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Study on the toxicity of acid rain to microbiota in soils

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Abstract— With simulated acid rain and acidification, the soils from both Nanning and Liuzhou municipalities, Guangxi Zhuang Nationality Autonomous Region have been studied to determine the counts of *azotobacter*, bacteria, *actinomyces* and the activity of urease in soils, and the changes in respiratory intensity of soil so as to identify the toxicity of acidic substances to microbiota and enzyme in such soils. The concept of the critical pH of toxicity has been developed and used to discuss the representation of the concept, the basis on which the soil treated with simulated acid rain can be taken as an object of study, and the criteria for the identification of toxicity. Based on that as mentioned above, it was found that acid rain behaved to have toxicity to the microbiota in soils from Liuzhou other than from Nanning. The findings may be regarded as an objective basis to study the toxicity of acidic substances to microbiota and enzyme in soils in this region.

Keywords: acid rain; soil microbiota; soil enzyme; toxicity.

INTRODUCTION

In the last decade, the serious subsequences caused by acid rain have been commonly concerned in the countries of the world. It was reported that the pollutants in acid rain can damage the natural ecosystem. They may damage forest vegetation, leach and loss the nutrients from soil, and may also cause a series of changes in chemical properties of soil (Abrabamsen, 1976). Furthermore, they may have undesirable effects on microbiota in soil (Tamm, 1979; Babich, 1978). No report, however, has been found on the quantitative methods in use for identifying the toxicities of acidic substances to microbiota in soils.

Soil microbiota is an integral part of soil and also plays an important role in carbon and nitrogen cycling in soil while being the indicator that is the most susceptible to acidic substances

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in soil. Therefore, the studies on microbiota and enzyme in soil and the developments of principles and criteria for identifying toxicity are of a great importance to the prediction or forecasting of the toxicities of pollutants in acid rainfall to microbiota in soil of the region studied.

This paper is intended to study the soils from Nanning and Liuzhou, Guangxi Zhuang Nationality Autonomous Region, treated with simulated acid rain and acidified treatment in a sequence of pH values in order to observe the changes in the critical pH values of toxicity to microbiota and enzyme in soils and to identify the toxicities of simulated acid rain, acidified soil, and the soils from Nanning and Liuzhou.

MATERIALS AND METHODS

General features of soils studied (Table 1)

Table 1 General features of soils

Treatment	Sampling sites / soils	Organic matter,%	pH	Sampling depth, cm	Sampling date
Simulated	Wuming Yingxu Forestry Centre in Nanning / mountain podzolic soil	1.82	4.44	0—5	April 25, 1987
	Tree Farm of the Liuzhou Forestry School /mountain red earth	1.52	4.56	0—5	April 28, 1987
Acidification	Wuming Yingxu Forestry Centre in Nanning /mountain red earth	2.15	4.31	0—5	August 20, 1987
	Tree Farm of the Liuzhou Forestry School /mountain red earth	2.60	4.33	0—5	August 20, 1987

Preparation of the compositions for leaching and acidifying and the treatment

1. Experiment of simulated acid rain

(1) Mixed solution of acid rain (Table 2)

Table 2 Composition of the mixed solution of simulated acid rain

Ions	F^-	Cl^-	NO_3^-	SO_4^{2-}	Na^+	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	H^+
Concentration, μeq/L	1.15	13.92	6.15	54.03	12.06	28.29	8.07	24.32	5.43	16.16

The simulated acid rain was formulated in an amount that was equivalent to the local annual rainfall of 1280.9 mm in Guangxi. Soil samples were leached for 4 weeks at the rate of 106 ml of the mixed solution each time and 3 times in every week.

(2) pH titrants

A solution prepared in the ratio of sulfuric acid: nitric acid=9:1 (by normality) was added dropwise to the mixed solution of simulated acid rain as mentioned above while monitoring the acidity of solution after titration by using a pH meter to allow the mixed solution to be regulated to have values of 5.6, 4.7, 4.2, 3.7 and 3.1. Similarly, NaOH solution was used to regulate the mixed solutions. These pH-regulated solutions were used to leach the soils.

(3) Leaching of soil columns

Soil samples were collected from the top layer of soil, and in turn, air dried, ground, passed through a screen of 2 mm mesh size, and then filled in a glass column with 5.4 i.d. by 5 cm height, having bottom plugged with glass fibre. Each column was filled with 150 g of soils. Every treatment was duplicated.

The above simulated experiment started with October 22 and finished on November 17,1987. Soil microbiota, activities of soil enzyme and their variations were detected.

2. Experiment of soil acidification

(1) Mixed solution of acid rain (Table 3)

Table 3 Composition of the mixed solution of acid rain

Ions	Cl^-	NO_3^-	SO_4^{2-}	Na^+	NH_4^+	K^+	Ca^{2+}	Mg^{2+}
Concentration, μeq/L	13.92	6.15	54.04	12.06	28.29	8.07	24.32	15.43

(2) Preparation of the mixture of nitric acid, sulfuric acid and the mixed solution of acid rain

9 ml of concentrated sulfuric acid was mixed in 1ml of concentrated nitric acid and the mixed acid was then added slowly to 90 ml of the mixed solution of acid rain to form the acid mixture.

(3) Procedure of soil acidification

Five portions of air dried soil samples with 160 g of soil portion were taken. 400 ml of distilled waters were added to each portion of soil samples (the ratio by weight of water to soil is about 2.5:1). Three of five resulting soil solutions were added dropwise thereto with the acid mixture of nitric acid, sulfuric acid and mixed solution of acid rain as prepared in 2(2) in order to be regulated to have pH values of 4.2, 3.7 and 3.1, respectively. The remaining 2 resulting soil solutions were similarly regulated with 10% aqueous $Ca(OH)_2$ solution to have pH 4.7 and 5.6, respectively. All of the pH-regulated soil solutions were then filtrated through qualitative paper filters. The wet soils over paper filters were pH-regulated once again by the same procedures as those mentioned above in the next day. Five portions of treated soils were collected from the paper filters, then placed in their respective culture dishes, incubated at the temperature of 28°C for 3 weeks, and then detected for microbiota and activity of enzyme in the soils.

3. Detection of microbiota and activity of enzyme in soils

Azotobacter was cultivated on the culture medium with mineral salts and sucrose(Alexander, 1965), bacteria and *actinomyces* were cultivated on the culture medium with albumen and sodium salt (Tanabe, 1966), and fungi were cultivated on the Martin's medium (Alexander, 1977). The Schffer and Twachtman method and the modified Conway-Bremner method were used to detect urease activity, and the closed standing culture method was used for the determination of the intensity of soil respiration (Committee, 1987).

RESULTS AND DISCUSSIONS

Counts of microorganisms, urease activity, and intensity of soil respiration, found in both the simulated acid rain treated soils and the acidified soils were taken as ordinates to be plotted against pH values as abscissas as shown in Fig.1, 2, 3 and 4.

1. The results for the simulated acid rain treated soils were shown in Fig. 1 and Fig. 2.
2. The results for the acidified soils were shown in Fig. 3 and Fig. 4.

From Fig. 1, 2, 3 and 4, it can be seen that, due to the toxicities and damages of acidic substances to the microbiota in soils, all of the curves for soil microbiota, soil enzyme and soil respiratory intensity have their respective single turning points appeared at certain pH value. The pH value at such a turning point is defined as the critical pH of toxicity. For the purpose of discussion, all of the critical pH values of toxicity have been summarized in Table 4.

It can be found from Table 4 that the critical pH values of toxicity ranged from 3.7 to 4.7 with the average of 4.30 for simulated acid rain treated soils from Nanning, from 4.3 to 5.6 with the average of 4.80 for acidified soils from Nanning, from 4.2 to 5.6 with the average of 4.96 for simulated acid rain treated soils from Liuzhou, and from 4.7 to 5.6 with the average of 5.24 for the acidified soils from Liuzhou. These above data show that the critical pH values of toxicity are higher for the acidified soils than for the simulated acid rain treated soils and are higher for the soils from Liuzhou than from Nanning.

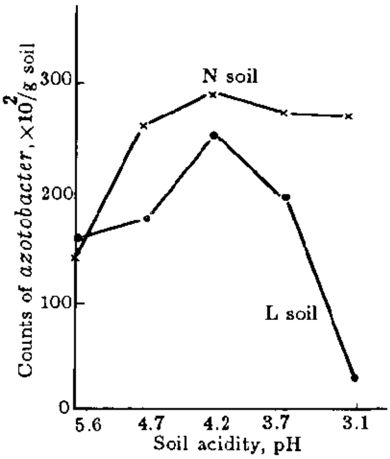

Fig. 1 Effect of acidic substances on *azotobacter* in the soils from Liuzhou and Nanning

L soil=the soil from Liuzhou; N soil=the soil from Nanning

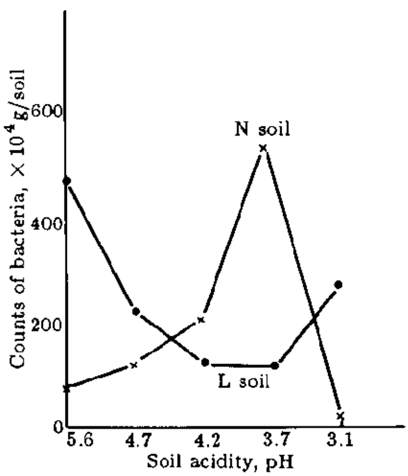

Fig. 2 Effect of acidic substances on bacteria in the soils from Liuzhou and Nanning

(The legend is the same as to Fig. 1)

The following three criteria are suggested by the authors of this paper to assess the critical pH values of toxicity and the toxicity of acid rain to the microbiota in soils from a certain region.

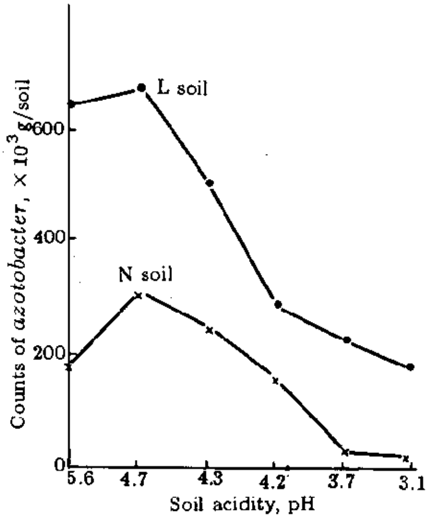

Fig. 3 Effect of acidic substances on *azotobacter* in the soils from Liuzhou and Nanning

(The legend is the same as to Fig. 1)

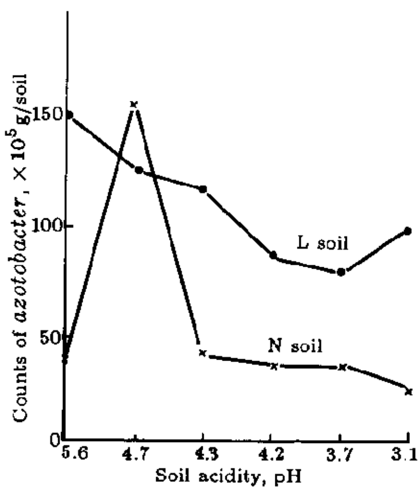

Fig. 4 Effect of acidic substances on bacteria in the soils from Liuzhou and Nanning

(The legend is the same as to Fig. 1)

(1) No toxic

The pH value of soil in the region is higher than the critical pH value of toxicity to the microbiota in soil from the same region.

Table 4 The critical pH values for the toxicities of simulated acid rain and acidification to microbiota, enzyme activity and respiratory intensity in the soils from Nanning and Liuzhou

Determining item	Critical pH value of toxicity			
	Soils from Nanning		Soils from Liuzhou	
	Acidification	Simulated acid rain	Acidification	Simulated acid rain
Azotobacter	4.7	4.2	4.7	4.2
Bacteria	4.7	3.7	5.6	5.6
Actinomyces	4.7*	4.2*	4.7*	5.6*
Fungi	—	3.7**	—	4.2**
Urease activity	4.3*	4.7*	5.6*	4.7*
Respiratory intensity in soil	5.6*	4.7*	5.6*	4.7*
Average	4.80	4.30	5.24	4.96

Note: — No detection
 * Detected without plotting
 ** Not included in the average

(2) Initially toxic

The pH value of soil in the region is equal to the critical pH value of toxicity to the microbiota in soil from the same region.

(3) Toxic

The pH value of soil in the region is lower than the critical pH value of toxicity to the microbiota in soil from the same region.

For example, the soils from Liuzhou have pH values in the range of 4.33—4.56 (Table 1) that are lower than 4.96 of the critical pH value of toxicity to the microbiota in soils from Liuzhou as determined above. Therefore, according to the criteria (3), acid rain should be toxic to the microbiota in soils from Liuzhou. In the cases of Nanning, however, the soils have pH values in the range of 4.31—4.44 that are higher than 4.30 of the critical pH value of toxicity to the microbiota in soils. Hence, according to the criteria (1), acid rain should be no toxic to the microbiota in soils from Nanning. Nevertheless, the pH values of soils are quite close to the critical pH value of toxicity.

Because the critical pH value of toxicity for acidified soil is not so much met with the situation in the real environment, it would be better to use the critical pH value of toxicity for simulated acid rain when an assessment will be based on the above three criteria. This can be explained by the fact that the acidified soil was made by 2 successive times to regulate pH value within 2 days so that the pH value was acutely changed in a short term and the physico-chemical properties of soil have only a small change. In this case, the microbiota has too short time to adapt itself to the acute change. On the other hand, the simulated acid rain treated soil was made by using the amount of acid rainfall as much as the local annual rainfall in Guangxi to leach the soil at a rate of 3 times each week during the period as long as one month. This is chronic treatment in a long term. Therefore, the acidic substances in a large amount can be physically or chemically combined with soils and the microbiota in soil can have a long time to adapt itself to such changes.

Fig. 5 shows the change in acidity of simulated acid rain treated soil after standing for 2 months. The curve for acid rain represents the pH values of the solution for leaching and the upper curves indicate the changes in pH values of the soils from Nanning (N soil) and from Liuzhou (L soil) after standing for 2 months. During the period, the acidic substances and the physico-chemical properties were changed that in turn caused the pH change. The soils from both regions have the same trend. Fig. 6 shows the change in acidities of the acidified soils after standing for 2 months. The curve for the acidified soil represents the initially regulated pH values and the two upper curves show the pH values of the soils from Nanning (N soil) and Liuzhou (L soil) after 2 months standing. The latter two curves have the trends different from those for the simulated acid rain treated soils. This may be due to the soils being subjected to the acute acidification in a short term. Both the soils from Nanning and Liuzhou have their

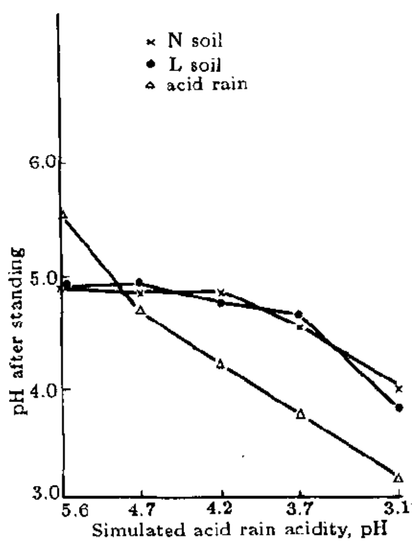

Fig. 5 Changes in acidities of the simulated acid rain treated soils after standing for 2 months
(The legend is the same as to Fig. 1)

own single small peaks at pH 4.2 and 3.7, respectively (Fig. 6). At the same time, it can be found that there is a peak of respiratory intensity occurred correspondingly at pH 4.2 for the soil from Nanning while a peak of the counts of *actinomyces* and a peak of urease activity are both appeared at pH 3.7 for the soil from Liuzhou. It is probable that at the respective pH values the soluble carbon and nitrogen compounds were released from the soils so that led to an increased in the number of microbe (Killham, 1983). The growth and propagation of microbe would also cause the change of pH values in soil.

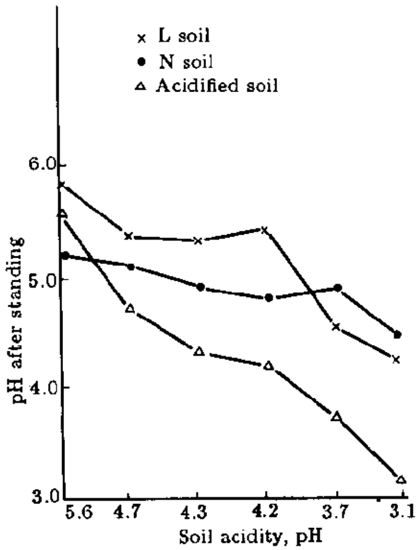

Fig. 6 Changes in acidities of the acidified soils after standing for 2 months (The legend is the same as to Fig. 1)

The physical and chemical properties of the soils from Nanning are different from those of the soils from Liuzhou, so that there is a difference in the appearance of critical pH values of toxicities between the soils from Liuzhou and Nanning. The critical pH value of toxicity to the microbe in the soils from Liuzhou is higher than that from Nanning.

In consideration of the representativeness of the microbe detected, respiratory intensity of soil was selected to stand for the microbe that can decompose carbon compounds; *azotobacter* and urease activity to stand for the microbes that have abilities to fix and to decompose nitrogen compounds, respectively and the remaining three types of microbe-becteria, fungi and *actinomyces* are common in soil.

CONCLUSIONS

The microbes in soil are susceptible to acidic condition. Acidic substances occurring in a certain concentration are toxic to the microbes in soil. Particularly, the counts of microbe in soil, the urease activity of soil and the respiratory intensity of soil are suddenly reduced and have their respective turning points at a certain pH value. This phenomenon is so called as the critical pH values of toxicity to the microbe in soil.

As there are a great diversity of microbes in soil, it is necessary to detect more species of microbes as possible, mainly related to the cycles of carbon and nitrogen and three general kinds of microbes. The critical pH value of toxicity is determined for every group of microbes detected and then averaged for all groups of microbes detected to have general representativeness.

For the determination of the critical pH value of toxicity, the soil treated with simulated acid rain is closer to the natural case than the soil acidified.

After understanding the pH value of soil in a region and determining the critical pH value of toxicity to the microbes in the region, three criteria can be used to diagnose the toxicity of acid rain to the microbes in soils in the region. By making such a diagnosis, it has been found that acid rain is toxic to the microbe in the soils from Liuzhou and has not been toxic to the microbe in the soils from Nanning although it will be possible to occur such a toxicity if the current trend continues.

The soils from different regions have different physical and chemical properties that relate closely to the critical pH value of toxicity to the microbes in soil. Therefore, there is a difference in the critical pH values of toxicity of acidic substances to the microbe in soils of different regions.

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