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COMPARISON OF THE EFFECTS OF COPPER AND LEAD ON SOIL MICROBIAL BIOMASS CARBON AND NITROGEN IN RED SOIL*

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Abstract: A laboratory incubation experiment was conducted to study the effect of copper as cupric sulfate ($CuSO_4 . 5H_2O$), and lead as lead acetate ($Pb(OAc)_2$) on the size of the microbial biomass in red soil. The metals were applied, separately at six different levels: Cu at 50, 100, 200, 300, 400, and 600 $\mu g \cdot g^{-1}$ soil and Pb at 100, 200, 400, 600, 800, and 1000 $\mu g \cdot g^{-1}$ soil. In comparison to uncontaminated soil, the microbial biomass carbon (C_{mic}) and biomass nitrogen (N_{mic}) decreased sharply in soils contaminated with Cu and Pb. The microbial biomass C:N ratio in the metal contaminated soil was observed to be considerably higher than that in untreated control. Between the two tested metals, Cu displayed greater biocidal effect on microbial biomass carbon and nitrogen than Pb, showing their relative toxicity in the order: Cu$\gg$Pb.

Key words: copper, lead, microbial biomass, red soil
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INTRODUCTION

Agricultural activities and human industrialization increase release of heavy metals into the environment. Since, heavy metals readily accumulate in soil, the amount in soil is likely to continue to increase. The metals in soil have deleterious effects on the metabolism of the soil microorganisms, which in turn affect the organic matter decomposition and microbial activities (Fritze et al., 1989).

Copper (Cu) is a heavy metal toxic to soil microorganisms, but it is also an essential nutrient cation at a trace level. Copper sulfate has been used as an algicide since the early 1900s in eutrophic lakes and is still widely used today. Cu mobility and displacement in soils is relatively lower, it is strongly bound by organic matter and clay minerals, so downward movement of Cu in clayey or silty soils is almost negligible.

Excessive heavy metal concentration in the soil was reported to cause a decrease in microbial population(Hicks et al., 1990; McGrath et al., 1995), changes in the population's structure (Bardgett et al., 1994) and physiological activity (Contrufo et al., 1995). The influence of heavy metals on the microbial biomass varied with the kind of heavy metal and with the soil type. Literature(Doelman, 1985) on the effects of Cd, Cr, Cu, Hg, Ni, Pb, and Zn on the microbial biomass showed that the effect of Hg, Cd, Cu was the most significant and that the effect of Pb was the least significant. Baath (1989) found similar tendency (Cd > Cu > Zn > Pb). The variation in the effect may be ascribed to differences in the metal toxicity to the soil microorganisms and the metal forms in soil. Lead has assumed greater significance because its dispersal now through anthropogenic activity has exceeded the inputs from natural sources by about 17 - fold(Nragu and Pacyna, 1988), so it is of great environmental concern to researchers and the public because of its great abundance and long persistence. Lead tends to accumulate in soils due to its low solubility and relative freedom from microbial degradation. It remains accessible to the food chain far into the future(Alloway, 1990, Merrington et al., 1993).

The addition of copper to soil was reported to significantly decrease the amount of microbial biomass, and to have a pronounced toxic effect on the size of the biomass compared to certain metals such as Pb and As (Aoyama and Nagumo, 1997). In general bacteria are more sensitive to

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copper than fungi(Huysman et al.,1994). Low doses of copper (10 – 100 $\mu g \cdot g^{-1}$) added to sandy and alluvial soils showed a markedly harmful influence on the growth of bacteria, actinomycetes, cellulolytics, and microflora and nitrification in both soils while Pb in the concentration of 500$\mu g \cdot g^{-1}$ had no effect on microorganisms(Maliszewska et al.,1985).

MATERIALS AND METHODS

A laboratory incubation experiment was conducted in a red soil. The red soil was developed on Quaternary red clay parent material and collected from Longyou County, Zhejiang Province, southeastern part of China. The fresh field soil immediately after collection, was brought to the laboratory, handpicked to remove plant residues and soil animals, passed through a 2 mm sieve, and homogenized. A sub-sample of the soil was taken, air-dried, ground, and analyzed for various physico-chemical properties listed in Table 1. The total metals (Cu, Pb) in soil were measured by an atomic absorption spectrophotometer (AAS) after digestion with a mixture of HNO_3-HCl(Soon and Abboud, 1993). The available metals (Cu, Pb) were extracted with EDTA solution and analyzed by AAS.

Table 1 Basic properties of the tested red soil

Characteristics	Unit	Value
Textural class	–	Clay loam
Water-holding capacity	$g \cdot kg^{-1}$	450
Total N	$g \cdot kg^{-1}$	1.8
pH	–	4.92
Cation exchange capacity	c $mol \cdot kg^{-1}$	18
Organic matter	$g \cdot kg^{-1}$	26.7
Total Cu	$mg \cdot kg^{-1}$	20
Total Pb	$mg \cdot kg^{-1}$	47.6
Available Cu	$mg \cdot kg^{-1}$	4.7
Available Pb	$mg \cdot kg^{-1}$	10.3

The fresh, pretreated soil in equivalent to 100 g oven-dry weight portions was transferred to 250 mL glass beakers. Two soil sample sets each containing 18 beakers were prepared at six levels of copper and lead, with three replications. At the same time, one set of three beakers was also prepared to keep as control without metal application. The moisture contents in the soil samples were first adjusted to 40% of the soil water-holding capacity (WHC) by adding distilled water and then the samples were pre-incubated at 25 ℃ for 7 days (conditioning period), after which, designated amounts of copper sulfate were applied to the first set of beakers to achieve copper concentrations of 50, 100, 200, 300, 400, and 600 $\mu g \cdot g^{-1}$ soil. To the second set of beakers designated amounts of lead acetate were added to obtain Pb concentrations of 100, 200, 400, 600, 800, and 1000 $\mu g \cdot g^{-1}$ soil. All the metal salts were applied in solution form adjusted to pH 4.92 (soil pH). After the treated soils' water contents were adjusted to 50% WHC. They were incubated at 25 ℃ for 14 days. The soil moisture was kept at the same level (50% WHC) by adding distilled water at regular intervals throughout the incubation period. At the end of the incubation, soil samples were taken and analyzed as follows.

SOIL ANALYSIS

Microbial biomass carbon (C_{mic})(Vance et al.,1987)

Soil samples taken at the end of incubation were fumigated with ethanol free $CHCl_3$ and extracted with 0.5 mol/L K_2SO_4. The C_{mic} was calculated based on a kc value of 0.45. The organic carbon in the soil extracts was measured using an automated TOC analyzer(Wu et al.,1990).

Microbial biomass nitrogen (N_{mic}) (Brookes et al., 1985b)

Soil samples taken at the end of incubation were fumigated with ethanol free $CHCl_3$ and extracted with 0.5 mol/L K_2SO_4, and the total nitrogen in the soil extracts was measured after Kjeldahl digestion (Brookes et al., 1985a) Statistix software(Costat 1990) was used for variance analysis of data.

RESULTS

Effect of copper on soil microbial biomass

Application of copper to the soil sharply reduced the microbial biomass (Table 2). The microbial biomass carbon (C_{mic}) and microbial biomass nitrogen (N_{mic}) decreased consistently with

Table 2 Effect of copper on microbial biomass in red soil

Cu level $\mu g \cdot g^{-1}$	Biomass C[a] $\mu g \cdot g^{-1}$	Reduction %	Biomass N[a)] $\mu g \cdot g^{-1}$	Reduction %	BiomassC:N -
Control	227.5 A		44.2 A		5.1
50	214.3 A	5.8	40.0 A	9.5	5.4
100	192.1 B	15.6	31.2 AB	29.4	6.2
200	178.5 BC	21.5	23.9 BC	45.9	7.5
300	162.5 C	28.6	19.2 CD	56.7	8.5
400	143.9 D	36.7	15.3 D	65.4	9.4
600	105.4 E	53.7	8.6D	80.5	12.2

[a)] Means differ significantly according to LSD at 1% of probability

increasing level of copper in the soil. Results (Fig.1) indicated that copper applied as copper sulfate at the levels of 50, 100, 200, 300, 400, and 600 $\mu g \cdot g^{-1}$ soil caused 5.8%, 15.6%, 21.5%, 28.6%, 36.7%, and 53.7% reduction in the C_{mic}, respectively compared with control. Copper treatments at levels of 50, 100, 200, 300, 400, and 600 $\mu g \cdot g^{-1}$ soil resulted in 9.5%, 29.4%, 45.9%, 56.7%, 65.4% and 80.5% reductions in the N_{mic} respectively, compared with the control (Fig.2).

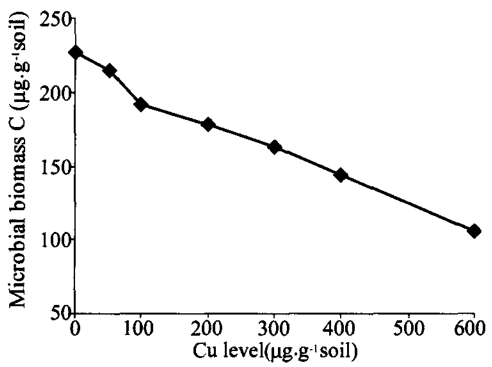

Fig.1 Effect of Cu on microbial biomass C

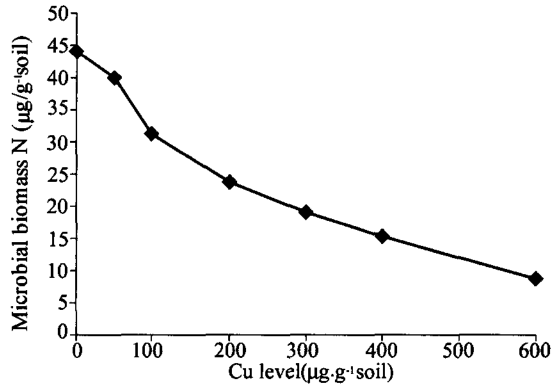

Fig.2 Effect of Cu on microbial biomass N

A marked increase in the biomass C: N ratio was noticed due to increase in the level of the applied copper (Fig.3). The biomass C: N ratio of 5.1 observed in the control was increased to 5.4, 6.2, 7.5, 8.5, 9.4 and 12.2 due to copper applications of 50, 100, 200, 300, 400, and 600 $\mu g \cdot g^{-1}$ soil, respectively.

Effect of lead on soil microbial biomass

The addition of lead to the soil also reduced considerably the size of soil microbial biomass (Table 3). Results (Fig.4) showed that lead ap-

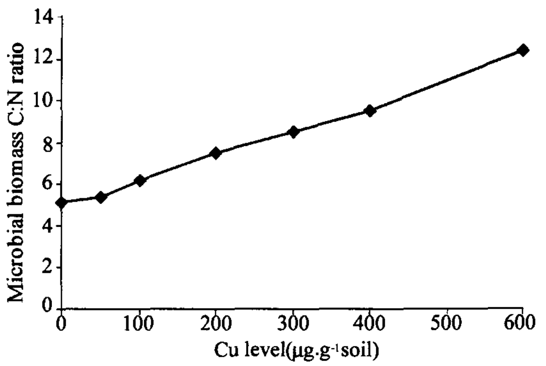

Fig.3 Effect of Cu on microbial biomass C:N ratio

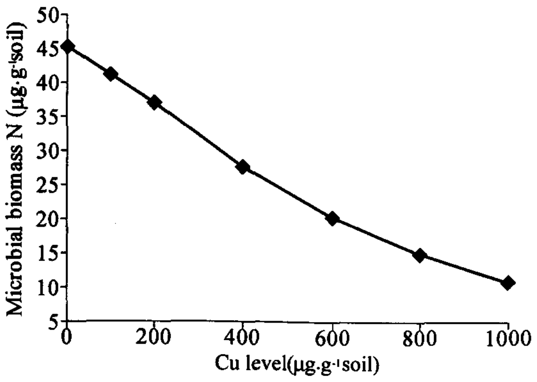

Fig.4 Effect of Pb on microbial biomass C

plied as lead acetate at the level of 100 $\mu g \cdot g^{-1}$ soil caused 3.6% reduction in the biomass carbon (C_{mic}) compared with control, whereas lead treatments levels of 200, 400, 600, 800, and 1000 $\mu g \cdot g^{-1}$ soil resulted in 6.7%, 15.3%, 25.6%, 34.7%, and 45.7% reductions in the C_{mic}, respectively, as against the control. Biomass nitrogen (N_{mic}) showed considerable reductions when various levels of lead were applied to the soil. Results indicated that the lead treatments at levels of 100, 200, 400, 600, 800, and 1000 $\mu g \cdot g^{-1}$ caused 9.2%, 18.3%, 39.3%, 55.1%, 66.7% and 76.6% reductions in the Nmic (Fig. 5) respectively, as against the control. A marked increase in the biomass C: N ratio was noticed due to increase in the level of applied lead (Fig.6). The biomass C: N ratio of 4.9 observed in the control was increased to 5.3, 5.7, 6.9, 8.3, 9.8 and 11.4 due to application of lead at levels of 100, 200, 400, 600, 800, and 1000 $\mu g \cdot g^{-1}$ soil, respectively.

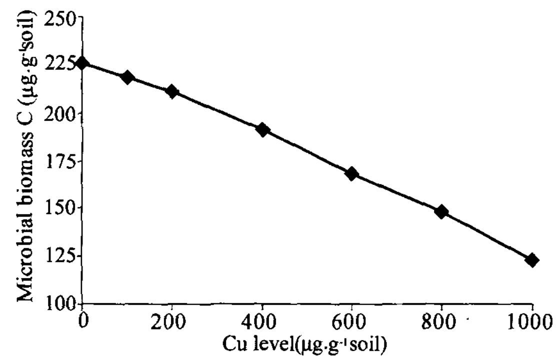

Fig.5 Effect of Pb on microbial biomass N

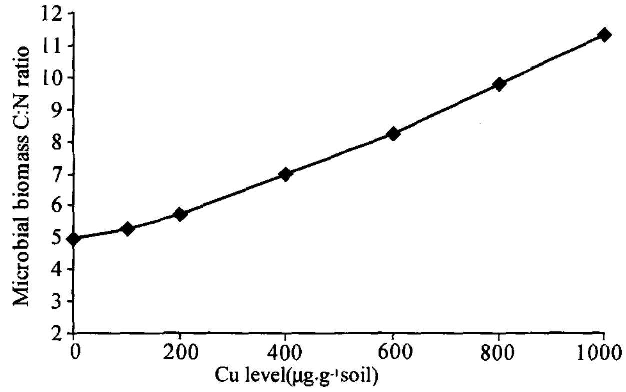

Fig.6 Effect of Pb on microbial biomass C:N ratio

Table 3 Effect of lead on microbial biomass in red soil

Pb level $\mu g \cdot g^{-1}$	Biomass C^{a} $\mu g \cdot g^{-1}$	Reduction %	Biomass $N^{a)}$ $\mu g \cdot g^{-1}$	Reduction %	Biomass C:N –
Control	226.3 A		45.4 A		4.9
100	218.1 AB	3.6	41.2 A	9.3	5.3
200	211.1 AB	6.7	37.1 AB	18.3	5.7
400	191.7 BC	15.3	27.6 BC	39.2	6.9
600	168.4 CD	25.6	20.4 CD	55.1	8.3
800	147.8 DE	34.7	15.1 D	66.7	9.8
1000	122.9 E	45.7	10.8 D	76.6	11.4

[a)] Means differ significantly according to LSD at 1% of probability

DISCUSSION

Although applications of both metals (Cu, Pb) reduced the microbial biomass significantly, the effect of Cu was more pronounced as compared to Pb at the same levels of application. The relative toxicity of metal compounds to microbial processes and populations decreased in the order of Cd > Cu > Zn > Pb (Bath, 1989; Maliszewska et al., 1985; Hattori, 1992; Leita et al., 1995; Aoyama et al., 1997; Khan et al., 1998 Huang et al., 1998). This suggested that the adsorption processes in the soil might be a large extent, control the toxicity of heavy metals to microbial biomass. The addition of copper to soil was reported to significantly decrease the amount of soil microbial biomass C and N as compared to certain metals such as Pb and As when compared on a molarity basis and the toxicity was in the order: Cu$\gg$Pb > As (Aoyama et al., 1997). These conclusions are in accordance with our findings.

The present study showed a significant depressing effect of lead on the soil microbial biomass. One reason for this depressing effect of lead observed in our study might be that in the previous studies, lead was added as lead chloride, whereas we applied the lead in the form of lead acetate. Since the solubility of lead acetate is about fifty times that of lead chloride (Cook et al., 1996) and as the accompanying anions could have a strong effect on the toxicity of the applied metal salt (Chet et al., 1991a and b; Khan et al., 1998), the present results showed more marked effect of the applied lead. Secondly, pH of the soil used in the present experiment was quite low (4.92), which might have decreased the lead adsorption in the soil due to its effect on the degree of lead hydrolysis. Therefore, the greater bioavailability of lead in the present case could have resulted in a stronger biocide effect of lead on the soil microbial biomass, which possibility had not been reported earlier.

The addition of Cu decreased significantly the amounts of biomass C and N relative to the control. The 20 mmol Cu·kg^{-1} soil treatment resulted in smaller biomass values, compared to these in the 10 mmol Cu·kg^{-1} soil treatment. The biomass values in the 10 mmol Pb.kg^{-1} treatment were significantly smaller than those in the control, but significantly larger than those in the 10mmol Cu·kg^{-1} soil treatment. These showed that Cu had a pronounced toxic effect on the size of the microbial biomass, while Pb had no or less toxic effect on it, when compared on a molarity basis (Aoyama et al., 1997). Similar findings were also reported by Hattori (1992).

The C: N ratio of the microbial biomass in the soils treated with Cu was higher than that in the soils treated with Pb. Higher C: N ratio of the microbial biomass implies that the proportion of the fungal biomass to the bacterial biomass was higher, since the C: N ratio of fungal cells is generally higher than that of bacterial cells (Jenkinson, 1976). Bacteria are more sensitive to copper than fungi (FlieBbach et al., 1994; Huysman et al., 1994). Low doses of copper (10 – 100 $\mu g \cdot g^{-1}$) added to sandy and alluvial soils showed a markedly harmful influence on the growth of bacteria, and microflora while the growth of fungi was stimulated by the addition of copper, especially in the soils with the highest doses of Cu (5 000 – 10 000 $\mu g \cdot g^{-1}$). Pb in 500 $\mu g \cdot g^{-1}$ soil concentration had no effects on microorganismsZ (Maliszewska et al., 1985). Hence, the observed increase in the C: N ratio in our results could be due to the increased fungal biomass in soil.

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