Journal of Environmental Sciences Vol. 11, No. 1, pp. 40—47, 1999 ISSN 1001—0742

Effects of heavy metal pollution on soil microbial biomass

K. S. Khan, Huang Chang-yong

College of Natural Resources and Environmental Sciences, Zhejiang Agricultural University, Hangzhou 310029, China

Abstract—This paper reviewed the effects of heavy metals on microbial biomass in metal-polluted soils. Laboratory and field investigations where metals were applied as inorganic or organic salts demonstrated a significant decline in the size of soil microbial biomass. In most of the cases, negative effects were evident at metal concentrations below the European Community's (EC) current permissible metal levels in the soil. Application of metal-enriched sludges and composts caused significant inhibition of microbial biomass at surprisingly modest concentrations of metals in the soil that were indeed smaller than those likely to decrease the growth of sensitive crop species. On the whole, relative toxicity of metals decreased in the order of Cd > Cu > Zn > Pb, but a few exceptions to this trend also existed. A significant decline in the biomass carbon to organic carbon ratio(C_{min}/C_{org}) in metal-polluted soils indicated that this parameter can serve as a good indicator of the toxicity of metals on soil microflora. The knowledge regarding the response of soil biota to metal interactions and the factors affecting metal toxicity to soil microorganisms is still very limited and warrants further study.

Keywords: heavy metal, pollution, soil, microbial biomass.

1 Introduction

1.1 Basic concept of microbial biomass

Soil microbial biomass is defined as the living part of soil organic matter, excluding plant roots and soil animals larger than about 5×10^3 μm^3 (Jenkinson, 1981). It consists of many species of bacteria and fungi, together with relatively larger soil organisms such as yeasts, algae and protozoa. Inspite of the fact that each species plays a particular role in the soil ecosystem, for many purposes the biomass is regarded as a single compartment(Brookes, 1985). Treating soil biomass in this way, as an undifferentiated whole, is useful in studying the flux of energy and material through the soil populations. Chloroform-fumigation method showed that soil microbial biomass is much larger in size than previously realized(Jenkinson, 1981)

1.2 Significance of microbial biomass in soil fertility

Because of the large amount of biomass in the soil and also because it is a much more labile components of soil organic matter, soil microbial biomass plays a significant role in the maintenance of long-term soil fertility. The contribution of microbial biomass to the plant-availability of nutrients arises mainly in two ways. Firstly, microbial biomass is an important reservoir of plant nutrients especially N, P and S. Secondly, microbial biomass acts as a catalyst in the cycling of C, N, P and S(Smith, 1991; Lee, 1992). Hence, the magnitude of the biomass pool directly affects the nutrient flux and bioavailability. In grassland soils, the annual release of N and P through soil microbial biomass have been shown equal to, or greater, the offtakes of these nutrients by the plants(Brookes, 1984). A recent study(He, 1997) reported highly significant correlations between the biomass carbon(C_{mic}), biomass nitrogen(N_{mic}) and the N uptake by plants. These evidences indicate that microbial biomass carbon and microbial biomass nitrogen are not only important soil fertility parameters but also important indices of soil sustainability.

1.3 Heavy metal pollution vs microbial biomass

Heavy metals are toxic to all organisms if present in high concentrations. Microorganisms are no different in this respect, and heavy metal exposure has, since the last century, been known to affect the microbial growth and survival(Baath, 1989). In the last several years, there has been increasing concern over the role of potential toxic metals in the degradation of natural environment. Meanwhile, the development of fairly simple and rapid laboratory methods encouraged scientists to study microbial biomass as a single compartment which was previously impossible due to methodological limitations. Since then a number of studies have been conducted aimed at evaluating the effects of metals on soil microbial biomass and microbial processes in the soil. Different aspects of heavy metal toxicity towards microorganisms and microbially mediated processes in soil have been reviewed earlier by Tyler(Tyler, 1981), Domasch(Domasch, 1981), Baath(Baath, 1989) and McGrath(McGrath, 1995), but none of them comprehensively reported the toxic effects of metals on soil microbial biomass. The main objective of the present review is therefore to summarize the recent advances in the studies of the effects of heavy metal pollution on microbial biomass in soils.

2 Effects of metal pollution on microbial biomass carbon in soils

2.1 Microbial biomass C(C_{mic})

Measurement of the microbial biomass carbon(C_{mic}) offers a mean of assessing the response of total microbial populations to the changes in soil management practices(McGrath, 1995; Roper, 1995). A number of studies reported the effect of metals on soil microbial biomass(Nordgren, 1983; Capone, 1983; Brookes, 1984; Ohya, 1988; Wilke, 1988; Schuller, 1989; Balzer, 1989; Baath, 1991; Chander, 1991; Patra, 1991; Kandeler, 1992; Speir, 1992; Aoyama, 1993; Chander, 1993; Post, 1993; Dar, 1994; Fließach, 1994; Bardgett, 1994; Leita, 1995; Aoyama, 1995; Speir, 1995; Chander, 1995; Aoyama, 1996; Dar, 1996; 1997; Kandeler, 1997; Hemida, 1997), but most of them dealt with the toxicity of metals added via sewage sludges.

Soil amendment with low-metal sludges has been shown to increase the C_{mic} significantly (Fließach, 1994; Dar, 1994; Chander, 1995; Dar, 1996; 1997). Fließach *et al*. (Fließach, 1994) found that addition of low-metal sludges at the rates of 100 and 300 $m^3/(hm^2 \cdot a)$ to an arable and a former woodland soils caused significant increase in the total C_{mic} compared to the unsludged control soil. Similarly, Dar(Dar, 1997) reported that sludge application at the rate of 0.75% to sandy loam, loam and clay loam soils caused an increase of 7% to 18% in the C_{mic}, more in the sandy loam and less in loam soils. The positive effect of sludge application in above studies was mainly due to the addition of organic carbon and nutrients contained in the sludges. However, in cases where high-metal sledges were applied or the sludge application continued for a long period of time, a significant decline in the C_{mic} was noted (Brookes, 1984; Fließach, 1994; Chander, 1995; Dar, 1996; 1997). Brookes and McGrath(Brookes, 1984) found that at Woburn and Luddington experimental sites, the C_{mic} was about 50% less in high-metal sludge or sludge-compost treated soils compared to soils receiving farmyard manure or inorganic fertilizers. However, biomass measurements at Luddington site indicated that the decline occurred only due to the addition of Cu- or Ni-enriched sludges, whereas the application of Zn- or Cr-enriched sludges did not affect the biomass. In another study, Fließach *et al*.(Fließach, 1994) observed a 26% reduction in the C_{mic} in high-metal sludge treated(300 $m^3/(hm^2 \cdot a)$) soils. It was concluded that application of sludges in larger amounts over a long period of time can lead to the accumulation of toxic metals in the soil that may results in adverse effects on the soil microflora and inhibition of the soil microbial biomass (Brookes, 1984; Balzer, 1989).

A major problem associated with metals is their long persistence in soil environment. Many workers studied the long-term effects of metals added by sewage sludges on soil microbial biomass (Brookes, 1984; Balzer, 1989; Chander, 1991; 1995). Brookes and McGrath(Brookes, 1984) found that the negative effects of metals on soil microbial biomass at Woburn experimental site were evident even more than 20 years after the last application of sludges or sludge-composts. Chander (Chander, 1991) observed a significant reduction in the C_{mic} in a sandy loam soil at Luddington and a silty clay loam soil at Lee Valley, where the sewage sludge, mainly enriched with single metals (Zn, Cu, Ni or Cd) were applied some 22 years ago.

Studies regarding the effects of metals at the European Community's (EC) current allowable total metal concentrations in soil(Cd: 1—3; Cu: 50—140; Cr: 100—150; Ni: 30—75; Pb: 50—300; Zn: 150—300 and Hg: 1—1.5 mg/kg soil) revealed that no single metal (Zn, Cu, Ni or Cd) in the sludge-treated soils at or below the current EC limits decreased the amount of soil microbial biomass(Chander, 1991; 1993; Chander, 1995). However, Cu at about 2.5 times of permitted metal limits reduced the biomass by about 40% in both a sandy loam and a silty clay loam soil. Zn at about the same concentration, decreased the biomass by about 40% in the sandy loam and 30% in the silty clay loam. Whereas, Cd at 6 mg/kg soil and Ni at 138 mg/kg soil(about 2—3 times of the current statutory limits) did not affect the amount of biomass in the silty clay loam soil (Chander, 1991). In a later study, Chander and Brookes(Chander, 1993) reported that Cu at about 4.9 times and Zn at about 2.3 times of permitted limits decreased the amounts of soil microbial biomass by 51% and 36% respectively. The soils containing either Cu or Zn separately at about 1.4 times of permitted limits had about 12% less C_{mic} than the control soil. In contrast, Cu and Zn in combination at about 1.4 and 1.2 times of permitted limits, respectively, decreased the biomass by about 29%, and soils containing Cu and Zn in combination at 1.8 and 1.4 times of the limits had 53% less biomass than the control soil(Chander, 1993). Thus, a combination of Zn and Cu in the above study decreased the amount of biomass at lower soil metal concentrations than either of the metal present singly, suggesting the effects were additive. In another experiment, Chander(Chander, 1995) found that Zn, Cu or Cd individually at approximately twice of the European Union (EU) metal limits decreased the C_{mic} by 20%. An exception to above results was reported by Knight *et al*. (Knight, 1997), who observed that Cu addition to a low pH(4.5) sludge-amended soil significantly reduced soil microbial biomass though its concentration was not above the current maximum allowable copper concentration in sludge-amended soils.

Compared to work on the toxicity of metals added by sewage sludges, very few studies measured the effects of metal pollution arising due to mining and smelting activities on soil microbial biomass (Nordgren, 1983; Schuller, 1989; Kandeler, 1992). The results of these studies have been quite conflicting. Schuller(Schuller, 1989) observed a non-significant effect of heavy metal contamination on microbial biomass in soils collected from an old Zn smelter and an old landfill site, while Baath *et al*. (Baath, 1991) and Kandeler *et al*. (Kandeler, 1992) reported a significant decrease in the C_{mic} with increasing heavy metal pollution in soils close to smelters. A significant inhibition of fungal biomass was also observed due to metal pollution in mining and smelting areas(Nordgren, 1983; Schuller, 1989). Nordgren(Nordgren, 1983) found about 70% decline in fungal biomass along a steep copper, zinc and lead gradient(up to 20000 μg of Cu, 20000 μg of Zn and 1000 μg of Pb/g dry soil) around a brass mill near Gusum in southern Sweden. Above 1000 μg of Cu/g, the decrease was clearly evident but below 1000 μg of Cu/g, no obvious effects were observed. However, there was a decreasing tendency for total mycelial length.

In soils receiving metals from the sewage sludge, mining and smelting activities or vehicle exhausts, the pollution is never due to one single metal. Instead it varies from mainly two to almost all possible metals. This makes conclusions regarding the relative toxicity and interactions between the metals hard to draw(Brookes, 1984; 1987; Baath, 1989). The laboratory experiments where the metals are added one by one can give such indications. A number of laboratory and filed experiments where the metals were applied as inorganic and organic salts indicated a strong biocidal effect of metals on soil microbial biomass. Wilke(Wilke, 1988) observed that Br, F, Ni and V added as inorganic salts 10 years earlier to a sandy Cambisol reduced microbial biomass at total elemental concentrations below the European Union's maximum recommended levels. Br had an inhibitory effect even below the concentration of 10 mg/kg soil. Patra *et al*. (Patra, 1991) found that Pb, Cd and Hg application to a sandy loam soil significantly reduced the soil microbial biomass. However, the lowest values for C_{mic} were observed in soils containing 20 ppm Cd and 10 ppm Hg. Similarly, Aoyama *et al*.(Aoyama, 1993) noted a significant inhibition of the microbial biomass in soils(Udifluvent and Melanudand) enriched with 100—1000 in 200—2000 mg Cu/kg soil, respectively.

Dar and Mishra(Dar, 1994) found that Cd application at 10 μg/g soil had little effect on soil microbial biomass, whereas significant reductions in the C_{mic} occurred at 25 and 50 μg Cd/g soil. In a later study by Dar(Dar, 1996), Cd additions at 10 and 50 μg/g soil showed the same trends as observed before. Speir(Speir, 1995) reported severe decline in the C_{mic} due to Cr addition at 5 and 50 μmol Cr(VI) /g soil. Leita *et al*. (Leita, 1995) in a study regarding the influence of Pb, Zn and Tl on microbial biomass survival indicated that soil applications of Zn and Tl significantly decreased the C_{mic} whereas, Pb had no effect. Among the tested metals, Zn displayed the greatest biocidal effect followed by Tl and Pb. Dar(Dar, 1997) reported that Pb addition of 100 μg/g soil as $PbCl_2$ caused no significant change in microbial biomass. However, Pb addition at the level of 250 μg/g soil significantly decreased the microbial biomass in sandy loam, whereas at 500 μg/g soil, a decrease of 16%—26% in microbial biomass occurred in both sandy loam and loam soils.

2.2 Ratio of biomass C to total organic C

Microbial biomass as a percentage of soil organic matter is one of the important parameters that can be used to assess the alteration of natural ecosystem caused by the metal pollutants(Brookes, 1995). Generally, there is a reasonably close, linear and positive relationship between organic carbon and the biomass carbon contents in uncontaminated soils(Jenkinson, 1981), but no such relationship was found in soils contaminated with heavy metals(Brookes, 1984). A decrease in the ratio of biomass C to total organic C(C_{mic}/C_{org}) with increasing concentrations of heavy metals in soil has been reported by many workers(Chander, 1991; Fließach, 1994; Bardgett, 1994). Chander and Brookes(Chander, 1991) found that the C_{mic}/C_{org} was much lower($<1.0\%$) in soils contaminated with Zn and Cu at about 2.5 times of current permitted European Union (EU) limits than in soils containing less metals. Chander and Brookes(Chander, 1993) observed that C_{mic}/C_{org} in soils contaminated with higher rates of Zn or Cu singly or with both metals in combination was less than half of that in the soils which received no sludge, uncontaminated sludge or sludge contaminated with lower rates of metals. Khan(Khan, 1997) reported that C_{mic}/C_{org} decreased significantly with the increasing level or toxicity of Cd in a red soil.

The decline in C_{mic}/C_{org} in metal contaminated soils occurs mainly due to reduction in the rate of carbon mineralization that in turn results in the accumulation of organic matter in the soil. Many workers reported reduced rates of organic matter decomposition in metal contaminated soils (Chander, 1991; Aoyama, 1993; Yeates, 1994; Dar, 1994). Chander and Brookes(Chander, 1991) found that Cu at about 2.5 times of permitted metal limits caused an increased accumulation of organic carbon, about 30% in sandy loam and 13% in silty clay loam soils. Zn at about the same concentration increased organic C by 9%—14%. Valsecchi *et al*. (Valsecchi, 1995) observed a close positive relationship among the metals and the organic carbon contents in soils. They ascribed it as an adverse effect of heavy metals on soil microflora, which appeared to increase the accumulation of organic matter as the heavy metals contents increased, probably because the biomass was less effective in mineralizing the soil organic matter under these conditions.

Another factor responsible for the reduction in C_{mic}/C_{org} is the low efficiency of the substrate conversion into biomass. Chander and Brookes (Chander, 1992), in a laboratory experiment observed that addition of soil inoculum plus glucose in repeatedly fumigated high and low metal containing soils increased microbial biomass in both soils, but the amount of biomass synthesized in the high-metal soil was about half of that developed in the low-metal soil. They suggested that conversion efficiency of substrate C to biomass C was lower in the high-metal soils than in the low-metal soils. Bardgett and Saggar (Bardgett, 1994) found that the microbial biomass ^{14}C formed following the addition of glucose was consistently lower in metal-contaminated soil than the control soil. More glucose-derived $^{14}CO_2$ was evolved from contaminated soil than from uncontaminated soil. The ratio of both total respired $^{14}CO_2$: biomass ^{14}C was greater in the contaminated soil than in uncontaminated soil. They concluded that the microbial biomass in soils contaminated with heavy metals were less efficient in utilization of substrate for biomass synthesis and needed to expend more energy for maintenance requirements.

3 Microbial biomass N and ratio of biomass C to N

The measurements of microbial biomass nitrogen (N_{mic}) provide another mean of assessing the effect of metals on soil microbial biomass. A few workers so far reported the effect of metals on biomass nitrogen and biomass C∶N ratio. Patra *et al*. (Patra, 1991) in an incubation experiment on a sandy loam soil, observed a decrease in the N_{mic} with increasing concentrations of Pb, Cd and Hg in soils. The lowest value of N_{mic} was found in soils with 20 ppm Cd and 10 ppm Hg. A further study by Patra *et al*. (Patra, 1992) indicated a significant decrease in the N_{mic} with increasing levels of Cr, Se, and Ni in the soil. Witter *et al*. (Witter, 1993) found that 60% reduction in the N_{mic} in soils collected from metal contaminated sludge-treated plots. The metal concentrations at which these reductions occurred were Zn 230, Cd 0.7, Cu 125, Ni 35, Pb 40 and Cr 85 mg/kg soil. Bardgett *et al*. (Bardgett, 1994) reported a significant decrease in the N_{mic} along a gradient of increasing Cu, Cr and As concentrations in a pasture soil. Recently Khan *et al*. (Khan, 1998a) reported an overall 13.5% to 81.6% decline in the biomass nitrogen due to increasing levels of Cd (5—100 μg/g), Pb (100—600 μg/g) and Zn (100—250 μg/g) in a red soil. The relative toxicity of the metals in decreasing the N_{mic} was in the order of Cd>Zn>Pb.

The C∶N ratios of microbial biomass have been shown to increase considerably with increasing metal toxicity in the soils (Patra, 1991; 1992; Khan, 1998a). Khan *et al*. (Khan, 1998a) observed that a biomass C∶N ratio of 4.6 in the uncontaminated control soil increased sharply due to soil contamination with Cd, Pb and Zn. An increase of 6.4 to 14.1 at Cd levels of 5 to 100 μg/g, 5.0 to 11.4 at Pb levels of 100 to 600 μg/g and 4.8 to 13.7 at Zn level of 50 to 250 μg/g soil was reported. It was concluded that the changes in the biomass C∶N ratio can be served as an indicator of the alteration in the microbial community structure caused by soil pollution with heavy metals.

4 Factors affecting metal toxicity to soil microbial biomass

An extensive literature exists on factors affecting the metal toxicity to microorganisms using laboratory media or simple model system, but knowledge regarding the effect of various factors on metal toxicity towards soil microbial biomass is still very limited (Baath, 1989). However, studies have shown a significant negative correlation between the biomass carbon and the available metal contents in soil (Aoyama, 1993; Dar, 1996). Thus it is expected that all those factors which affect the metal bioavailability in soil might influence the toxicity of metals to soil microbial biomass.

Addition of organic matter is an important factor affecting the metals availability and hence their potential toxicity in the soil environment(Alloway, 1990), but studies indicated that sewage sludge or farmyard manure application did not affect the metal toxicity to soil microbial biomass. Dar and Mishra(Dar, 1994) observed that significant inhibitory effects of Cd on microbial biomass at concentrations of 25 and 50 μg/g soil were slightly reduced but not mitigated by sludge addition to the soil. Similarly, Aoyama and Itaya (Aoyama, 1995) reported that addition of farmyard manure or orchard grass at 20g/kg soil did not reduce the Cu toxicity to microbial biomass in two Cu polluted soils.

On the other hand, soil texture has been shown to significantly affect the metal toxicity to soil microbial biomass. Dar(Dar, 1996) found that negative effects of Cd to microbial biomass at 50 μg/g soil were more pronounced in sandy loam than in loam or clay loam soils. In another study, Dar(Dar, 1997) observed that Pb addition at 250 μg/g soil significantly decreased microbial biomass only in sandy loam but not in loam or clay loam soils, whereas at 500 μg Pb/g soil, a significant reduction of 16%—26% occurred in all the soils, more in sandy loam and less in loam.

Another important factor affecting the metal toxicity to microbial biomass is the pH of soil. A decrease of soil pH from 7.0 to 4.5 caused about 75% reduction in microbial biomass in Cu polluted soils(Knight, 1997). Recently Khan *et al*. (Khan, 1997) found that presence of acetate in a Cd-contaminated low pH(4.51) red soil caused a significant increase in Cd toxicity to soil microbial biomass. In another study, Khan *et al*. (Khan, 1998b) reported that acetate addition to a Cd-spiked red soil at 900 and 2700 μg/g caused two- to six-fold more reductions in biomass carbon compared to same Cd levels with no acetate. The increased Cd toxicity was attributed to the adsorption of free acetate in the soil that in turn reduced the Cd adsorption, increasing its bioavailability and hence toxicity to the microbial biomass.

5 Conclusion

Metal contamination of soils caused significant reductions in the size of soil microbial biomass. In most of the cases, decline occurred at quite low metal concentrations and persisted for several years. This implies that heavy metal concentrations in soils near the current EC limits can probably led to a considerable inhibition of soil microbial biomass with long-term effects on soil productivity. However, the critical toxicity levels for soil microbial biomass are difficult to assess because of the range of values found in the literature. This fluctuation mainly arised due to differences among the metals, their sources and the physico-chemical properties of the soils. A part of the variation also resulted from the differences of the methodology and experimental conditions. The relative toxicity of metals, however, is fairly constant and the following degree of toxicity appears to be most commonly found: Cd>Cu>Zn>Pb.

There are several areas which warrant further investigation. For example, under natural conditions, pollution never occurs due to one single metal. Instead combinations of several elements, often together with other pollutants are commonly present. A few efforts have been made so far to study the response of soil microbial biomass to such combinations of pollutants. Similarly, there is very little information about soil properties and other factors affecting the metal toxicity to soil microbial biomass. Also the data regarding the effects of metals on microbial biomass nitrogen and phosphorus is clearly lacking. So these areas should be given due importance in the future research.

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(Received for review December 22. Accepted February 17, 1998)