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Effects of Copper Toxicity on Soil Microbial Biomass

Ayman M. El-Ghamry, Abid Subhani, Wael Moh'd, Huang Changyong and Xie Zhengmiao
College of Natural Resources and Environmental Sciences, Zhejiang University, Hangzhou 310029, China

Abstract: The wide utilization of copper in agriculture and industrial processes in addition to many other uses associated with human activities causes both point and non-point source pollution of the environment with copper. Although copper is relatively non-toxic to mammals and essential micronutrient necessary for a wide variety of prokaryotic and eukaryotic metabolic activities, is toxic to all organisms if present in elevated concentrations. Copper pollution in agricultural posing soils can adversely affect the living part of soil organic matter serious threat to sustained food and fiber production.

Key words: Copper toxicity, Microbial biomass, Enzymes, Agricultural soils

Introduction

Previous investigations carried out on the effects of heavy metals on soil microorganisms invariably indicated the adverse effects of these elements on the soil microbial activity, number and population (Olson and Thornton, 1982; Duxbury and Bicknell, 1983; Maliszewska *et al*., 1985; Hiroki, 1992; Chander and Brookes, 1991, 1993; Hattori, 1992; Huysman *et al*., 1994; Doelman and Haanstra, 1984; Aoyama and Nagumo, 1996; 1997). For example excessive heavy metal concentration in the soil has been reported to cause a decrease in microbial population (Hicks *et al*., 1990; McGrath *et al*., 1995), changes in populations structure (Chaudri *et al*., 1993; Bardgett *et al*., 1994; Huysman *et al*., 1994) and physiological activity (Bitton and Dutka, 1986; Cotrufo *et al*., 1995; Valsecchi *et al*., 1995). The role of microbial biomass in soil fertility by serving as a reservoir of plant nutrients (esp. N, P and S) and as catalyst in the cycling of C, P and S (Smith and Paul, 1990; Lee and Pankhurst, 1992) make microbial biomass indispensable in soil nutrient flux and bioavailability. Toxicity of heavy metals in plants and animals have been well established but little is known about their adverse effects on soil microbial biomass (Baath, 1989; McGrath *et al*., 1995). Of these metals, copper is extensively used in agriculture in form of metal containing fertilizers, bactericides, fungicides, algicides and in form of metal contaminated composts, sewage sludges, as well as feed additive in antibiotics, drugs, growth promoters etc, (Adriano, 1986). Its abundance in the earth crust (24-55 $\mu g\ g^{-1}$) and soil (20-30 $\mu g\ g^{-1}$) (Alloway, 1990) makes it a pollution problem in most agricultural soils. Copper is highly toxic to microorganisms if present in excess concentration (Flemming and Trevors, 1989; Maliszewska *et al*., 1985; Alloway, 1990) which consequently cause change in soil biological equilibrium with adverse effect on both soil fertility, plant development and yield (Huisingh, 1974). A recent study by He *et al*. (1997) indicated a highly significant correlation between the biomass-C, N and the N uptake by plants indicating that microbial biomass-C and N are not only important soil fertility parameters but also important indices of soil sustainability. This paper therefore reviews the effect of copper toxicity on soil microbial biomass with particular attention to microbial biomass-C, N and enzyme activity.

Effect of copper on soil microbial biomass: Soil microbial biomass consists of many species of bacteria and fungi together with larger soil organisms such as yeast, algae and protozoa. However, inspite of the specie specific role in soil, microbial biomass is often considered as a single compartment (Brookes *et al*., 1985). Previous studies carried out on microbial biomass in metal polluted soils indicated decrease in soil biomass e.g., (Brookes and McGrath, 1984; Brookes *et al*., 1986; Zibilske and Wagner, 1982). Maliszewska *et al*. (1985) put the relative toxicity of metal compounds on soil microorganisms in the following order: Hg (metallic) > $CuSO_4$ > $HgCl_2$ > Hg_2Cl_2 > HgO > As_2O_3 > $ZnSO_4$ > $NaHAsO_4$ > Pb $(NO_3)_2$.

Copper is known to be very toxic to microorganisms in the free ionic form especially Cu^{2+} and $CuOH^+$ (Zevenhuizen *et al*., 1979). The addition of copper to soil was reported to significantly decrease the amount of microbial biomass and exert a pronounce toxic effect on the decrease size of the biomass compared to certain metals such as Pb and As when compared on a molarity basis (Aoyama and Nagumo, 1997). Also he concluded that in apple orchard soils with heavy metal accumulation Cu was the primary factor affecting microbial biomass. Knight *et al.* (1997) conducted a study of the effect of Cd, Cu and Zn on microbial biomass and reported that only Cu showed a notable reduction in microbial biomass and reduced the metabolic potential of soil microbial population. In general bacteria are more sensitive to Cu than fungi and most Cu resistant bacteria are oxidase positive and 50% of the strains are pigmented (Huysman *et al*., 1994). Low doses of Cu (10-100 $\mu g\ g^{-1}$) added to sandy and alluvial soil showed a marked harmful influence on the growth of bacteria, actinomycetes, cellulytic, microflora and nitrifiers in both soils (Maliszewska *et al*., 1985).

Kobus and Kabata-Pendias (1977) and Wilson (1977) had earlier observed inhibition of growth of azobacter and nitrifiers by the presence of high concentration of Cu in soil. Hattori (1992) also reported a marked decrease in soil bacterial colonies by addition of 10 $\mu mol\ g^{-1}$ soil Cu to Gley and Andosol soil. He further observed that addition of 20 $\mu mol\ L^{-1}$ of Cu decreased the number of colonies to one tenth of the control. Hemida *et al*. (1997) found that application of $CuSO_4$ to clay soil for one week resulted in the reduction of bacterial count ($p < 0.05$) with 200 and 2000 $\mu g\ g^{-1}$ soil but same doses enhanced bacterial count in sandy soil one and four weeks after application although the count was only significant after four weeks of application.

The addition of Cu to a paddy soil, up to 1600 $\mu g\ g^{-1}$ soil, was reported to increase fungal population size, proportional to the Cu concentration added, yet decreased fungal diversity by 40%. (Hiroki *et al*., 1985). The number of fungi in gley soil was shown to reduce with Cu increase by more than 100 times compared to control (Hattori, 1992). Similar fungal reduction in excess Cu was earlier observed (Schnurer, 1989). The increase in fungal population was related to the inhibition of CO_2 evolution from soil (Hattori, 1992). Hiroki *et al*. (1985) had reported a positive correlation coefficient between the Cu content and the number of fungal colonies in the soil and that Cu tolerant fungi especially the genus *penicillium* to be dominant in Cu polluted soils. However, a contradiction was reported by Maliszewska *et al.* (1985) where growth of fungi was found to be stimulated by the addition of Cu in soils with high doses (5000-10,000 $\mu g\ g^{-1}$ Cu). (Hemida *et al*., 1997). The authors observed a non significant effect on

thermophillic and *thermotolerant fungi* in clay soil and a significant reduction in sandy soil one week after application but three weeks later the count significantly increased. They reported the count of *actinomyces* isolated in the two soils to be very low or not measurable during the first four weeks of application.
Zibilske and Wagner (1982) on the contrary reported that *penicillium* and *paecilomyces* decreased with increase Cu concentration in soil while *Trichoderma*, *Rhizopus* and *Asprgillus* increased. However, Hiroki *et al*. (1985) argued that it is quite probable that *penicillium* is the dominant genus in copper polluted soils because in their study most 1000 μg Cu ml^{-1} tolerant fungi were *penicillium* and the growth or *sporulation* of some of the *penicillium* isolated from the soil were stimulated in medium containing Cu and about 65% of all the isolates at high Cu concentrations were *penicillium*. Chander and Brookes (1993) observed that C_{mic}/C_{org} in soils contaminated with higher rates of Cu was less than half of that in the soils which received no sludge, uncontaminated sludge or sludge contaminated with lower rates of metals. 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 soil. Chander and Brookes (1991) have reported reduced rates of organic matter decomposition in metal contaminated soils and they found that Cu at about 2.5 times the permitted metal limits caused an increased accumulation of organic carbon, about 30% in sandy loam and 13% in silty clay loam soils. Witter *et al*. (1993) found about 60% reduction in the N_{mic} in soils collected from metal contaminated sludge-treated plots. The metal concentration at which this reductions occurred was 125 μg g^{-1} soil Cu. Also Bardgett and Saggar (1994) reported a significant decrease in the N_{mic} along a gradient of increasing Cu concentrations in a pasture soil.

Effect of copper on microbial enzyme activity: Microbial enzyme activity has also been previously studied in relation to heavy metal pollution in soil (Mathur *et al*., 1980; Nordgren *et al*., 1986). The activities of dehydrogenase (Wilke, 1988; Schnurer, 1989), phosphatase (Speir *et al*., 1992) and urease (Hemida *et al*., 1997) were found to decrease in a wide range of Cu polluted soils and could be used as indicators of the toxic effects of Cu on soil microbial populations. However, due to highly significant correlation between the microbial biomass and the dehydrogenase activity (Wilke, 1988), C-mineralization and dehydrogenase activity (Serra-Wittling *et al*., 1995) and 0.1M $CaCl_2$-extractable Cu and dehydrogenase activity, the measurement of dehydrogenase activity was regarded as the most sensitive parameter for the detection of harmful effects of Cu on the soil microflora (Wilke, 1987,1988; Aoyama and Itaya, 1995). Several workers indicated the effect of Cu contamination on soil microbial enzymes, for example: Tyler (1974). Tyler (1981) showed lower enzyme activity with low levels of Cu concentration. Mathur *et al*. (1980) studied several hydrolytic enzymes in relation to Cu content of cupriferous bogs and found that although differences existed in the degree of inhibition, most enzymes were affected in a similar way. They also noticed that enzyme activities were all found to be lower in soil containing higher Cu. Yet in another study carried out by Hemida *et al*. (1997) copper was found to reduce urease activity in soils at the rate of 200 μg g^{-1}soil in both clay and sandy soil. The authors also observed similar effect on nitrate reductase although much weaker than that of urease. However, they found a marginal effect on amidase activity, which was only significantly affected at high application rate of 2000 μg C g^{-1} soil. A somewhat similar observation had been made by Frankenberger and Tabatabai (1981) who reported that metal ion does not strongly inhibit amidase activity. High concentration of Cu was observed to markedly inhibit the activity of dehydrogenase in sandy and alluvial soils (Maliszewska *et al*., 1985; Chander and Brooks 1991; Chander *et al*., 1995). It was found a significant decline in the dehydrogenase activity in soils amended with metal-enriched sludges (Chander *et al*., 1995). Further study by Chander and Brookes (1991) indicated that the main reason of decreased dehydrogenase activity was the abiological reaction between TPF and Cu, rather than a biological effect. All these observation agreed with the early study conducted by Tyler (1974) who observed general inhibition of soil enzymes by excess Cu contamination.

Effect of copper on microbial activity.: Measurement of soil respiration rate was largely used over the years in relation to heavy metal pollution (Baath, 1989). Most heavy metals pollution exhibited little effect on CO_2 evolution at low levels of contamination, but with higher doses, a significant decrease in soil respiration rate was reported (Capone *et al*., 1983; Nordgren *et al*., 1983; Speir *et al*., 1992; Aoyama *et al*., 1993; Cotrufo *et al*., 1995).
However, there were a few exceptions that reported increase in the respiration rate due to metal addition to soils (Bond *et al*., 1976; Capone *et al*., 1983). These differences suggested that measurement of soil respiration alone could not be efficient in determining the toxic effects of metals on the size and activity of the soil microbial biomass (McGrath *et al*., 1995). However, soil respiration rate was easy to be measured and appears to be a sensitive measurement with which to detect heavy metal pollution (Baath, 1989). The standardization of soil water content minimized the variability among the samples and can detect changes in respiration rate at lower contamination levels than soil samples with field moisture content (Tyler, 1974). Combining microbial activity and the population measurement, such biomass specific respiration appears to provide a more sensitive indication of the soil pollution by heavy metals (Brookes and McGrath, 1987; Brookes, 1995). Fliebach *et al*. (1994) measured respiration in soil of low and high metal sludge and observed that soil respiration especially the respiration per unit biomass (specific respiration) increased with the increasing heavy metal concentrations in soil. Recent study (Bardgett *et al*., 1994) measured the specific respiration (respiration per unit biomass) in the top 5 cm of a pasture soil and reported a significant increase in the ratio of respired C to biomass C along a gradient of increasing Cu concentration. In addition to other reports that indicated the negative effect of high doses of copper on CO_2 evolution (Jackson and Watson, 1977; Mathur *et al*., 1980; Nordgren *et al*., 1986). Mathur *et al*. (1980) reported in a muck (sapric) sample containing 2922 μg g^{-1} of total Cu lost carbon through aerobic soil respiration at half the rate of a muck sample containing 1159 μg g^{-1} Cu. Similarly the soil respiration rate of the peat (hemic) sample containing 797 μg g^{-1} Cu was half that of the peat sample with 408 μg g^{-1} Cu. Hattori (1992) found that copper exerted an inhibitory effect of CO_2 evolution from the Gley soil and less conspicuous effect in light colored Andisol. Earlier studies have also indicated the effect of heavy metals on N-mineralization in the field (Brooks *et al*., 1986) and N-mineralization had been indicted to be a very sensitive measurement of metal pollution (Tyler, 1981). Premi and Cornfield (1969) observed that a relatively large doses of Cu stimulated the activity of nitrification and ammonification in soils with 100 μg g^{-1} Cu and a distinct inhibition of this process with 10000 μg g^{-1} Cu. Brookes *et al*. (1986) found a 50% decrease in N_2-fixation by green algae in sludge amended soil at a very low level of Cu contamination (15 μg g^{-1} Cu).
Copper was also observed to inhibit N_2-fixation by free living *heterotrophic bacteria* in the soil and reduced that of cyanobacteria by 50% at the concentration of 37 μg g^{-1} Cu. It

reduced that of *Rhisobium leguminosarim* by several orders of magnitude at soil Cu concentration of 27-48 μg g^{-1} Cu (McGrath *et al*., 1988). Several other reports indicated similar negative effect of Cu on N_2-fixation (Porter and Sheridan, 1981; McGrath *et al*., 1988).

Conclusion

Most studies indicated the toxicity of excess Cu to size of soil microbial biomass, their activity and enzymes. The few contradictions may be attributed to different properties of soils, experimental methods, conditions etc. However, the effect of Cu contamination on soil microbial biomass seriously affects the recycling of nutrients in the soil and thus plant growth and yield. The combined effect of Cu interaction with other metals, microbial adaptation and subsequent tolerant to Cu by microorganisms, different soils organic matter content etc. suggest the need for a well plan standard study of the effect of copper toxicity on soil microbial biomass.

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