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Influences of Cadmium on soil Nitrification ; Relation between Nitrification Rate and the Population of Nitrifying Autotrophs in Cd Contaminated Soil.

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硝化作用に及ぼすカドミウムの影響，とくにカドミウム汚染土壌中の硝化活性と硝化菌数の関係

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Summary

The influences of Cd on nitrification rate and the population of nitrifying autotrophs in sandy clay loam soil were studied. The soil was treated with 0, 5, 50 or 500 μg of Cd per g dry soil and preincubated. After preincubation for 32 days, 100 μg of NH_4^+－N per g dry soil was applied to the preincubated soil, and the oxidation of ammonium and changes in number of nitrifying autotrophs were assayed. The determination of NH_4^+－N and (NO_2^-+NO_3^-)－N revealed that, though the rate of nitrification was not decreased below the control level by the application of 5 and 50 μg Cd/g dry soil, the nitrification was markedly retarded by 500 μg Cd/g dry soil. However, the number of ammonium-oxidizing autotrophs in the soil treated with 500 μg Cd/g dry soil was larger than those found in the other soils. The number of nitrite-oxidizing autotrophs was smallest in the soil treated with 500 μg Cd/g dry soil. These facts suggest that nitrification rate is controlled, in certain environmental conditions, not only by the number of the nitrifying autotrophs but also by the nitrifying activity of the individual microorganisms.

Introduction

A fair number of investigations have been reported on the effects of Cd on nitrification (Tyler et al. 1974, Morrisey et al. 1974, Liang and Tabatabai 1978). The effects of the metal on some microorganisms or microbial ecology in soil were also reported (Babich and Stotzky 1978). However, the influences of the metal on the population of nitrifying autotrophs in soil remains unknown. It has been considered that the nitrifying activity in soil has a firm connection with the number of nitrifier living in the soil, so that the number of nitrifying autotrophs has expediently been evaluated by nitrifying activity in soil (Sakai 1975, Watanabe 1974). The objective of this study is to find out whether a correlation between

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the nitrifying activity and the number of the nitrifying autotrophs exists or not in Cd contaminated soil.

Materials and Methods

The physical and chemical properties of the soil used were shown in Table 1. The soil was passed through a 2-mm sieve, and 300 g of the soil was adjusted to 16% moisture contents and also to 5, 50, or 500 μg of Cd per g dry soil with a $CdCl_2$ solution. The Cd extracted with 0.1*N*-HCl from the 0, 5, 50 and 500 μg Cd/g dry soil added soil were 0.2, 5.2, 48.3 and 488.9 μg/g dry soil respectively. These soils and control soil were kept in 250 ml Erlenmyer flasks and preincubated at 28°C. After 32 days, 100 μg NH_4^+-N/g dry soil as $(NH_4)_2SO_4$ was added to the preincubated soil and the moisture contents were adjusted to 20% (50% of MWC). Aliquots (10 g) of each soil sample were put into test tubes and incubated for 4, 8, 16 and 24 days. The top of each tubes was covered with aluminum foil to prevent from drying of the soil.

Table 1　Some Physical and chemical properties of the soil used

pH	5.7(KCl 1 : 2.5)
Organic C	1.5%
Total-N	0.11%
NH_4^+-N	0.46mg/100g dry soil
$(NO_2^-+NO_3^-)$-N	1.73mg/100g dry soil
Texture	SCL
Corse sand	52.4%
Fine sand	12.7%
Silt	12.7%
Clay	17.3%
M.W.C*	40.0%

*maximum water holding capacity

Nitrification rate was assayed by the determination of NH_4^+-N and $(NO_2^-+NO_3^-)$-N concentrations in the soil extracts using Conway's micro-diffusion method at the end of a specific incubation periods. The NH_4^+-N and $(NO_2^-+NO_3^-)$-N in the soils were extracted with 2*N*-KCl in a 1 : 5 soil : liquid(w/v) ratio (Bremner 1965). The soil pH determination was made on 1 : 2.5 soil : *N* KCl(w/v) suspension. The number of nitrifying autotrophs was estimated by MPN procedure : 1 ml portions of the tenfold serial dilution of the sample were introduced into test tubes containing 4 ml of a medium adequate to support the development of ammonium- and nitrite-oxidizing autotrophs, and the mixtures were incubated at 28°C for 4 weeks and then for the estimation of nitrifying activity using Griess-Ilosvay colorimetric method (Alexander and Clark 1965).

Results and Discussion

As shown in Fig. 1, the oxidation of ammonium to nitrite was not inhibited in soils treated with 5 and 50 μg Cd/g dry soil, but the treatment with 500 μg Cd/g dry soil markedly retarded nitrification. On the other hand, the number of ammonium-oxidizing autotrophs in soil treated with 500 μg Cd/g dry soil was significantly larger than those in control soil and in the soils treated with 5 and 50 μg Cd/g dry soil (Fig. 2). The number of nitrite-oxidizing autotrophs was smallest in the 500 μg Cd/g dry soil treated soil.

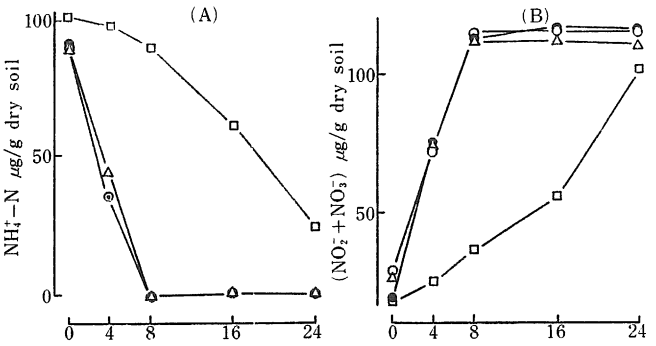

Figure 1 Changes in concentration of NH_4^+-N (A) and $(NO_2^- + NO_3^-)$−N (B) during a 24 days incubation period after the application of NH_4^+-N in soils treated with 5, 50, and 500 μg Cd/g dry soil.
● Control, ○ 5 μg Cd/g, △ 50 μg Cd/g, □ 500 μg Cd/g

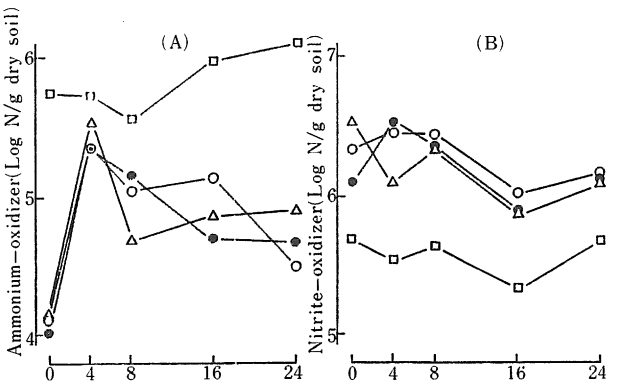

Figure 2 Changes in numbers of ammonium-oxidizing autotrophs (A) and nitrite-oxidizing autotrophs (B) in soils treated with 5, 50 and 500 μg Cd/g dry soil.
● Control, ○ 5 μg Cd/g, △ 50 μg Cd/g, □500 μg Cd/g

Nitrification is exclusively carried out by nitrifying chemoautotrophs which derive energy from ammonium or nitrite oxidation to fix carbon. Therefore, it has been supposed that nitrification rate and the number of nitrifying autotrophs are closely related. However, it was found in the present study that the addition of 500 μg Cd/g dry soil to soil decreased nitrification rate, despite the number of ammonium-oxidizing autotrophs being increased.

Table 2 Changes in pH of soils treated with Cd after the application of 100 μg NH_4^+-N/g dry soil

Cd added	Days after the application of NH_4^+-N				
(μg/g dry soil)	0	4	8	16	24
0	6.1	5.9	5.8	5.8	5.9
5	6.0	5.9	5.7	5.8	5.9
50	6.0	6.0	5.7	5.8	5.9
500	6.0	6.0	6.0	5.9	5.9

It is well known that nitrification is strongly affected by soil pH value. The values observed in the cases of the 5, 50 and 500 μg Cd/g dry soil, however, were nearly the same as control throughout the experiments (Table 2). Shindhu and Cornfield (1967) showed that nitrate production is not affects by addition of chlorides (up to 1%) to soils. These

facts indicate that the observed effects on nitrification in this study resulted from an increase in Cd concentration rather than a change in pH or an increase in chloride concentration. Though the cause or mechanism of the observed phenomenon is yet sufficiently clarified, the results would be explained in terms of either (1), (2) or (3) given below. (1) Method for enumeration of nitrifying autotrophs was not appropriate. (2) In some condition, considerable nitrification was carried out by heterotrophic organisms. (3) The nitrifying activity of *Nitrosomonas* was extremely decreased in certain environmental conditions.

In any event, it is obvious that the method (Sakai 1975) for the evaluation of the nitrifying autotrophs in a farm land by means of the determination of nitrification activity is not always applicable, since the nitrification rate in soil is controlled not only by the number of nitrifying autotrophs but also the nitrifying activity of individual microorganisms in certain environments such as in soils polluted with heavy metals.

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摘　　要

土壌中における硝化作用に及ぼすカドミウムの影響，とくにカドミウム汚染土壌中での硝化活性と独立栄養硝化細菌数との関係について検討した．土壌に 5, 50, 500 μg/g 乾土となるようにカドミウムを混入して32日間前培養を行い，その後アンモニア態窒素を添加して硝化活性と硝化菌数（アンモニア酸化細菌数，亜硝酸酸化細菌数）の推移を測定した．5 μg/g 乾土区および 50 μg/g 乾土区の硝化活性は非汚染土壌と同様な推移を示したが，500 μg/g 乾土区では著しく抑制された．一方，硝化菌数のうちアンモニア酸化細菌数は，硝化活性が最も低かった 500 μg/g 乾土区において最も多く計数され，土壌の条件によっては，硝化活性を，硝化を司どる微生物の多寡と関連づけられない場合があることが示唆された．このことは，土壌中の硝化活性を測定することによって硝化菌数の多寡を評価する簡便法には限界があり，慎重に取り扱う必要があることを示している．