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Biological Effects of Reformative Red Soils Degenerated from Heavy Metal Pollution

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Pot experiment on red soil (garden soil) contaminated by heavy metal was conducted in Guixi, Jiangxi, China. The results indicate that using lime to tune soil pH to light alkalescence could improve net photosynthesis significantly, increase soil microbe C (especially for cellulose-decomposing bacterium), reduce the available content of heavy metal for the growth of plants, and serves as a viable treatment of soil under the condition in existence.

Key words*:* pollution of heavy metals; physiological and ecological function; red soil.

Soil, the most important element in the farming ecosystem, is closely related with human health. The pollution of the environment for farming produces a direct threat to human existence. Researchers have investigated into the relation between the heavy metal content and the function of the plant mechanism (Anderson *et al*, 1993; Burken and Schnoor, 1996; Pence *et al*, 2000; Gu *et al*, 2005; Bao *et al*, 2008). Since it costs too much to alter or cleanse the polluted soil, it is therefore both economically and scientifically recommendable to take reformative measures to reduce the effect of pollutants on the growth of plants. Cao and many other research groups studied the use of lime and organic fertilizer for an increased organic-matter-cation exchange as well as an improved pH value of the soil (Cao *et al*., 1992; Zong and Ding, 2001; Qian *et al*., 2007). Their study, which achieved a whole series of changes in the composition of heavy metals, should be highly evaluated for the possibility it has provided of improving soil with heavy metals.

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MATERIALS AND METHODS

Guixi Smeltery, the biggest of its kind in China, lies in the rural area of Bingjiang, Guixi City. Sulfide oxidation and powder were released into the air and such heavy metals as Cu, Pb, Cd, Zn and As into the water. About 410 km2 farmland had been heavily polluted. The selected location for the experiment is characterized by red sandy shale and typical red soil. The vegetable plot is 200 meters east of a copper-smelting plant and receives sewage from the latter that contains such heavy metals as Cu, Pb and Cd.

The polluted sample soil: 100 kg of earth taken from surface cultivation layer (0 - 20 cm) of the vegetable plot. It is air-dried, ground and sieved with 10-mm holes.

Treatment: 1) C1(contrast), treated without lime and organic fertilizer; 2) C2, treated with 1.20 g kg^{-1} of lime only; 3) C3, treated with 2.40 g kg^{-1} of lime; 4) C4, treated with organic fertilizer (hollow swine feces) 6.0 g kg^{-1}; 5) C5, treated with organic fertilizer (Hollow swine feces) 12.0 g kg^{-1}. All of the above (from C1 to C5) are each treated with 0.20 g kg^{-1} of the car amide (amounting to 0.09 g kg^{-1} of N), 0.40 g kg^{-1} of phosphoric acid (with N amounting to 0.06 g kg^{-1}, and P_2O_5 to 0.18 g kg^{-1}) and 0.30 g kg^{-1} of potassium chloride (amounting to 0.18 g kg^{-1} of K_2O). All the treatments follow the literature of The Chemical Approach to Agriculture (Northwest Agriculture Academy and South-China Agriculture University, 1987). Each treatment has 4 exact copies with 3.0 kg of earth in a pot grown with sweet potato. The pots are provided with the amount of water in accordance with the actual water content in the field by adopting the measurement specified in literature (Chinese Soil Agricultural Chemistry Committee, 1983): maintaining a 35% standard dampness by watering the pots once or twice a day. Grown in each pot are 2 sweet potato plants with cross-pot similarities in the size of leaves (around 7.5 g) and lengths. One is pulled out 20 days after the planting.

Chemical analyze

The pH value of the soil is taken in the liquid form and measured by the potential. The measurement of the total and working contents of organic substances in the soil and the organic fertilizer (Carbon, action exchange, nitrogen and phosphorus) refer to the way of Chinese Soil Agricultural Chemistry Committee (1983) and Lu (1999).

Analyses of heavy metal content in soil and plant

After the pre-test treatment (Earth Chemistry Criterion Reference Research Group of the Chinese Geology and Mineral Department, 1988), the values of heavy metal contents in the earth and the sweet potato specimen are read out with ICP-AES (French JOINYNON Corporation). This measurement uses 0.05 M HCL as the elicitor of heavy metals with a water-earth ratio of 1: 5 in the test matter after 2 hours' oscillation.

Measurement of physiological and ecological function

Three measurements were taken in May and June, each lasting 3 days consecutively from 8:00 to 18:00 at 2 hours' intervals. CL-301PS (Photo synthesis System Cl301PS) is used for measuring the ratio of clean photosynthesis of the leaves of the pot plants. We also adopted the plant meter law to measure the leaf face product index number, and the harvesting method the measurement of the clean biomass.

Measurement of carbon and nitrogen

The amounts of the microbe carbon and microbe nitrogen are taken through suffocation with TOC-500 (Shimadzu Corporation) measurement (Yu *et al.*, 1997).

Soil microbe amount measurement

Dilute monotonous law (Microbe Department of Nanjing Soil Research Institute of the Chinese Science Academy, 1985) is adopted for measuring bacterium, antinomies and fungi with the culture medium of beef cream albumen peptone, GAO Shi one and Ma Ding's agar-agar.

In addition, data were analyzed using the general method of statistical analysis such as difference test, correlation analysis. They were performed on the discussion part of heavy metals' distribution and physiological and ecological changes.

RESULTS AND DISCUSSION

Physical and chemical nature of the earth

In the way of fertility, the soil chosen for the pot-plant experiment contains 6.10 g kg^{-1} of pH (H_2O), 24.82 g kg^{-1} of organisms, 1.28 g kg^{-1} of whole nitrogen, 0.96 g kg^{-1} of whole phosphorus, 8.35 g kg^{-1} of whole kalium, 115.04 mg kg^{-1} of Hydrolysis nitrogen, 308.75 mg kg^{-1} of quick result phosphorus, 69.29 mg kg^{-1} of quick result potassium, and 78.58 mmol kg^{-1} of organic carbon. After lime is used, PH (H_2O) of the soil of C2 and C3 reached respectively the slightly alkali value from 7.40 to 7.54 and the almost neutral value from 6.51 to 6.76. They reached their maximum values within two weeks and slightly fell thereafter. The subsequent addition of organic phosphorus in the form of the swine feces changed the contents of whole N, whole P, whole K and organic phosphorus in the dried soil samples respectively into 29.64 g kg^{-1}, 3.93 g kg^{-1}, 68.79 g kg^{-1} and 158.77 mg kg^{-1}. The significantly-changed contents of nutrients (particularly quick result nutrients) soluble organic carbon have a strong effect on the solution and transfer of heavy metal elements and their absorption by plants.

The distribution of heavy metals in soil and plants

According to our measurement, the total mount of Cu in the pot soil is 376.7 mg kg^{-1} and that of Cd 1.512 mg kg^{-1}. In order to understand the growth mechanism of the plants, we would provide here a brief discussion of the valid distribution of heavy metals.

The available contents of Cu of the

contrasted treatments all display the highest ratio against the total amount of heavy metals (RP). The valid Cd contents and averaged RP increased more or less and, even considerably in the soil treated with organic fertilizer. However, the valid Pb's content and RP value for C3 treatment dropped significantly without any other considerable changes. In short, the use of higher amounts of lime for a slightly alkali soil has produced a similar effect on the decrease of Cu, Cd and Pb to that produced by the experiment of Chen Huaiman, Walker *et al*.(Chen *et al*.,1996; Walker *et al*., 2003, 2004). Because of the influence of acid soil on the validity of Cu, an increase in pH can improve absorption of Cu by oxides in the soil and hence reduce its effect (Cotter-Howells, 1996). This gives us an option for the improvement of the soil.

Tests at the root, stalk and leaves show a regular distribution of such elements as Cu and Cd with a considerable difference in their contents between the locations of the root and the leaf. Lime-treated C3 plants show a considerable decrease in Cu, Cd and Pb especially at the root. Such a discovery tends to bespeak a reduced influence of heavy metals on plants in the slightly alkali soil. Organic-fertilizer-treated C5 generally shows less content of Cu in the plants but a slight increase of Cd. The shorter growth cycle of plants and different fertility of the soil did not show a similar difference in those contents between the root and the leaf.

Table 1. The available content of soil heavy metal and its differencing check on pot experiment

Handle	Cu		Cd		Pb	
	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%
C1	235.23c	62.44	0.79d	52.91	4.75a	18.30
C2	231.20c	61.38	0.82d	54.23	4.60bc	17.76
C3	207.93c	55.2	0.84d	55.56	3.26e	12.56
C4	222.63c	59.10	1.38c	71.43	4.29c	16.56
C5	225.26c	59.80	1.66b	90.61	3.79b	14.60

The contrasts marked with the same letter in the same column are not significantly different from each other. Duncan multiple comparison is 0.05.

Besides, the pot experiment produced significant difference between treatments with organic fertilizer and with lime, especially in the root systems. The C3 treatment of the soil in vegetable garden produced lower contents of the three heavy metals in the plants than in the contrast pot, but it increased the absorption of Cu and Pb, which means that it is not a recommendable method. Thus, our preliminary conclusion is that the use of lime (especially in larger amounts) to a slightly alkali soil can effectively decrease the effect of the available heavy metals in the soil on the plants, which is both economic and effective as an alternative solution to the problem.

Table 2. The available content of plant heavy metal and its differencing check on pot experiment

Handle	Cu			Cd			Pb		
Disposal	Piece mg kg^{-1}	Stalk mg kg^{-1}	Foliage mg kg^{-1}	Piece mg kg^{-1}	Stalk mg kg^{-1}	Foliage mg kg^{-1}	Piece mg kg^{-1}	Stalk mg kg^{-1}	Foliage mg kg^{-1}
C1	168.87a	31.53c	26.66a	3.43c	0.87d	0.86ab	5.28d	3.76bc	6.19a
C2	156.30b	41.50b	24.33b	3.65bc	0.51f	0.43g	3.80e	5.53a	2.04f
C3	94.13fg	18.30g	20.23cd	2.22e	0.43g	0.33h	3.99e	2.89f	4.57e
C4	147.43c	51.56a	20.20cd	3.73b	0.81de	0.48fg	5.64cd	3.86b	5.19cd
C5	140.13d	24.23d	21.77c	5.51a	1.22b	0.88a	5.61cd	3.32de	5.37bc

The contrasts marked with the same letter in the same column are not significantly different from each other. Duncan multiple comparison is 0.05.

Physiological and ecological changes related with the photosynthesis

During the three measurements taken in May and June the ratio of air temperature (T), relative humidity and the total radiation of sunlight (ΣEg) is 1 : 1.04 : 1.09 : 1.02, and 1 : 1.06 : 0.95. The daily effective radiation (PAR) is normally 1050 - 1230 µmo1 m^{-2} s^{-1}. It is therefore believe that different pot treatments and different stages of plant growth may influence net photosynthesis rate (P).

Table 3. The net biomass (dry weight), the leaf area index and the average net photosynthesis of plants on pot experiment

Treatment	Net biomass g	Leaf area index	Net photosynthesis µmo1 m^{-2} s^{-1}
C1	7.20	0.82	2.75
C2	10.02	1.20	3.78
C3	10.81	1.41	3.66
C4	7.31	1.01	2.97
C5	6.88	0.90	2.86

The research shows that the rate of net photosynthesis and leaf area index are correlated with net biomass, with the coefficients being 0.957 and 0.949 respectively. Application of high amounts of lime yields the highest leaf area index and net photosynthesis. Treatments with organic fertilizer produced second highest leaf area index and net photosynthesis. Under the same conditions of use of chemical fertilizer and watering, use of higher amounts of lime can significantly increase the physiological and ecological functions of the photosynthesis of the leaves, which speaks for a clear correlation of physiological and ecological indexes with the soil productivity. Thus the conditions of the soil can be analyzed in terms of the physiology and ecology of the plants.

Physiological and ecological changes of microbes

Our experiment also shows that the application of lime and swine feces increased both soil microbe quantity (C) and biomass quantity (N) considerably against the contrast soil. Specifically, the microbe quantity for the treatment with organic fertilizer is higher than that for the treatment with lime and increases with the quantity of organic fertilizer used. For example, for the treatment with lime, Bc increased from 171.52 mg kg^{-1} to 200.23 mg kg^{-1} and, for the treatment of organic fertilizer, to 369.81 mg kg^{-1}, which is over 100% higher than that of the contrasts (Fig.1).

From Table 4, we can see that the treatment with organic fertilizer significantly increased bacteria and microbes in the soil because the fertilizer itself carries some microbes and organic substances. Among the samples, C5 produced the biggest increase in bacteria and microbes without much influence on the change of quantities of Epiphyte and Actinomycete. We may ascribe the last effect to the different adaptability to the environment. Among all the treatments, C3 displays the highest percentage of soil bacteria, possibly because most bacteria like the slightly alkali soil (Hao and Cao, 1987). Thus the application of lime can change the flora composition without changing the microbe quantity (Bertocchi *et al*., 2006; Gray *et al*., 2006).

The cellulose-decomposing bacterium fungus is a microbe Flora that can decompose fibers. Our experiment shows that there is a small number of cellulose-decomposing bacteria (only 4000 per gram of dry soil) for the contrast treatment. Just as existing researches have shown, they increased significantly after the application of organic fertilizer and very significantly (75 - 361 times more) after the application of lime. C3 with the highest amounted of used lime achieved the biggest increase. Such changes all point to it that cellulose-decomposing bacterium can be adopted as a sensitive microbe indexer for the pH values and fertility of the soil in the improvement of the heavy-metal-polluted soil. Thus, from photosynthetic, physiological and ecological viewpoints, the soil treatment with lime is a highly recommendable measure for solving the heavy metal pollution problem.

Table 4. Soil microbial population distribution on pot experiment in dry soil

Handle	Bacterium			Epiphyte			Antinomies			Microbe Quantity	Fiber Decom-
Bacterium	$\bar{A}$ 10^4 g^{-1}	SD	CV	10^4 g^{-1}	SD	CV	10^4 g-1	SD	CV	104 g^{-1}	posing 10^4 g^{-1}
C1	2798.67	113.03	0.04	21.33	0.75	0.04	163.77	20.86	0.13	2964.60	0.40
C2	2749.40	193.14	0.07	20.90	1.80	0.09	196.23	20.42	0.10	2947.70	30.40
C3	2851.30	343.44	0.12	12.73	1.50	0.12	61.27	7.35	0.12	2913.90	134.80
C4	3367.17	114.48	0.03	10.60	0.36	0.03	165.83	18.69	0.11	3534.10	5.70
C5	4154.23	373.15	0.09	20.30	1.64	0.08	197.37	25.51	0.13	4353.60	9.30

In the table, SD and CV separately means Arithmetic mean, Standard deviation, Coefficient of variation.

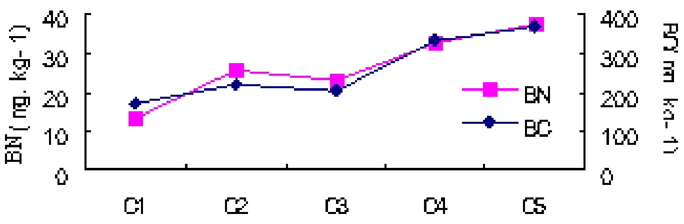

Fig. 1. Soil microbial biomass diversification on pot experiment

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