Study on Microorganism Distribution Characteristics of Three Types of Typical Acidified Soils

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***Abstract*—The acidified soil caused by the acid rain has become a global problem, however, its effects to the microorganisms in the soil is not well understood. The authors employed a series of physiological and biochemical experiments to test and analyze three typical acidified and the remediated soil like grassland soil on barren hills, masson pine soil, and mixed coniferous and broad-leaved forest soils. The observation shows that the number and categories of microorganisms decreased in the order bacteria> epiphyte >actinomycetes, and the number of microorganisms in different depth of soil decreased in the order 0～20cm>20～40cm>40～60cm.Moreover, separation and identification of bacteria indicates that preponderant bacteria in different soils were distinguishing, so were the soil before and after remediation with the change of soil pH.The results enable us to further understand the characteristics of the remediated acidified soil from the aspect of microbiology.**

Keywords-acidified soil; biological activities; bacteria identification; remediated soil.

I. INTRODUCTION

Microorganism is one of the significant components in soil ecosystems. The soil microorganism including bacteria, epiphyte and actinomycetes, involved in almost all biochemical reactions in soils can play an important role in maintaining soil quality, decomposing relicts of propagation as well as organic and harmful substances in soil, and regulating biochemical cycling and formation process of soil structure, and serves as a significant indicator of soil fertility [1], [2]. Generally, most bacteria and actinomycetes live in the environment with neutral or alkaline pH, while the suitable living environment for epiphyte is acidic and most actinomycetes and epiphyte are limited under aerobic conditions [3]. The study on quantity, type and activity of microorganism in acidified soil contribute to exploring the mechanism of soil acidification and soil fertility status and therefore exhibits vital reference value to the remediation of acidified soil.

II. METHODS, RESULTS AND DISCUSSION:

A. *Soil sample*

i) Soil before remediation

The soil samples were collected from Longli ecological park with small watershed named LongliYangjichong as a unit in the upper branch of Sanyuan River in Wujiang River System where serious sulfuric acid rain happens due to the high level of sulfate radical. This area is located in Guizhou, China where the most typical acid soil is found. The acidified soils in this area were categorized into three fields: (1) Acid soil developing from sandstone, mostly covered by grassland on barren hills; (2) Acid soil developing from sandstone, mostly covered by masson pine forest; (3) Acid soil developing from limestone, mostly covered by mixed broadleaf-conifer forest (mixed wood).

TABLE I. PRIMARY PHYSICAL AND CHEMICAL PROPERTIES OF THE SOIL SAMPLES

Soil type	*Depth*(cm)	*pH*	*Exchangeable cation*(mmol/kg)						*CEC* (mmol/kg)	*BS*(%)	*A1S* (%)
			Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	H^{+}	Al^{3+}			
Masson Pine Forest	0~20	4.33	5.13	0.91	1.41	0.26	8.53	98.06	114.30	6.74	85.79
	20~40	4.45	4.70	0.43	0.98	0.28	7.29	83.24	96.92	6.59	85.88
	40~60	4.68	3.65	0.47	1.45	0.23	3.01	43.52	52.33	11.08	83.16
Mixed Wood	0~20	5.68	18.30	6.43	1.79	0.42	6.31	18.75	51.99	51.81	36.06
	20~40	5.87	12.97	7.87	1.03	0.46	5.63	14.53	42.48	52.55	34.20
	40~60	5.89	11.25	6.97	1.20	0.41	5.99	20.54	46.36	42.77	44.31
Grassland on Barren Hills	0~20	4.21	3.41	2.53	1.77	1.01	3.72	4.19	54.48	85.48	7.69
	20~40	4.43	2.74	1.47	1.39	0.21	3.27	3.85	50.89	86.02	7.57
	40~60	4.59	2.03	0.93	1.39	0.23	3.30	1.16	46.44	90.40	2.50

Annotate: CEC—Cation Exchange Capacity; BS—percentage content of base cation in CEC, namely Base Saturation；A1S—percentage content of A1 in CEC, namely A1 Saturation: A1S (%) =100∑ (A13+)/CEC.

ii) Remediated soil

The research of remediating the three types of acidified soil was performed through the model of soil columns equipment in laboratory. According to the characteristics of the acid rain in the experiment area, remediation agent were added to the acidified soil in Guizhou, under simulated Acid-rain Leaching. By observing the remediation effect, we find the best way to remediate the three types of acidified soil as follows: (1) 150g of dolomite and 150g of magnesite for per square meter of grassland soil, (2) 300g of magnesite for per square meter of masson pine soil, (3) 300g of dolomite for per square meter of mixed coniferous and broad-leaved forest soil. In addition, all of the best adding approaches was to dissolve these powders into 500L water and to leach the soils.

B. Means of microorganism test and analysis

With the method of dilution-plate coating, we carried out gradient dilution respectively to three soil depth (0～20cm, 20～40cm and 40～60cm) of three types of soils taken from the three types of the acid soils. The microorganisms were cultured on culture medium plate of corresponding physiological groups that were smeared by 0.1ml of soil suspension with appropriate dilution degree at 30°C.The bacteria, epiphyte and actinomycetes were respectively counted 1~2days later[4].

i) Isolation and purification of bacteria

The strain was isolated with plate streaking method and dilution-plate method. We chose five kinds of dominant bacteria from every type of soil before and after remediation, and performed culture rotation regularly[4].

ii) Experiment of morphological character of soil bacteria

Experiment of Gram stain[5]: Bacteria were categorized into gram-negative bacteria (G-) and gram-positive bacteria (G+) according to the differences in bacterial cell wall structure and composition.

Motility experiment: After cultivation, the measured strain was named negative when the thalligrowth was limited in the growth line and with clear edge while the mobile strain was named positive when the thalli proliferated nebulously.

iii) Three types of acidified soil bacteria's physiological and biochemical experiment

Physiological and biochemical experiments were performed on dominant bacteria selected from the three types of soils, including aerobic experiment, sugar fermentation experiment, nitrate reduction experiment, citrate utilization experiment, starch hydrolysis experiment, fat hydrolysis experiment, urea hydrolysis experiment, litmus milk experiment, oxidase experiment, contact enzyme (catalase) activity experiment, gelatin liquefaction experiment, methyl red (MR) experiment, acetyl methyl alcohol (VP) experiment, produce hydrogen sulfide experiment, produce indole experiment.

C. Counting statistical analysis of microorganism in three types of soil

Plate count was carried out on total number of microorganism, bacteria, actinomycetes, epiphyte in different depths of three types of acidified soils. The results are shown in Figure1,2,3and 4.

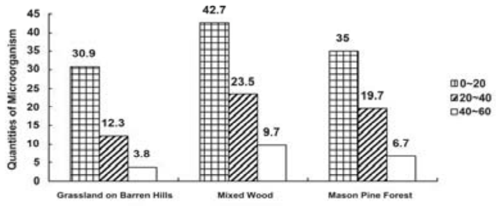

Figure 1. Quantities of microorganisms

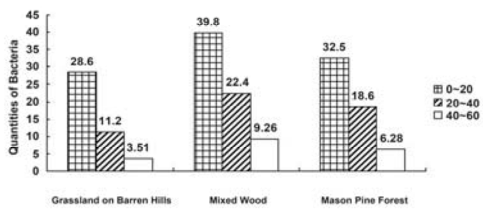

Figure 2. Quantities of bacteria

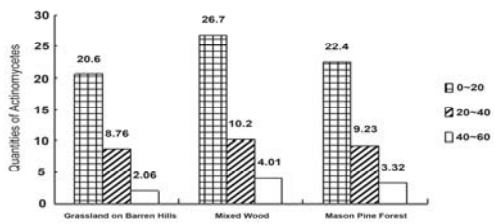

Figure 3. Quantities of actinomycetes

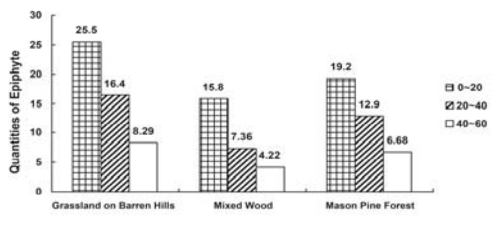

Figure 4. Quantities of epiphytes

The above four figures indicate that in the three types of soils, microorganic quantity declines sharply along with the deepening of the soil. In surface soil, the decomposed leaves and weed can transform into the soilthatcan produce a thick humus layer rich in organic matter, the total N and hydrolytic N that can be conducive to microbial activity. At the same time, soil surface temperature and ventilation conditions are conducive to microbial survival and reproduction, therefore, the quantity of soil microorganism in the surface soil is the largest.Furthermore, the difference in the number of soil microorganism between the depth of 20~40cm and 40~60 is smaller than that between the depth of 0~20 and 20~40cm, which can be due to that with the soil depth, soil structure, nutrients, moisture, temperature, ventilation condition become stable that can result in the slight difference in the number of underlying microorganism.

The antiacid ability of the three types of soil is as follows: mix wood >masson pine forest > grassland on barren hills due to more bacteria and actinomycetes exist in neutral or alkaline environment while acidic environment is suitable for epiphyte. Therefore, more bacteria and actinomycetes exist in mix wood while more epiphyte in grassland on barren hills where soil acidification is more serious. The horizontal comparison of the microorganic quantity in three types of soil indicates that the acid resistance of the microorganisms is positively correlated to the biological.

D. *Result and analysis*

The 15 dominant bacteria in the three types of acidified soil were selected after separation and purification from the plates. Another five kinds of dominant bacteria in the three types of remediated soil were selected after separation and purification. The results of character experiment are as follows.

i) The experiment result of bacterial morphological character

The result of Gram stain experiment shows that before and after remediation of the three types of acidified soil, dominant bacteria in soil are almost negative from the viewpoint of strain's polarity, and morphology of strain changes slightly from the viewpoint of morphology. In details: In the soil of grassland on barren hills, strains were mainly rod-shape, spiral shape and club-shape; In the soil of masson pine forest, strains were mainly rod-shape, club-shape and short and thick rod-shape; In the soil of mix wood, strains were mainly rod-shape and round shape.

TABLE II. RESULT OF MOTILITY EXPERIMENT ON DOMINANT STRAINS IN THREE TYPES OF TYPICAL ACIDIFIED SOIL BEFORE AND AFTER REMEDIATION

SN	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
M	+	+	+	+	+	—	+	+	+	+
SN	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
M	—	+	—	+	+	—	—	—	+	+
SN	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9	Z10
M	+	—	+	+	—	+	—	+	+	—

Annotate: SN-Serial number; M-motility;+-positive;—-negative.

We can find that almost strains in grasslands on barren hills can move while 60 percent of the strains can move in masson pine forest and mix wood.

ii) Identification result of strains in grassland on barren hills

Table 3 indicates that the five kinds of dominant strains were identified before the remediation of soil in grassland on barren hills, in which there were acidophilic Alcaligenes and Bacillus except Pseudomonas, Spirillum and Agrobacteriumcommon in soil. When the Alcaligeneswas in acidic environment,it could produce alkali to maintain the acid-basicity of growth environment. The Bacillus acidocaldarius and Bacillus pantothenticus belonging to the Bacillus can grow in acidic environment, especially bacillus acidocaldarius can grow in distinct acidic water and soil which can indicate that the acid-basicity of soil can have a direct impact on diversity of bacterial colony.

TABLE III. IDENTIFICATION RESULT OF DOMINANT STRAINS IN SOIL OF GRASSLAND ON BARREN HILLS BEFORE REMEDIATION

SN	H1	H2	H3	H4	H5
becteria genus	Pseudomonas Migula 1894,237	Spirillum Ehrenberg 1832,38	Agrobacterium Conn 1942,359	AlcaligenesCastellani& Chalmers,1919,936	Bacillus Cohn 1872,174
S/M	0.5-1×1.5-4	0.25-1.7	0.8×1.5-3.0	0.5-1.2×0.5-2.6	0.3-2.2×1.2-7.0
GT	4-43℃	30℃	25-30℃	20-37℃	——
G+C	58-70	38-65	59.6-62.8	57.9-70	32-62
GP	7.0-8.5	——	6.0-9.0	7.0	5.5-8.5

Annotate: SN-Serial number;S/M-Size/micron;GT-Growth temperature;G+C-G+C content in DNA/mol%;GP-Growth pH, similarly hereinafter.

TABLE IV. IDENTIFICATION RESULT OF DOMINANT STRAINS IN SOIL OF GRASSLAND ON BARREN HILLS AFTER REMEDIATION

SN	H6	H7	H8	H9	H10
becteria genus	AcinetobacterBrisou&Prevot 1954,727	Spirillum Ehrenberg 1832,38	Agrobacterium Conn 1942,359	AlcaligenesCastellani& Chalmers,1919,936	Pseudomonas Migula 1894,237
S/M	1.0-1.5×1.5-2.5	0.25-1.7	0.8×1.5-3.0	0.5-1.2×0.5-2.6	0.5-1×1.5-4
GT	30-32℃	30℃	25-30℃	20-37℃	4-43℃
G+C	40-47	38-65	59.6-62.8	57.9-70	58-70
GP	7.0	——	6.0-9.0	7.0	7.0-8.5

TABLE V. IDENTIFICATION RESULT OF DOMINANT STRAINS IN SOIL OF MASSON PINE FOREST HILLS BEFORE REMEDIATION

SN	M1	M2	M3	M4	M5
bacteria genus	AzotobacterBeijerinck 1991,567	Derxia Jensen, Peterson, De & Bhattacharya, 1960,193	BrucellaMeyer&Shaw 1920,173	Agrobacterium Conn 1942,359	Bacillus Cohn 1872,174
S/M	2×5	1.0-1.2×3.0-6.0	0.5-0.7×0.6-1.5	0.8×1.5-3.0	0.3-2.2×1.2-7.0
GT	20-30℃	25-35℃	20-40℃	25-30℃	——
G+C	63-66	70.4	56-58	59.6-62.8	32-62
GP	5.5-8.5	5.5-9.0	6.6-7.4	6.0-9.0	5.5-8.5

TABLE VI. IDENTIFICATION RESULT OF DOMINANT STRAINS IN SOIL OF MASSON PINE FOREST HILLS AFTER REMEDIATION

SN	M6	M7	M8	M9	M10
bacteria genus	AzotobacterBeijerinck 1991,567	Clostridium Prazmowski 1882,23	BrucellaMeyer&Shaw 1920,173	Agrobacterium Conn 1942,359	Pseudomonas Migula 1894,237
S/M	2×5	0.6-1.2×3.0-7.0	0.5-0.7×0.6-1.5	0.8×1.5-3.0	0.5-1×1.5-4
GT	20-30℃	25-37℃	20-40℃	25-30℃	4-43℃
G+C	63-66	23-43	56-58	59.6-62.8	58-70
GP	5.5-8.5	——	6.6-7.4	6.0-9.0	7.0-8.5

TABLE VII. IDENTIFICATION RESULT OF DOMINANT STRAINS IN SOIL OF MIX WOOD BEFORE REMEDIATION

SN	Z1	Z2	Z3	Z4	Z5
bacteria genus	BeijerinckiaDerx 1850,145	Sporosarcinaureae(Beijerinck) Kluyver&vanNiel 1936,401	Agrobacterium Conn 1942,359	AlcaligenesCastellani& Chalmers,1919,936	NitrobacterWinogradskr 1892,127 nom. cons
S/M	0.5-1.5×1.7-4.5	φ1.2-2.5	0.8×1.5-3.0	0.5-1.2×0.5-2.6	0.6-0.8×1.0-2.0
GT	10-35℃	22-30℃	25-30℃	20-37℃	5-40℃
G+C	50.4-60.7	40-43	59.6-62.8	57.9-70	60.7-61.7
GP	3-9.5	6.4-9.4	6.0-9.0	7.0	6.5-8.5

TABLE VIII. IDENTIFICATION RESULT OF DOMINANT STRAINS IN SOIL OF MIX WOOD AFTER REMEDIATION

SN	Z6	Z7	Z8	Z9	Z10
Bacteria genus	Rhizobium Frank 1889,338	Sporosarcinaureae (BeijerinckKluyver &vanNiel)1936,401	Agrobacterium Conn 1942,359	AzomonasWinogradsky 1938,391	NitrobacterWinogradskr 1892,127 nom. cons
S/M	0.5-0.9×1.2-3.0	φ1.2-2.5	0.8×1.5-3.0	φ2	0.6-0.8×1.0-2.0
GT	25-30℃	22-30℃	25-30℃	20-30℃	5-40℃
G+C	59.1-65.5	40-43	59.6-62.8	53-59	60.7-61.7
GP	5.0-8.5	6.4-9.4	6.0-9.0	7.0-7.5	6.5-8.5

Table 4 indicates that in the soil after remediation, the dominant strains were different from those before remediation indicating that the microorganic environment of had improved to a certain extent.

iii) Identification result of strains in masson pine forest

Table 5 indicates the existence of Derxia and Bacillus in the soil of masson pine forest due to the stronger acidity before remediation which is suitable for the acid-resistant colonies.

Table 6 shows that in the soil of masson pine forest after remediation, the dominant strains are different from that before remediation Clostridium and Pseudomonas had dominated rather than Derxia and Bacillus growing in acidic environment. After the remediation of acidic soil, dominant strains altered because of the change of hydroniums and nutrients in soil and the change of soil's pH which shows that soil remediation technology can improve the characteristics of the soil.

iv) Identification result of strains in mix wood

Table 7 indicates that the dominant strains in the soil of mix wood contain the common Agrobacterium and Nitrobacter. In the dominant strains, there were Beijerinckia (Beijerinckia that can produce acid in neutral and alkalinous environment, alkaline matter in strong acidic culture medium to improve the pH value of environment is widespread in intertropical acidic soil)[11] and Alcaligenes who can maintain acid-basicity of environment.

After remediation, the dominant strains in the soil of mix wood changed due to the change of soil pH, the improvement of acidic environment, the recovery of leaching ion and the improvement of ion and nutrients. Beijerinckia and Alcaligenes were replaced by Rhizobium and Azomonas.

III. CONCLUSIONS

From the quantitative analysis of three types of soil microorganism, the microorganic activity is as follows: soil in mix wood > soil in masson pine forest > soil in grassland on barren hills which can demonstrate that in the three types of soil, the soil in mix wood possesses the best acid resistance and buffer capacity.

The identified genus can indicate that dominant strains changed because of soil acidification. The acid-resistant strains or those which can produce alkali began to dominate. However, in the soil after remediation, instead of the genus that is acid-resistant or adapted to acidic environment, there were some strains of the common genus in soil that are adapted to neutral environment. In the remediated soil, the common genus adapted to neutral environment could replace the acid-resistant genus and those strains well-adapted to the acid environment.

The separation and identification of soil microorganism can lay the foundation for further research of soil acidification mechanism and the results of this study will provide a meaningful guidance for the remediation of acidified soil.

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