microbial metabolic diversity under different petroleum pollution levels, conventional soil detection technology and Biolog® technology were used to study the soil chemical properties and microbial community characteristics of the clean soil in the north Shaanxi oilfield under four different petroleum pollution gradients. The results showed that the soil after petroleum pollution had higher total organic carbon content, higher carbon nitrogen ratio and lower total nitrogen content. Petroleum had a significant effect on the metabolic capacity of soil microbial communities from carbon sources and their utilization patterns. There were significant differences in microbial community metabolism in petroleum-contaminated soils at different concentrations, with a decreasing trend with increasing contamination. At the same time, the microbial activity that could utilize carboxylic acid compounds and polymers was higher in the contaminated soil, while the microbial activity that utilized amino acids and carbohydrates was higher in the clean soil. With the increase of pollutant concentration, the metabolic pattern of soil microbial community to carbon sources changed from sugar-based to polymers-based. This indicates that oil pollution has changed the use of some preferred carbon sources by soil microorganisms, and long-term pollution will stabilize the carbon sources available to microorganisms and promote the development of microbial communities that prefer polymeric substances as carbon sources. This provides theoretical guidance and data support for the bioremediation and treatment of petroleum-contaminated soil in loess hilly areas.

Palabras clave: contaminación por petróleo, microorganismos del suelo, fuente de carbono, actividad metabólica, Shaanxi del Norte.

RESUMEN

Con el fin de explorar los cambios de las propiedades químicas del suelo y la diversidad metabólica microbiana bajo diferentes niveles de contaminación por petróleo, se utilizó la tecnología convencional de detección de suelos y la tecnología Biolog® para estudiar las propiedades químicas del suelo y las características de la comunidad microbiana del suelo limpio en el yacimiento petrolífero de Shaanxi norte bajo cuatro gradientes diferentes de contaminación por petróleo. Los resultados mostraron que el suelo después de la contaminación por petróleo tenía mayor contenido de carbono orgánico total, mayor relación nitrógeno carbono y menor contenido de nitrógeno total. El petróleo tuvo un efecto significativo en la capacidad metabólica de las comunidades microbianas del suelo a partir de fuentes de carbono y sus patrones de utilización. Hubo diferencias significativas en el metabolismo microbiano comunitario en suelos contaminados con petróleo en diferentes concentraciones, con una tendencia decreciente con el aumento de la contaminación. Al mismo tiempo, la actividad microbiana que podía utilizar compuestos de ácido carboxílico y polímeros era mayor en el suelo contaminado, mientras que la actividad microbiana que utilizaba aminoácidos e hidratos de carbono era mayor en el suelo limpio. Con el aumento de la concentración de contaminantes, el patrón metabólico de la comunidad microbiana del suelo a las fuentes de carbono cambió de base de azúcar a base de polímeros. Esto indica que la contaminación por petróleo ha cambiado el uso de algunas fuentes de carbono preferidas por los microorganismos del suelo, y la contaminación a largo plazo estabilizará las fuentes de carbono disponibles para los microorganismos y promoverá el desarrollo de comunidades microbianas que prefieren las sustancias poliméricas como fuentes de carbono. Esto proporciona orientación teórica y datos de apoyo para la biorremediación y el tratamiento de suelos contaminados con petróleo en áreas montañosas con suelos loess.

INTRODUCTION

Soil microorganisms are the main decomposers in the ecosystem, and their types, numbers and functions are extremely important to the health and function of the ecosystem. However, this series of characteristics are also affected by biotic and abiotic factors. The metabolic function of the soil microbial community refers to the microbial activity, substrate metabolic capacity and the functions related to the conversion of N, P, S and other nutrient elements in the soil. Generally, some transformation processes in the soil are determined by analysis, such as organic carbon and nitrogen. The functional characteristics of the microbial community are generally investigated by analyzing and measuring some transformation processes in the soil, such as organic carbon, soil nitrogen fixation rate, nitrification and enzyme activity (Donnison et al. 2000). To analyze the utilization function of soil microbial carbon source, the Biolog microplate method was used, reflecting the information about the overall activity and metabolic function of microbial community (Wartiainen et al. 2008). The diversity of soil microbial community structure and composition helps maintain the stability and sustainability of the soil ecosystem (Zuo et al. 2020), and at the same time improves the buffering capacity of microbes against the deterioration of the soil micro-ecological environment (He et al. 2020, Piao et al. 2009). Due to the high sensitivity of soil microbes to environmental changes and the high heterogeneity of soil, as well as the complex relationship between soil microbes and plants, there are still many uncertainties in the impact of environmental factors on the composition of soil microbial communities (Rinnan et al. 2007).

In recent decades, environmental pollution caused by petroleum and petrochemical products has attracted much attention (Duarte et al. 2020). Petroleum is a complex mixture containing thousands of chemical compounds, including saturated alkanes, aromatic hydrocarbons, asphaltenes and non-hydrocarbon compounds (Kavamura and Esposito 2010, Xu et al. 2012, Zeng et al. 2013). Because petroleum hydrocarbon (APH) is a common organic pollutant with high hydrophobicity and persistence, it causes a large amount of enrichment in the soil, and has a continuous negative impact on the function of the soil, and a negative impact on the microorganisms and plants of the entire ecosystem. Also, it causes different degrees of harm to the animals living in the area (Weghe et al. 2006, Zhang et al. 2012). At present, the research on oil-contaminated soil microorganisms mainly focuses on soil microbial remediation effects, such as the screening of high-efficiency degrading bacteria and the study of degradation characteristics, while the research on the structural and functional diversity of oil-contaminated soil microbial communities are very limited (Zhang et al. 1999, Julio et al. 2014, Sun et al. 2015). Soil microorganisms are very sensitive to any alterations due to the disturbance of pollutants, especially, they will change rapidly in their community diversity and activity (Schloter et al. 2003). The microbial community is of great significance to the stability of the entire underground ecosystem (Brohon et al. 2001, Eibes et al. 2006, Wu et al. 2011).

This project takes the oil well area in northern Shaanxi as the research object, and study its oil pollution status and microbial characteristics. By exploring the changes in soil chemical properties and microbial metabolic diversity under different levels of oil pollution in the study area, the stability of the soil ecosystem under different pollution levels will be revealed, thus providing a scientific and theoretical basis for the evaluation of soil environmental quality and the bioremediation and treatment of contaminated soil in the area.

MATERIALS AND METHODS

Study area

Yan'an, the study area, is located in a typical soil area of the Loess Plateau, with an average elevation of about 1089 meters. It is a typical loess plateau hilly area in northern Shaanxi. The annual average temperature is 8.8 ºC. The average temperature in the coldest month (January) is about -7.2 ºC, the extreme minimum temperature is -23.6 ºC, the hottest month is July (36.8 ºC), and the annual sunshine hours are 2397.3 hours. The early frost starts in early October and the late frost ends in late April. The frost-free period is 143 to 162 days. The average annual rainfall is about 328.4 mm, accounting for about 63% of the annual rainfall. Spring is dry and less rainy, summer temperature is hot and humid, drought and rain are alternating, autumn is cool and rainy, temperature drops quickly, frost and snow come early, and winter is cold and dry. Natural disasters mainly include drought, frost, rainstorm and hail. The soil is mainly loess developed on the parent material of loess after the erosion of black loess soil, with low soil fertility and low productivity.

Sample collection and processing

Sample collection

Soil samples were collected from a typical oil production area in Baota District, Yan'an City, northern Shaanxi Province, in an area not contaminated by oil near 200 m of oil production wells, following the principles of random, equal and multi-point mixing, at a depth of 0-30 cm. Three sampling points were selected to collect equal samples, and the samples from three sampling points were combined into one sample, passed through a 2 mm sieve and stored at low temperature after removing impurities. The samples were brought back to the laboratory for indoor simulation and sample testing. The geographic information of the sample collection sites is shown in **Table I**.

TABLE I. GEOGRAPHICAL INFORMATION RELATED TO SOIL SAMPLE COLLECTION SITES.

Sample number	Elevation	Longitude	Latitude	Place names
YA1	1058	109º58′63″	36º81′13″	Qinghuazhen Town
YA2	1176	109º61′72″	36º81′34″	Qinghuazhen Town
YA3	905	109º37′92''	36º41′98''	Yao Dian Town

Indoor simulation test

The indoor simulated petroleum contaminated soil had five different gradients, which were 0%, 0.5%, 1%, 2%, and 4%. The crude oil was added to the collected soil samples according to the mass oil content of 0%, 0.5%, 1%, 2% and 4%, respectively, and three replicates were set up for each treatment, mixed thoroughly, left to age for one week and incubated for 10 days under constant conditions, i.e., constant temperature (27º) and field water holding capacity (20-24%). A portion of the soil was stored at 4º to determine the metabolic characteristics of the microbial community; the other portion was air-dried, grounded and sieved to determine the chemical properties of the soil (Wang et al. 2009, Zhen et al. 2015).

Sample determination

The chemical properties of the soil were measured by conventional methods. The total soil carbon was measured by potassium dichromate oxidation-external heating method, total nitrogen by Kjeldahl method, the soil moisture content by the drying method (105ºC), and the pH value by the potentiometric method (water: soil = 2.5:1). Data were analyzed using one-way ANOVA. Multiple comparison tests were performed using LSD with a significance level of $p < 0.05$. Statistical analysis and regression analysis were performed using SPSS 20.0 software. (Song et al. 2013).

A Biolog-Eco® with 31 carbon sources was used to analyze the diversity of metabolic functions of the microbial community. For the preparation of the inoculum of the ECO plate we followed the method of Zheng et al. (2013). First, the soil sample was activated at 25 ºC for 24 h, 3 g of soil was placed into 27 mL of 0.85 mol/L NaCl solution, and shaked at 200 r/min for 30 min. Three mL of supernatant were added to 27 mL of NaCl solution, the final dilution ratio was 1:1000. 150 µL of diluent were added into each hole of ECO plate, and the diluent of each soil sample was set in three parallel groups and placed in a 25 ºC thermostatic incubator, at 24 h, 36 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h. The measurement wavelength was 590 nm (color+turbidity) and 750 nm (turbidity) (Yue et al., 2011). Microbial metabolic activity was represented by the absorbance value at 590 nm minus the absorbance value at 750 nm. If the value was less than 0.06, it was treated as 0 (Barragán et al. 2008).

The calculation formula of Average Color Change Rate of Hole (AWCD) is:

$$AWCD = \frac{\Sigma(\mathrm{C} - \mathrm{R})}{n} \quad (1)$$

Where C is the optical density difference between the two bands of each carbon source hole; R is the optical density value of the control hole; n is the number of medium carbon source types.

RESULTS

The influence of petroleum pollution on soil chemistry

It can be seen from **Table II** that there is no significant change in soil pH under different oil pollution treatments, except for the concentration of 4%. The soil pH under other pollution treatments is higher than that of unpolluted soil. This indicates that petroleum contamination increases soil alkalinity by within a certain contamination concentration range, but not significantly (Wang et al. 2010). The water content of oil contaminated soil was significantly increased under the high concentration treatments of 2% and 4%. Soil organic matter content increased with increasing petroleum contamination, except between 0.5% and 1%. All treatments reached significant levels, and organic matter content was significantly and positively correlated with petroleum content, which is due to the fact that crude oil contains a variety of substances such as hydrocarbons, organic matter and inorganic salts. The increased organic carbon content in soil, led to soil C/N imbalance and affected the growth and metabolism of various microorganisms (Ding et al. 2020). The total nitrogen in soil decreased first and then augmented with the increase of oil pollution concentration, but was not significant. It can be seen that there was no obvious relationship between soil nitrogen content and petroleum hydrocarbon content, which was consistent with the research results of Wang et al. (2010).

Effect of petroleum pollution on soil microbial metabolism activity

During the 240 h incubation period, the AWCD values of soil microbial communities under different petroleum pollution concentration treatments followed the same trend (**Fig. 1**), increased with incubation time, and remained stable at the end.

TABLE II. VARIATION CHARACTERISTICS OF SOIL CHEMICAL PROPERTIES UNDER DIFFERENT PETROLEUM POLLUTION CONCENTRATIONS.

Treatment	pH	Water content (%)	Organic matter (%)	Total nitrogen (%)
Control	8.68 ± 0.02a	14.09 ± 0.11b	0.7449 ± 0.33c	0.0215 ± 0.03a
0.5%	8.75 ± 0.02a	15.83 ± 0.16ab	1.717 ± 0.35c	0.0213 ± 0.02a
1.0%	8.74 ± 0.01a	16.06 ± 0.03ab	1.7875 ± 0.56bc	0.0191 ± 0.05a
2.0%	8.69 ± 0.03a	17.25 ± 0.08a	2.7475 ± 0.59b	0.0211 ± 0.01a
4.0%	8.55 ± 0.01a	17.01 ± 0.15a	4.4756 ± 0.30a	0.0213 ± 0.03a

In the first 24 h, AWCD values were at the same level among treatments, and after 120 h, differences appeared, in descending order of CK > 0.5% > 2% > 1% > 4%, reaching the lowest level at 4% treatment.

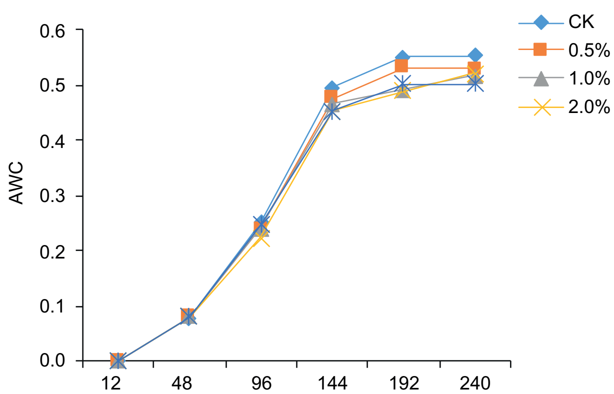

Fig. 1. Variation characteristics of soil microbial community AWCD value with culture time under different petroleum pollution concentrations.

Kinetic characteristics of different types of carbon sources used by microorganisms in petroleum-contaminated soil

The results showed that the soil microbial communities had different utilization rates of six carbon sources (carbohydrates, carboxylic acids, phenolic compounds, polymers, amino acids, amines) (**Fig. 2**). The efficiency of carbon source utilization by bacteria in the incubation phase was "S" shaped. It was relatively low at first, then changed at a higher rate and finally, stabilized. In clean soil, the efficiency of carbohydrates and amino acids utilization by microbial community was significantly higher than that of contaminated soil, and the utilization of carboxylic acids and polymers by soil microbial community increased after contamination by low concentration (0.5%, 1%) of petroleum pollution. The dominant population of soil changed to polymer metabolism at medium and high concentrations (2%, 4%) of petroleum pollution treatment (Liu et al. 2014).

CONCLUSION

Soil pH had no significant change under different gradients of petroleum pollution. Soil water content and organic matter content increased significantly with increasing petroleum pollution , and soil total nitrogen content decreased but did not at a significant level. This indicates that the polluted soil has higher total organic carbon content, higher carbon nitrogen ratio and lower total nitrogen content., which is consistent with the results of previous studies (Zhen 2015).

The changes of AWCD of soil microbial community under different petroleum pollution gradients were determined by Biolog-Eco microplates. The trend line of AWCD values with incubation time reveal the different carbon utilization ability of soil microorganisms in different treatments. The results showed that the AWCD values of the microbial community under different pollution treatments showed an "S" shaped trend with incubation time and remained stable at the end, with a decreasing trend with increasing pollution concentration. This may be due to the fact that the soil C:N:P of 120:10:1 is conducive to the degradation of pollutants by microorganisms, and the C:N:P imbalance after soil contamination by petroleum. Additionaly, the kinetic characteristics of the six carbon sources were determined, and it was concluded that the utilization efficiency of carbohydrates and amino acids by microbial communities was significantly higher in clean soil than in contaminated soil, and the utilization of carboxylic acids and polymers by soil microbial communities increased after contamination in low concentrations of petroleum pollution, and the dominant soil population changed to the polymers metabolism group when treated with medium and high concentrations of petroleum pollution. In general, the microbial activity that can utilize carboxylic acid compounds and polymers is higher in contaminated soil, while the microbial activity that utilizes amino acids and carbohydrates is higher in clean soil (Xu et al. 2012, Sun et al. 2015). Petroleum significantly affected both the metabolic capacity of soil microbial communities and their utilization patterns of carbon sources, and there were significant differences in the metabolism of microbial communities in petroleum-contaminated soils at different concentrations, and the metabolic patterns of soil microbial communities for carbon sources changed from sugar-based to polymers-based with increasing pollutant concentrations. This is the same as the results of Zhen et al. (2015), where petroleum pollution changed the utilization of certain preferred carbon sources by soil microorganisms, and long-term pollution would stabilize the carbon sources available to microorganisms and promote the development of microbial communities that prefer polymeric substances as carbon sources. This investigation pro-

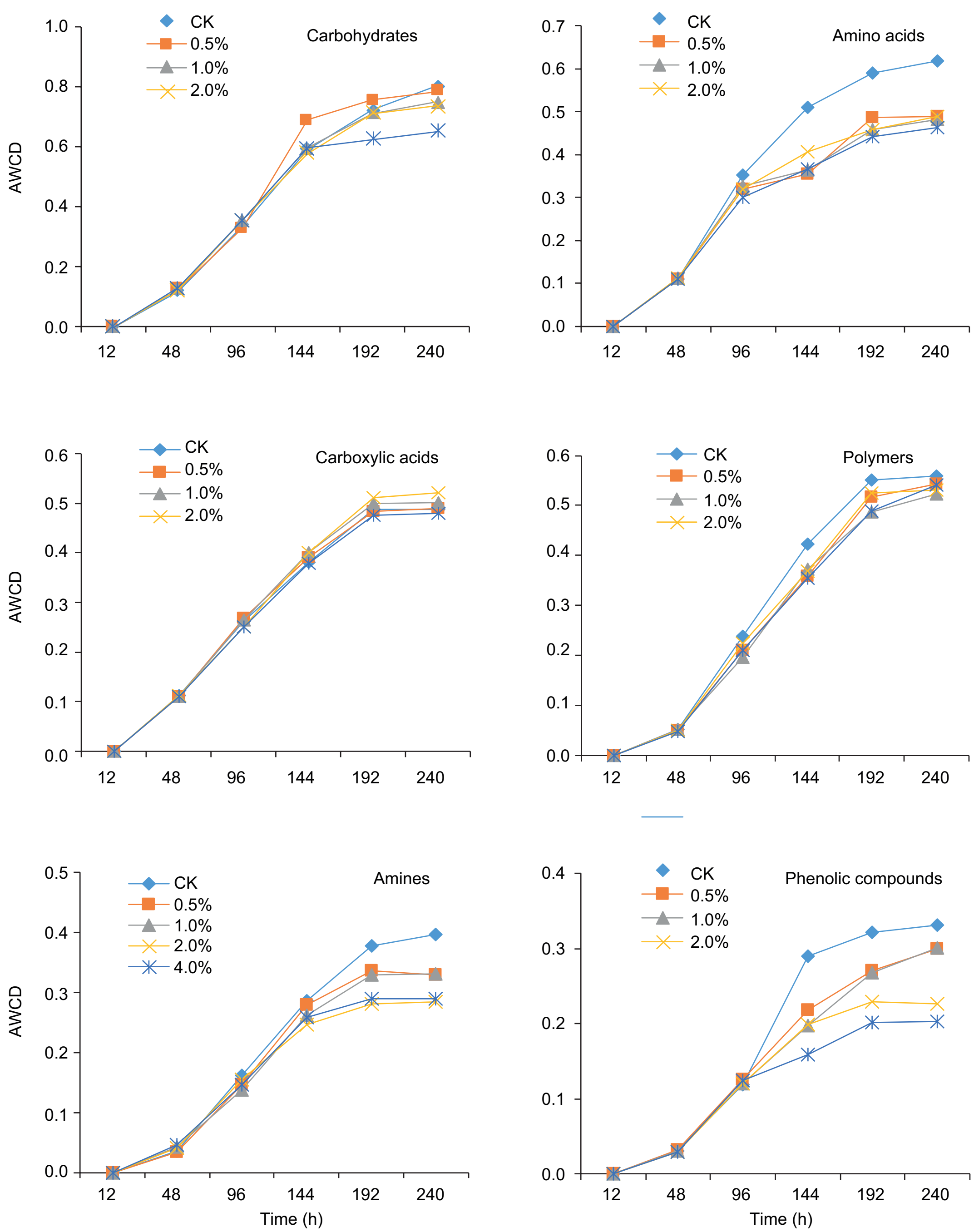

Fig. 2. Variation characteristics of soil microbial utilization of six types of carbon sources (AWCD value) with cultivation time.

vides data support and theoretical basis for the future continuation of quality evaluation and remediation treatment of petroleum-contaminated soils in ecologically fragile areas of the Loess Plateau.

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