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Effect of acetate on lead toxicity to microbial biomass in a red soil*

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Abstract—A laboratory incubation experiment was conducted to elucidate the effect of acetate on lead bioavailability and toxicity to microbial biomass in a red soil. Results indicated that the application of acetate along or at the lower lead levels of 100 and 200 μg/g soil stimulated the soil microbial biomass. The addition of acetate at the higher lead levels of 300, 450 and 600 μg/g soil caused significantly greater reductions in the biomass carbon(C_{mic}) and the biomass nitrogen (N_{mic}), compared with the control or the same lead levels applied individually. A greater increase in the biomass C:N ratio occurred by acetate addition at the same lead levels. The concentration of 0.01 mol/L $CaCl_2$-extractable lead was considerably higher in the lead plus acetate treatments than at the same lead levels with no acetate. Based on these results, it was concluded that the application of acetate might have suppressed the lead adsorption in the soil which in turn resulted in its more bioavailability and hence more toxicity to the soil microbial biomass.

Keywords: acetate, lead toxicity, microbial biomass, red soil.

1 Introduction

Environmental pollution by lead, resulting from mining and smelting activities, sewage sludge usage in agriculture and contamination from vehicle exhausts is well established (Adriano, 1986; Nriago, 1988). This fact underscores the need for an intensive research effort aimed at understanding the toxic effects of lead in the soil environment. The negative effects of lead on plant growth and its possible entry into the food-chain has been well documented (Solgaard, 1978; Koeppe, 1981) but a little is known about its adverse effects on soil microbial biomass (McGrath, 1995). A few studied have so far measured the microbial biomass in lead-spiked soils and yielded conflicting results (Patra, 1991; Leita, 1995; Dar, 1997; Khan, 1998). Leita *et al*. (Leita, 1995) found a non-significant decline in the biomass carbon at 200 μg/g soil lead. While, Khan *et al*. (Khan, 1998) and Dar (Dar, 1997) reported a significant reduction of microbial biomass at 200 and 250 μg/g Pb soil, respectively. These contradictions in the current literature urge for further evaluation of the toxic effects of lead on soil microflora.

Several factors such as the pH, inorganic ions(CO_3^{2-}, PO_4^{3-}), clay minerals, hydrous metal oxides and organic matter may influence the lead bioavailability and hence its potential toxicity in the soil environment. Among these, an important factor is the presence of organic substances capable of forming soluble chelate complexes with the lead (Adriano, 1986; Cook, 1996). Studies have shown the mobility of lead to be increased significantly by complexation with some organic ligands in soils (Stevenson, 1979; Reddy, 1995; Zhenbin, 1997). However, no attempt has been made to study the effect of such organic ligands on lead bioavailability and toxicity to the soil microbial biomass. Acetate is one of the most abundantly present organic ligands in the soil (Stevenson, 1972). Moreover, a significant amount of acetate may enter the soil through the application of manures and other organic wastes. The present work was, therefore, undertaken to: (1) study the effect of lead on the size of the soil microbial biomass, and (2) elucidate the effect of acetate on lead bioavailability and toxicity to soil microbial biomass.

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2 Experimental

2.1 Soil

A red soil (Ultisol) was collected at 0—15 cm in depth from the Longyou County, Zhejiang Province, China. The field fresh soil immediately after the collection was brought to the laboratory, hand-picked to remove stones, discrete plant residues and large soil animal(earthworms etc.), passed through a 2 mm sieve, and homogenized. A subsample of the soil was taken, air-dried, ground, and analyzed for various physico-chemical properties listed in Table 1. The total lead in soil was measured by atomic absorption spectrophotometer(AAS) after aqua regia digestion (Soon, 1993). The available lead was extracted with 0.01 mol/L $CaCl_2$ solution and analyzed by ASS.

Table 1 Physico-chemical properties of the red soil used in the experiment

Texture	Clay loam	pH	4.51
Water holding capacity, g/kg	421.80	Cation exchange capacity, cmol/kg	15.00
Total organic carbon, g/kg	10.08	Total Pb, mg/kg	52.25
Organic matter, g/kg	17.40	Available Pb, mg/kg	0.05

2.2 Incubation

The moist soil in portions equivalent to 100 g oven-dry weight was transferred to 250 ml glass beakers. Three sets each containing 18 beakers were prepared to maintain six lead levels with or without three levels of the acetate. All the treatments were replicated thrice. The soil samples were first adjusted to 40% of the soil water-holding capacity(WHC) by adding distilled water and then pre-incubated at 25℃ for 7 days(conditioning period). After conditioning, to the first set of beakers designated amounts of $Pb(OAc)_2$ were applied to achieve the lead concentrations of 0 (background), 100, 200, 300, 450 and 600 μg/g soil. No acetate was added to this set of treatment. To the second and third sets of beakers same lead levels were maintained as mentioned above but one set was adjusted with acetate to 900 μg/g, and the other set to 2700 μg/g soil, by adding acetic acid(CH_3COOH) solution adjusted to pH 4.51 (soil pH). The water contents in the treated soils were adjusted to 50% WHC and the soil samples were incubated at 25℃ for 30 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 described below.

2.3 Soil analyses

Microbial biomass carbon(C_{mic}): 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(Vance, 1987). The organic carbon in soil extracts was measured using an automated TOC analyzer (Wu, 1990) and the C_{mic} was calculated by a k_c value of 0.45.

Microbial biomass nitrogen (N_{mic}): Soil samples were fumigated with ethanol free $CHCl_3$ and extracted with 0.5 mol/L K_2SO_4(Brookes, 1985a). The total nitrogen in soil extracts was measured after Kjeldahl digestion (Brookes, 1985b) and the N_{mic} was calculated by a k_N value of 0.54.

Available lead(Pb): Soil samples were extracted with 0.01 mol/L $CaCl_2$ solution (Zhenbin, 1997) and lead contents in the extracts were determined by atomic absorption spectrophotometer.

2.4 Statistical analyses

Experimental data were examined by analysis of variance and unweighted least squares linear regression using statistics 4.1 software.

3 Results

The addition of acetate alone to the soil markedly enhanced the soil microbial biomass(Fig.1 and Fig.2). There was a non-significant increase of 2.6% in the biomass carbon(C_{mic}) and 4.1% in the biomass nitrogen (N_{mic}) at acetate level of 900 μg/g soil. A significant ($P=0.01$) increase of 40.9% in the C_{mic} and 22.1% in the N_{mic} was observed at acetate addition of 2700 μg/g soil. The C_{mic} to N_{mic} ratio showed a significant increase at acetate level of 2700 μg/g soil (Fig. 3).

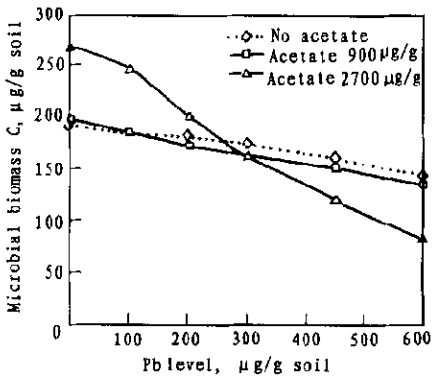

Fig. 1 Effect of acetate on lead toxicity to microbial biomass carbon in a red soil

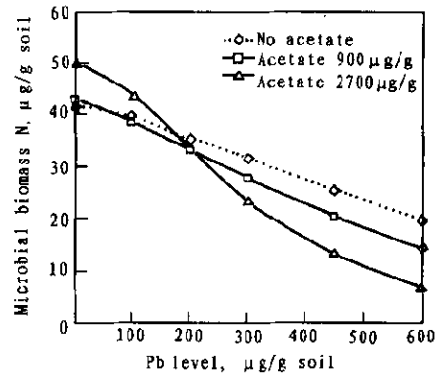

Fig. 2 Effect of acetate on lead toxicity to microbial biomass nitrogen in a red soil

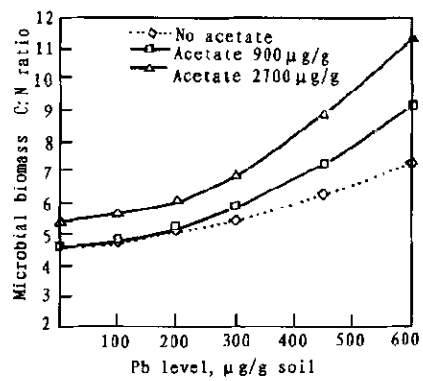

Fig. 3 Effect of acetate on lead toxicity to microbial biomass C:N ratio

The applicaiton of lead, without acetate, slightly reduced the soil microbial biomass. A significant ($P=0.01$) decline of 8.7% in the biomass carbon (C_{mic}) occurred at lead concentration of 300 μg/g soil (Fig.1). The higher levels of 450 and 600 μg Pb/g soil caused a significant ($P=0.01$) reduction of 16.4% and 24.3% in the C_{mic}, respectively, compared with the control. The biomass nitrogen (N_{mic}) was significantly ($P=0.01$) reduced by 14.1% at lead concentration of 200 μg/g soil (Fig.2). The other lead levels of 300, 450 and 600 μg/g soil exhibited 22.9%, 37.7% and 51.3% reductions in the N_{mic}, repectively, relative to the control. A significant increase in the C_{mic} to N_{mic} ratio was observed at levels of 300, 450 and 600 μg/g soil(Fig.3).

The toxicity of lead was considerably increased by the addition of acetate at 900 μg/g soil. The application of 200 μg Pb/g soil with 900 μg/g soil acetate caused a significant ($P=0.01$) reduction of 9.6% in the C_{mic}, compared with the control (Fig.1). The other treatments of 300, 450 and 600 μg/g soil with acetate (900 μg/g soil) resulted in a greater reductions of 13.8%,

20.9%, 29.8% in the C_{mic}, respectively. The biomass nitrogen (N_{mic}) showed a significant decline of 19.2% at lead concentration of 200 μg/g soil with 900 μg/g soil acetate (Fig. 2). A highly significant reductions of 32.1%, 49.6% and 64.5% in the N_{mic} were observed at 300, 450 and 600 μg Pb/g soil. The C_{mic} to N_{mic} ratio was significantly increased at lead concentrations of 200, 300, 450 and 600 μg/g soil, in the presence of 900 μg/g soil acetate (Fig. 3).

The addition of 2700 μg/g soil acetate stimulated the soil microbial biomass at lower lead levels of 100 and 200 μg/g soil. A significant ($P = 0.01$) increase of 29.7% in the biomass carbon (C_{mic}) occurred at 100 μg Pb/g soil with 2700 μg/g soil acetate (Fig. 1). The 200 μg Pb/g soil applied with 2700 μg/g soil acetate showed a non-significant increase of 5.2% in the C_{mic}, compared with the control. The biomass nitrogen (N_{mic}) exhibited a non-significant increase of 5.8% at 100 Pb μg/g soil with 2700 μg/g soil acetate (Fig.2).

A marked decline in the biomass carbon (C_{mic}) and biomass nitrogen (N_{mic}) occurred at the higher lead levels, when applied with 2700 μg/g soil acetate. The C_{mic} exhibited a highly significant ($P=0.01$) reductions of 14.3 %, 36.4% and 56.6% at lead concentrations of 300, 450 and 600 μg/g soil, respectively, with 2700 μg/g soil acetate (Fig. 1). The N_{mic} showed a highly significant ($P=0.01$) declines by 18.7%, 42.3%, 66.7% and 82.2% at lead levels of 200, 300, 450 and 600 μg/g soil, respectively, with 2700 μg/g soil acetate (Fig. 2). A sharp increase in the C_{mic} to N_{mic} ratio was noted at all the lead levels with 2700 μg/g soil acetate (Fig. 3). Linear regression analysis revealed the following relationship between the biomass carbon (Y_1, μg/g soil), biomass nitrogen (Y_2, μg/g soil), biomass C:N ratio (Y_3) and the concentrations (μg/g soil) of acetate (Ac) and lead (Pb) applied to the soil.

$$Y_1 = 184.101 + 3.038 \times 10^{-2}(Ac) - 5.164 \times 10^{-2}(Pb) \quad 9.633 \times 10^{-5}(Ac \times Pb), \quad (1)$$
$$F = 295.90^{**}$$

$$Y_2 = 41.629 + 2.640 \times 10^{-3}(Ac) - 3.629 \times 10^{-2}(Pb) - 1.407 \times 10^{-5}(Ac \times Pb), \quad (2)$$
$$F = 591.49^{**}$$

$$Y_3 = 4.156 + 1.495 \times 10^{-4}(Ac) + 5.160 \times 10^{-3}(Pb) + 1.910 \times 10^{-6}(Ac \times Pb), \quad (3)$$
$$F = 212.71^{**}.$$

The effect of acetate on lead bioavailability is presented in Fig.4. The 0.01 mol/L $CaCl_2$-extractable Pb in the soil increased significantly with the increasing level of the soil applied lead. A considerable increase in the 0.01 mol/L $CaCl_2$-extractable Pb occurred at acetate level of 900 μg/g soil. A much greater increase in the 0.01 mol/L $CaCl_2$-extracatable Pb was seen as the acetate concentration was increased to 2700 μg/g soil. The relationship between the 0.01 mol/L $CaCl_2$-extractable Pb(Y_4, μg/g soil) and the acetate (Ac) and lead (Pb) treatments (μg/g soil) is as follows:

$$Y_4 = 0.025 - 2.742 \times 10^{-5}(Ac) + 2.300 \times 10^{-3}(Pb) + 1.019 \times 10^{-6}(Ac \times Pb), \quad (4)$$
$$F = 138.37^{**}$$

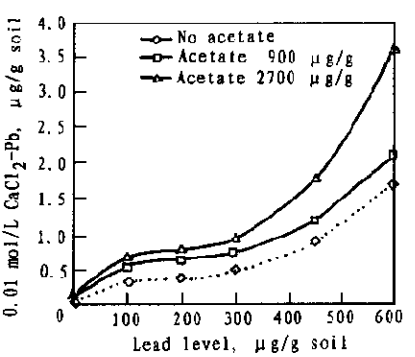

Fig. 4 Effect of acetate on lead bioavailablity in a red soil

4 Discussion

The microbial biomass carbon (C_{mic}) and microbial biomass nitrogen (N_{mic}) contents in the tested soil were 192.2 μg/g and 41.4 μg/g soil, respectively. These values fall within the range, 100—417 μg/g soil for the biomass carbon, and 18—51 μg/g soil for the biomass nitrogen, reported in the soils of China(Wang, 1996). In the estimation of N_{mic} by the fumigation-extraction method, the possibility of error due to NH_4^+-fixation in the soil was neglected, as a previous study (Chen, 1997) revealed the fumigation-extraction method to be reliable for estimating the microbial biomass nitrogen in red soils.

The results demonstrated a positive effect of acetate on the soil microbial biomass. This was probably because the acetate being an organic carbon compound acted as a substrate for the soil microorganisms(Alexander, 1977). Finding a readily available energy source, microbes became active and a part of the added acetate was converted into soil microbial biomass. Similar results showing microbial biomass stimulation by glucose additions have been reported earlier by Bardgett and Saggar(Bardgett, 1994) and Tsai *et al*. (Tsai, 1997). Loka Bharathi *et al*.(Loka, 1990) also found an increased growth of the sulfate-reducing bacterium in the presence of acetate. A significant increase in the biomass carbon to biomass nitrogen ratio at the higher level of acetate revealed less assimilation of nitrogen by the growing microbial populations in the soil. As the soil was naturally low in available nitrogen and also it was not applied externally, the less uptake of nitrogen by the microbial biomass resulting in higher biomass C:N ratio is quite justifiable.

The lead application without acetate showed a relatively less toxicity towards the soil microbial biomass, causing a significant inhibition only at the higher lead levels. This might be due to a very high chemical affinity of lead towards the soil constituents(Adriano, 1986; Evans, 1989; Cook, 1996), which rendered most part of the added lead unavailable in the soil. As only the soluble fraction of lead (Pb^{2+}) can react with biological membranes to have a direct toxic effect, the reduced lead levels in the soil solution might be the major reason for less toxicity of the added lead.

The stimulation of microbial biomass at the lower and levels in the presence of acetate was mainly because of the favorable effect of acetate on the soil microbial populations. Regression analysis indicated clearly that acetate had a positive effect on the soil microbial biomass. Therefore, the toxic effect of lead at low levels was mitigated by the stimulating effect of the acetate. This agrees with the findings of Loka Bharathi *et al*. (Loka, 1990) who observed that the inhibitory concentrations of mercury and lead had a stimulatory effect on the sulfate-reducing bacterium when acetate was used as a substrate. However, comparison of the above mentioned lead plus acetate treatments with those of the acetate alone at the same concentration demonstrated that the stimulation of soil microbial biomass, following the acetate addition was considerably lower in the presence of lead. This is in line with the observations of Chander and Brookes(Chander, 1992), and Bardgett and Saggr(Bardgett, 1994), who found that the efficiency of substrate conversion into microbial biomass was considerably lower in metal-contaminated soils.

A significant increase in lead availability by acetate addition at the higher lead levels was probably because of the ability of acetate to suppress lead adsorption by the soil constituents. The presence of acetate at higher concentrations can reduce the metal adsorption due to (1) an increase in the system ionic strength which in turn diminishes the heavy metal adsorption (Garcia-Miragaya, 1976); (2) a high ligand/metal ratio which may result in preferential adsorption of the free ligand with a resulting loss in the capacity of soil to adsorb the metal(Elliot, 1982; Benjamin,

1981). Since, acetic acid is weakly acidic ($pK_a = 4.75$), the un-ionized form, CH_3COOH, predominates for pH values < 4.75. This uncharged form experiences no repulsion by the negatively charged soil surfaces and thus might be adsorbed by H-bonding between the carboxyl group of acetate and the hydrolyzed surface functional groups (e.g. MOH, where M = Si, Al or Fe). As the pH of the soil used in this experiment was quite low(4.51), the addition of acetate might have resulted in the adsorption of free acetate, thus reducing the capacity of soil to adsorb lead. Elliott and Denneny (Elliott, 1982) reported suppression of cadmium adsorption in the presence of acetate under acidic soil conditions. Therefore, increased concentrations of lead in the soil solution could be the main cause of the greater toxicity of lead in the presence of acetate. Another possible reason for the higher toxicity of lead applied with acetate might be the more absorption of the acetate-lead complexes by the soil microorganisms. Lightart (Lightart, 1980) found that cadmium toxicity to *Pseudomonas* was increased significantly in the presence of citrates. He speculated that the bacterium could exclude the metal ion, but citrate facilitated the transport of the toxic metal across the membrane. Similar results showing increased toxicity of tin in the presence of serine and hydroxylflavone were later reported by Hallas *et al*. (Hallas, 1982).

An increase in the C_{mic} to N_{mic} ratio with the increasing lead toxicity in the soil resulted probably due to changes in the microbial community structure. Several investigators have reported an increase in the fungal to bacterial ratio with the increasing metal stress in soils (Maliszewska, 1985; Hattori, 1992). Anderson and Domsch (Anderson, 1980) found that C : N ratio of the fungal biomass was considerably higher than that of the bacteria. Therefore, an increase in the biomass C:N ratio evident from our results might be because of the increased fungal biomass in the soil. Patra *et al*. (Patra, 1991) and Khan (Khan, 1998) also reported similar results.

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